US 20230159626A1

(19) **United States**

(12) **Patent Application Publication**

OGEMBO et al.

(10) **Pub. No.: US 2023/0159626 A1**

(43) **Pub. Date: May 25, 2023**

(54) **EPSTEIN-BARR VIRUS ANTIBODIES AND USES THEREOF**

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(21) Appl. No.: **17/816,188**

(22) Filed: **Jul. 29, 2022**

Related U.S. Application Data

(63) Continuation of application No. 16/609,078, filed on Oct. 28, 2019, now Pat. No. 11,401,323, filed as application No. PCT/US2018/030030 on Apr. 27, 2018.

(60) Provisional application No. 62/491,945, filed on Apr. 28, 2017.

Publication Classification

(51) **Int. Cl.**

C07K 16/08	(2006.01)
A61K 47/68	(2006.01)
A61K 47/65	(2006.01)
A61K 35/763	(2006.01)
A61K 38/16	(2006.01)
A61K 39/245	(2006.01)

(52) **U.S. Cl.**

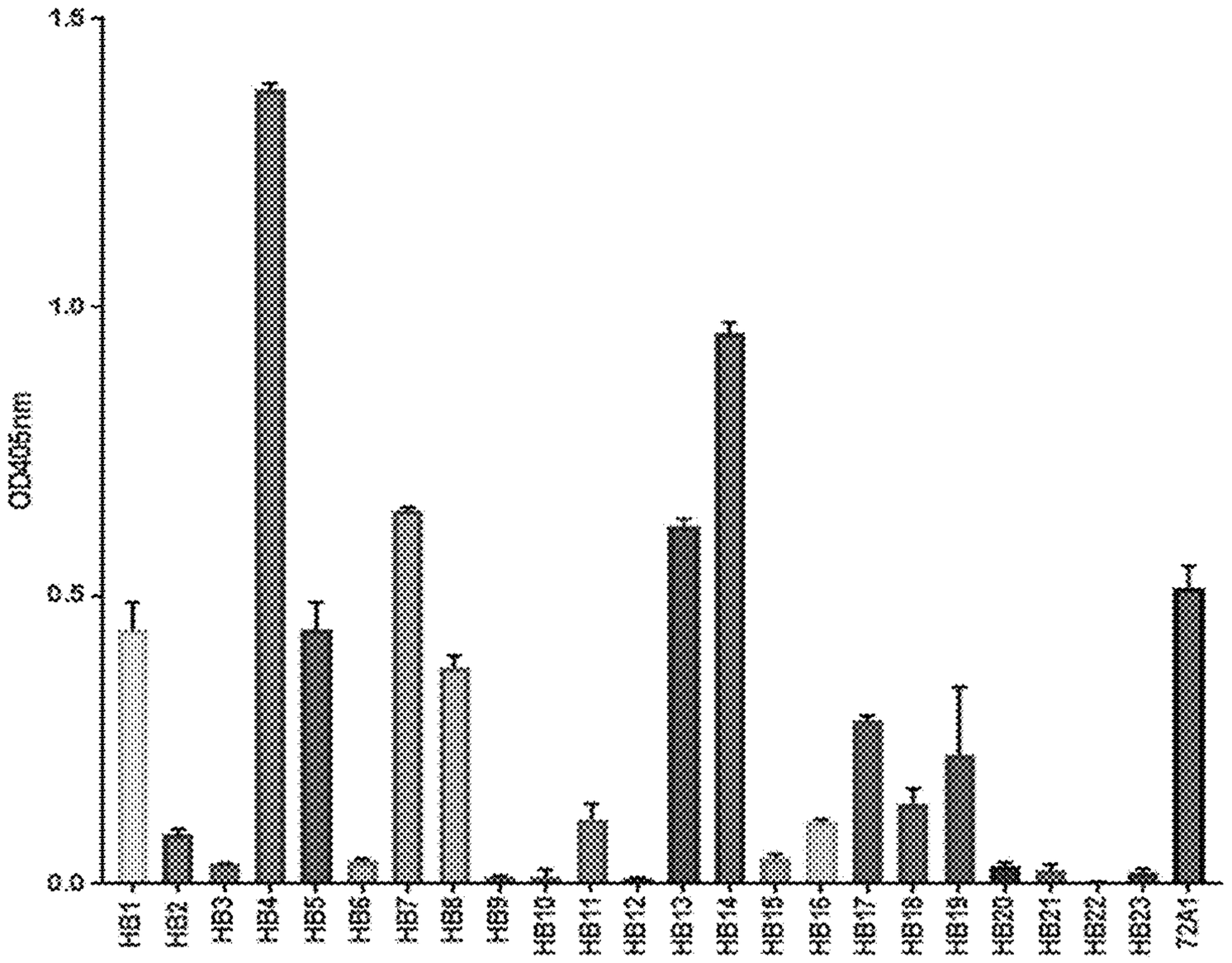
CPC ***C07K 16/085*** (2013.01); ***A61K 47/6811*** (2017.08); ***A61K 47/65*** (2017.08); ***A61K 47/6839*** (2017.08); ***A61K 35/763*** (2013.01); ***A61K 38/162*** (2013.01); ***A61K 39/245*** (2013.01); *C07K 2317/24* (2013.01); *C07K 2317/34* (2013.01); *C07K 2317/76* (2013.01); *C12N 2710/16222* (2013.01); *A61K 39/42* (2013.01)

(57) **ABSTRACT**

Disclosed herein are antibodies or immunogenic fragments thereof that specifically bind to Epstein-Barr virus (EBV) glycoprotein 350 (gp350) or 220 or one or more epitopes of EBV gp350 and neutralize EBV infection. Also disclosed are immunogenic peptides comprising one or more gp350 epitopes, EBV antibody-small molecule conjugates and pharmaceutical compositions comprising the antibody or an immunogenic fragment thereof, one or more epitopes of EBV gp350, one or more immunogenic peptides, or the EBV antibody-small molecule conjugate. The antibodies, epitopes, immunogenic peptides, conjugates, and pharmaceutical compositions can be used to treat or prevent EBV infections and EBV-associated conditions and diseases.

Fig. 4C

Fig. 1A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HB2
HB3
HB4
HB5
HB6
HB7
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HB9
HB10
HB11
HB12
HB13
HB14
HB15
HB16
HB17
HB18
HB19
HB20
HB21
HB22
HB23
72A1

Fig. 1B

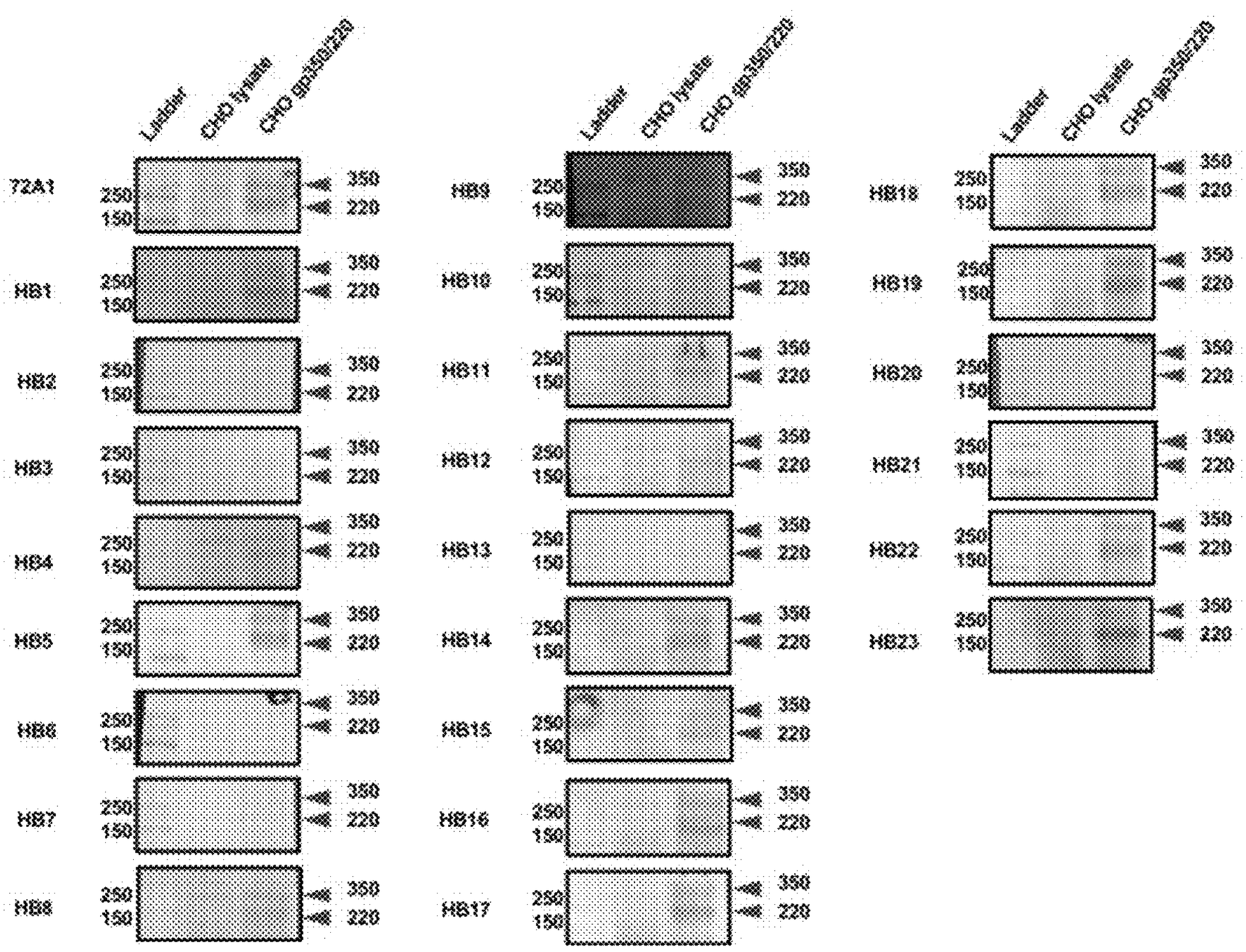

Ladder
CHO lysate
CHO gp350/220
72A1
HB1
HB2
HB3
HB4
HB5
HB6
HB7
HB8
HB9
HB10
HB11
HB12
HB13
HB14
HB15
HB16
HB17
HB18
HB19
HB20
HB21
HB22
HB23
250
150
350
220

Fig. 1C

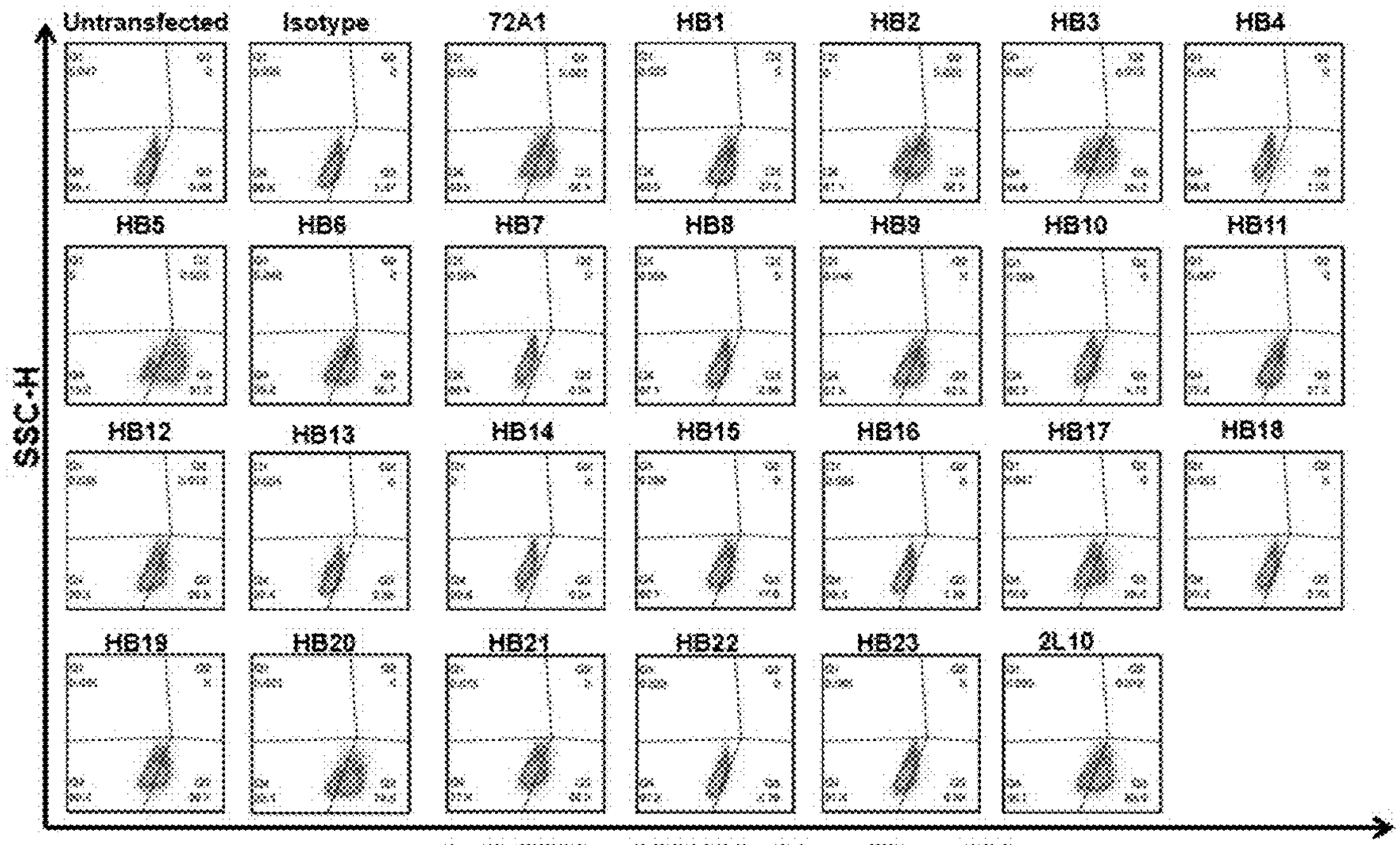
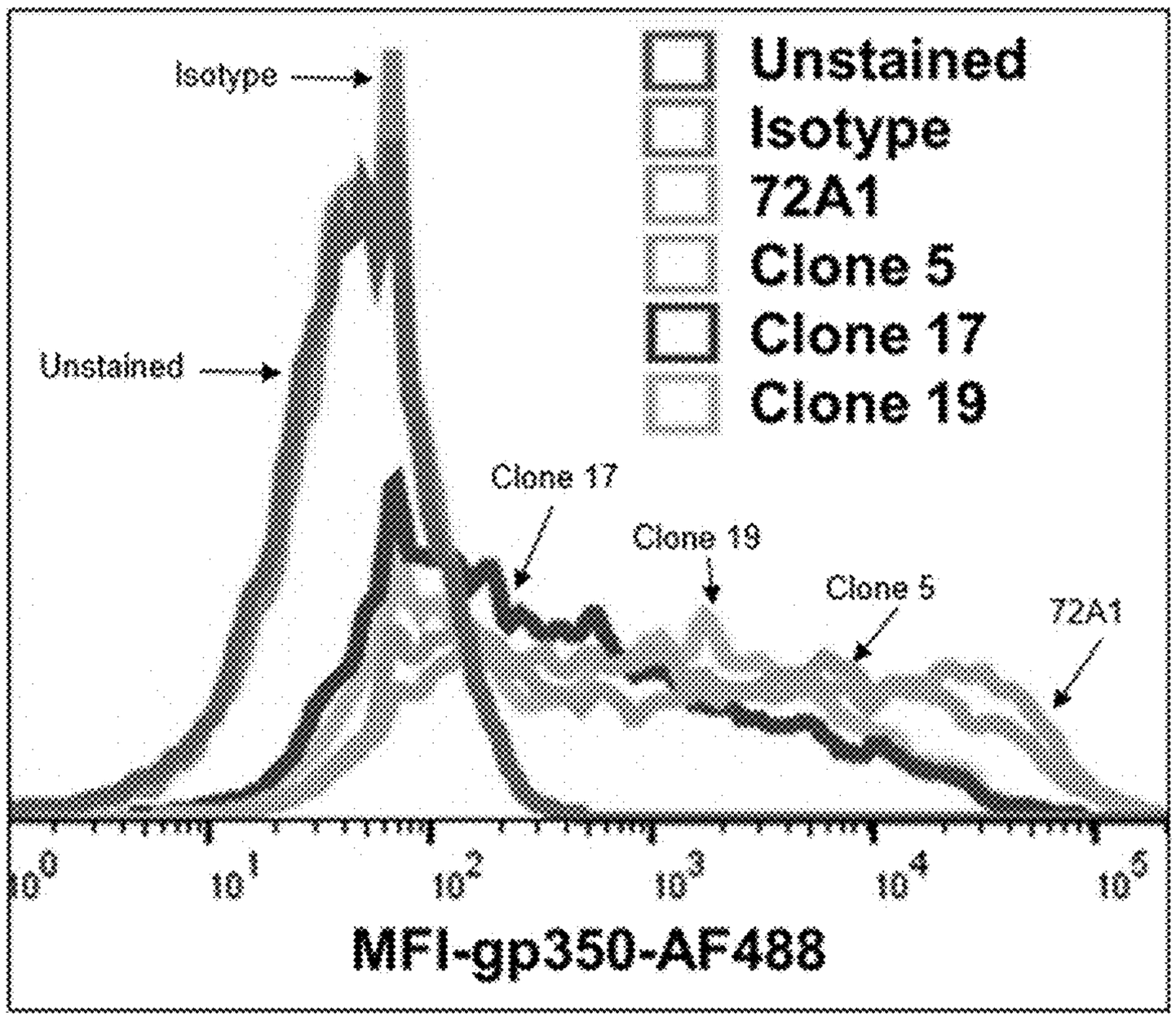

Untransfected
Isotype
72A1
HB1
HB2
HB3
HB4
HB5
HB6
HB7
HB8
HB9
HB10
HB11
HB12
HB13
HB14
HB15
HB16
HB17
HB18
HB19
HB20
HB21
HB22
HB23
2L10
SSC-H
Anti-EBV gp350/220- Alexa Fluor 488

Fig. 1D


Unstained
Isotype
72A1
Clone 5
Clone 17
Clone 19
Isotype
Unstained
Clone 17
Clone 19
Clone 5
72A1
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10^2
10^3
10^4
10^5
MFI-gp350-AF488

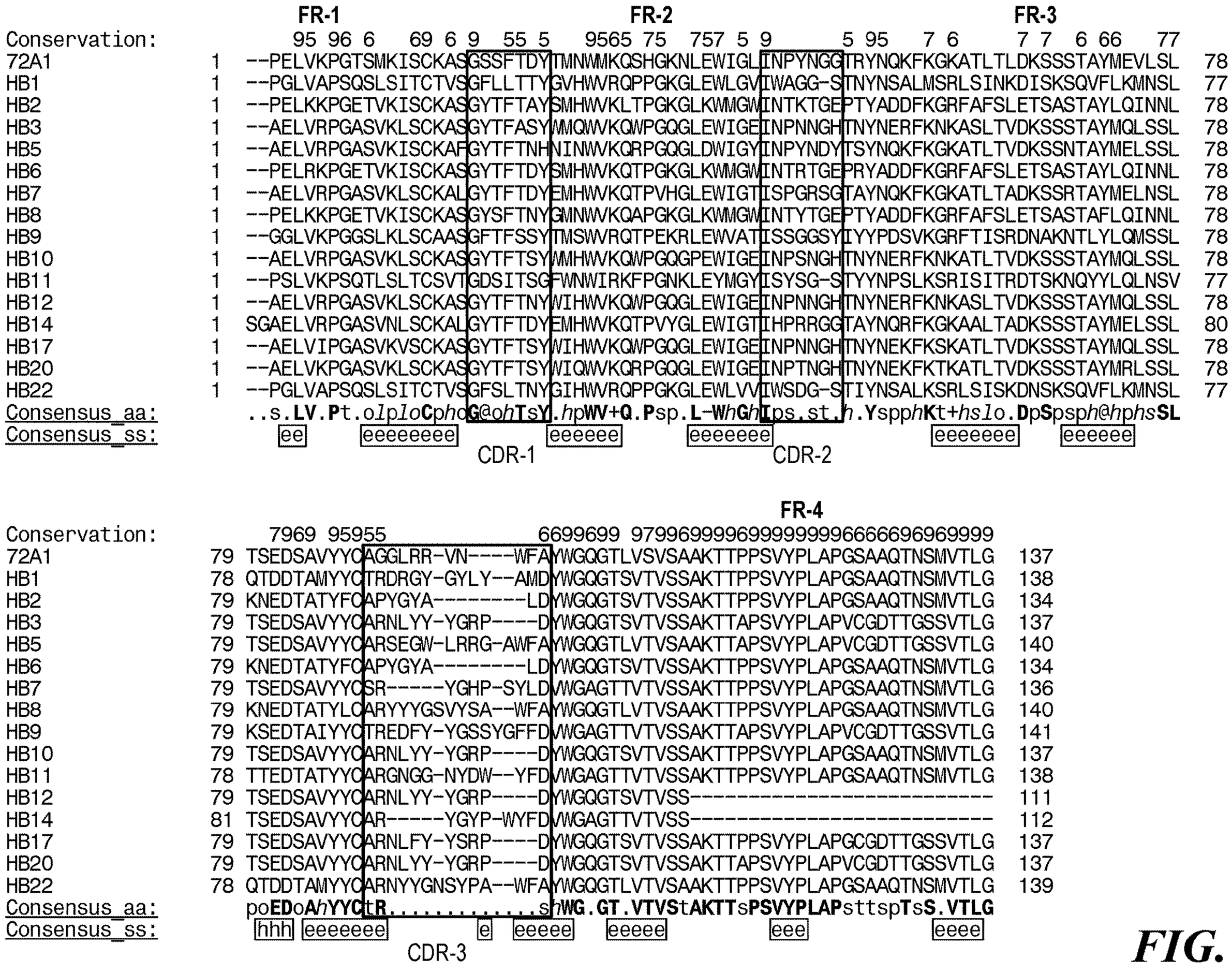
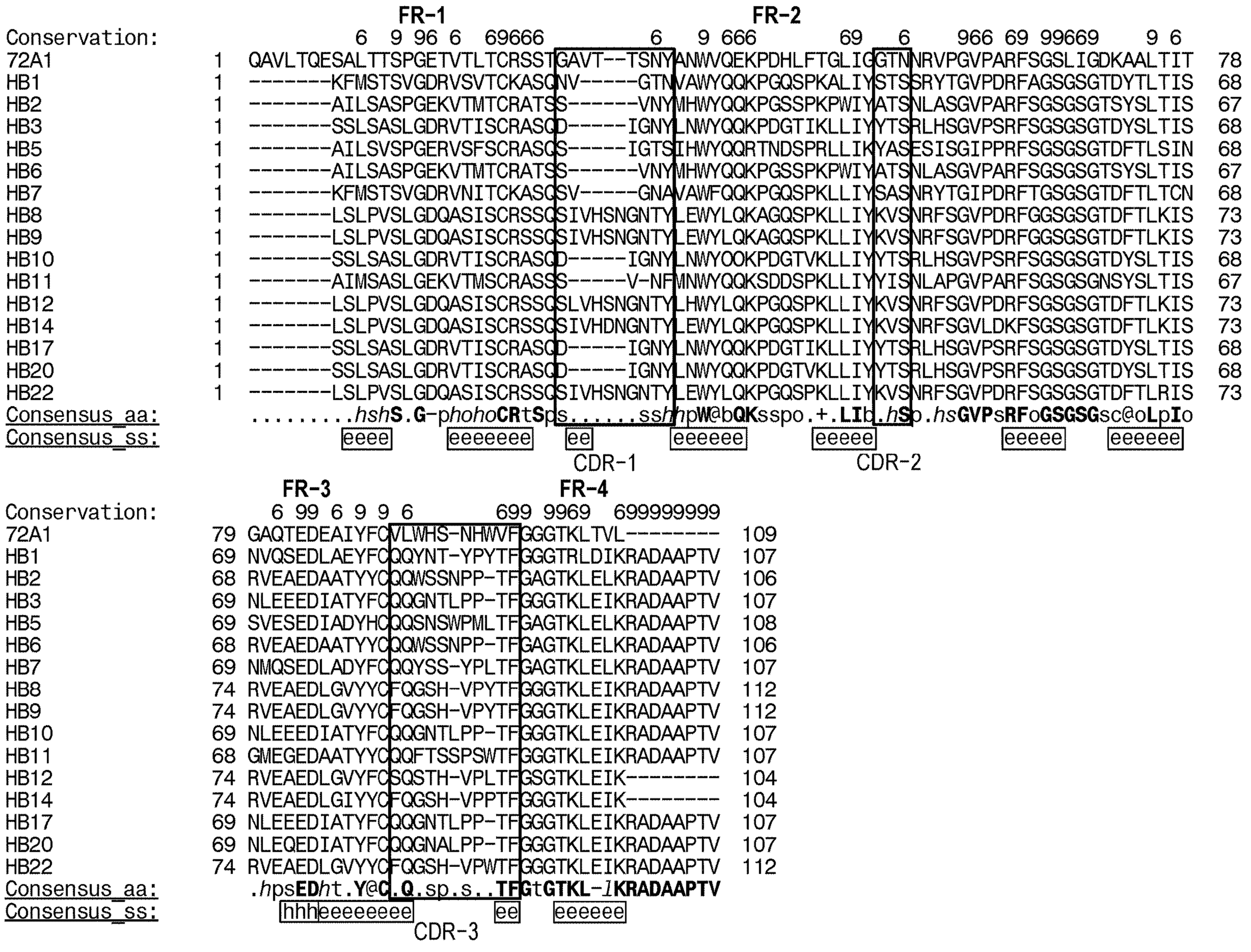

FR-1
FR-2
FR-3
FR-4
CDR-1
CDR-2
CDR-3
Conservation:
72A1
HB1
HB2
HB3
HB5
HB6
HB7
HB8
HB9
HB10
HB11
HB12
HB14
HB17
HB20
HB22
Consensus_aa:
Consensus_ss:

FIG. 2A


FR-1
FR-2
FR-3
FR-4
CDR-1
CDR-2
CDR-3
Conservation:
72A1 1 QAVLTQESALTTSPGETVTLTCRSSTGAVT--TSNYANWVQEKPDHLFTGLIGGTNNRVPGVPARFSGSLIGDKAALTIT 78
HB1 1 -------KFMSTSVGDRVSVTCKASQNV-----GTNVAWYQQKPGQSPKALIYSTSSRYTGVPDRFAGSGSGTDYTLTIS 68
HB2 1 -------AILSASPGEKVTMTCRATSS------VNYMHWYQQKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTIS 67
HB3 1 -------SSLSASLGDRVTISCRASQD-----IGNYLNWYQQKPDGTIKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIS 68
HB5 1 -------AILSVSPGERVSFSCRASQS-----IGTSIHWYQQRTNDSPRLLIKYASESISGIPPRFSGSGSGTDFTLSIN 68
HB6 1 -------AILSASPGEKVTMTCRATSS------VNYMHWYQQKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTIS 67
HB7 1 -------KFMSTSVGDRVNITCKASQSV-----GNAVAWFQQKPGQSPKLLIYSASNRYTGIPDRFTGSGSGTDFTLTCN 68
HB8 1 -------LSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKAGQSPKLLIYKVSNRFSGVPDRFGGSGSGTDFTLKIS 73
HB9 1 -------LSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKAGQSPKLLIYKVSNRFSGVPDRFGGSGSGTDFTLKIS 73
HB10 1 -------SSLSASLGDRVTISCRASQD-----IGNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIS 68
HB11 1 -------AIMSASLGEKVTMSCRASSS-----V-NFMNWYQQKSDDSPKLLIYYISNLAPGVPARFSGSGSGNSYSLTIS 67
HB12 1 -------LSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKIS 73
HB14 1 -------LSLPVSLGDQASISCRSSQSIVHDNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVLDKFSGSGSGTDFTLKIS 73
HB17 1 -------SSLSASLGDRVTISCRASQD-----IGNYLNWYQQKPDGTIKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIS 68
HB20 1 -------SSLSASLGDRVTISCRASQD-----IGNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTIS 68
HB22 1 -------LSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLRIS 73
Consensus_aa:
Consensus_ss:
72A1 79 GAQTEDEAIYFCVLWHS-NHWVFGGGTKLTVL-------- 109
HB1 69 NVQSEDLAEYFCQQYNT-YPYTFGGGTRLDIKRADAAPTV 107
HB2 68 RVEAEDAATYYCQQWSSNPP-TFGAGTKLELKRADAAPTV 106
HB3 69 NLEEEDIATYFCQQGNTLPP-TFGGGTKLEIKRADAAPTV 107
HB5 69 SVESEDIADYHCQQSNSWPMLTFGAGTKLELKRADAAPTV 108
HB6 68 RVEAEDAATYYCQQWSSNPP-TFGAGTKLELKRADAAPTV 106
HB7 69 NMQSEDLADYFCQQYSS-YPLTFGAGTKLELKRADAAPTV 107
HB8 74 RVEAEDLGVYYCFQGSH-VPYTFGGGTKLEIKRADAAPTV 112
HB9 74 RVEAEDLGVYYCFQGSH-VPYTFGGGTKLEIKRADAAPTV 112
HB10 69 NLEEEDIATYFCQQGNTLPP-TFGGGTKLEIKRADAAPTV 107
HB11 68 GMEGEDAATYYCQQFTSSPSWTFGGGTKLEIKRADAAPTV 107
HB12 74 RVEAEDLGVYFCSQSTH-VPLTFGSGTKLEIK-------- 104
HB14 74 RVEAEDLGIYYCFQGSH-VPPTFGGGTKLEIK-------- 104
HB17 69 NLEEEDIATYFCQQGNTLPP-TFGGGTKLEIKRADAAPTV 107
HB20 69 NLEQEDIATYFCQQGNALPP-TFGGGTKLEIKRADAAPTV 107
HB22 74 RVEAEDLGVYYCFQGSH-VPWTFGGGTKLEIKRADAAPTV 112
Consensus_aa:
Consensus_ss:

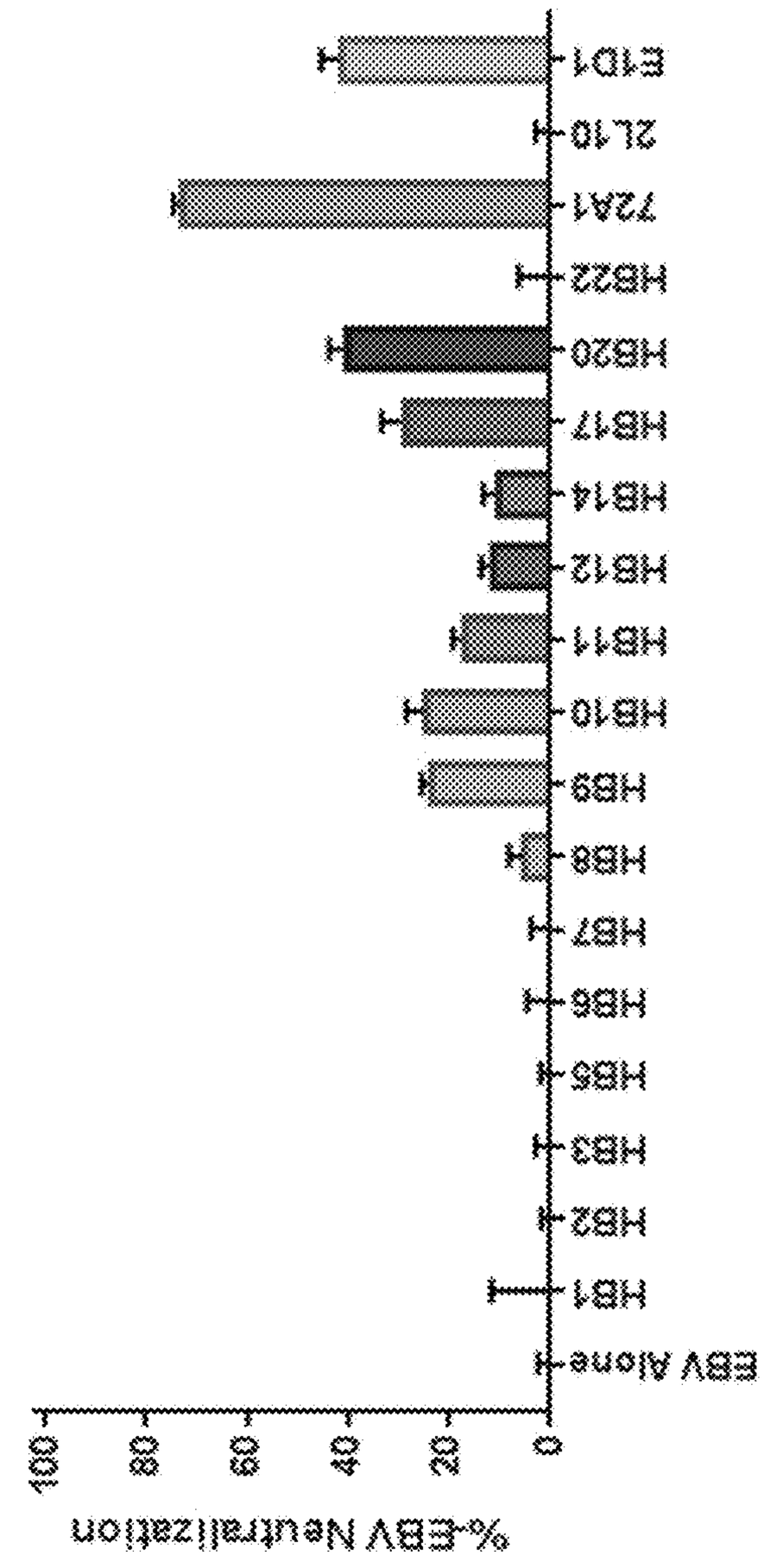

EBV Alone
HB1
HB2
HB3
HB5
HB6
HB7
HB8
HB9
HB10
HB11
HB12
HB14
HB17
HB20
HB22
2L10
72A1
E1D1
0
20
40
60
80
100
%-EBV Neutralization

Fig. 3A

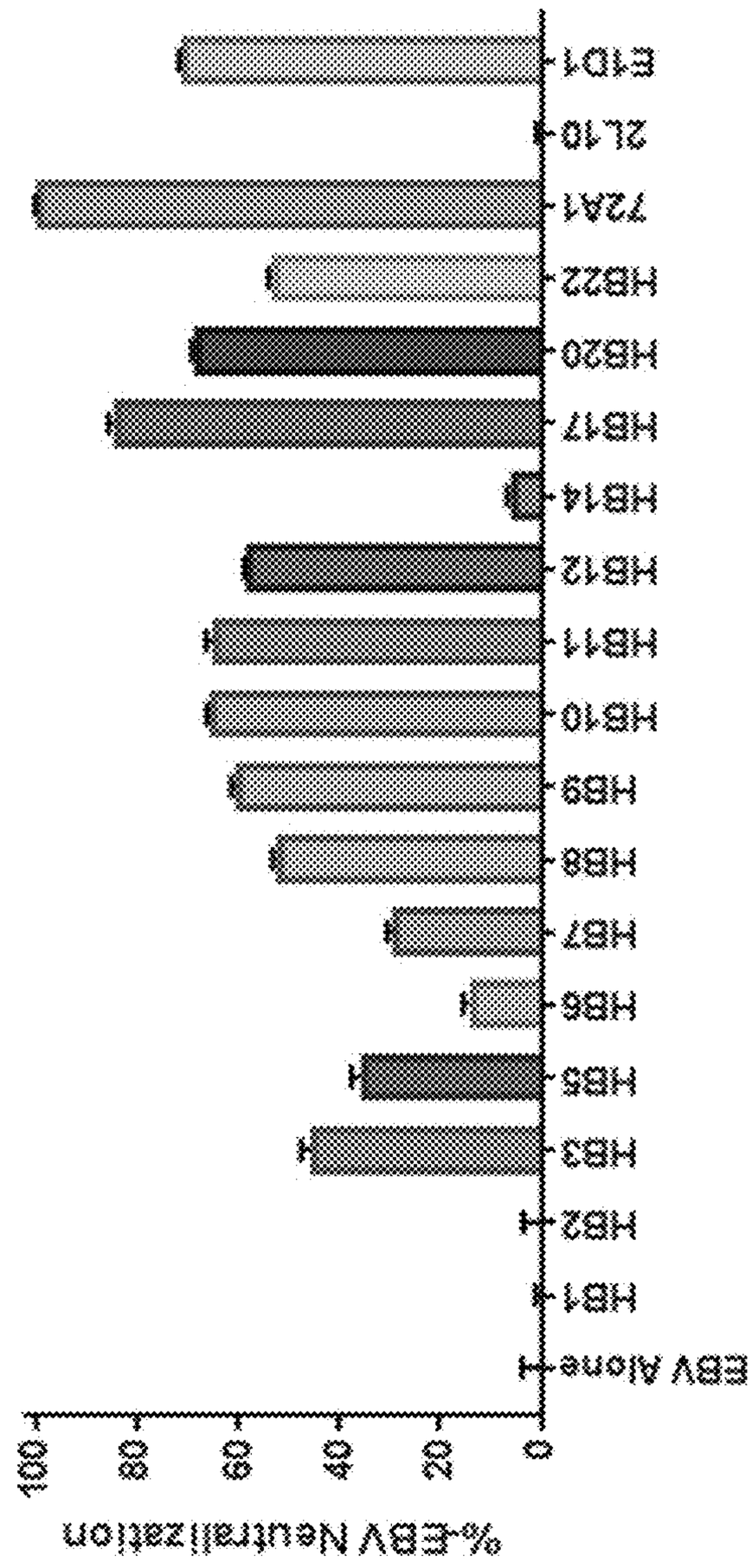

EBV Alone
HB1
HB2
HB3
HB5
HB6
HB7
HB8
HB9
HB10
HB11
HB12
HB14
HB17
HB20
HB22
2L10
72A1
E1D1
0
20
40
60
80
100
%-EBV Neutralization

Fig. 3B

Fig. 4A

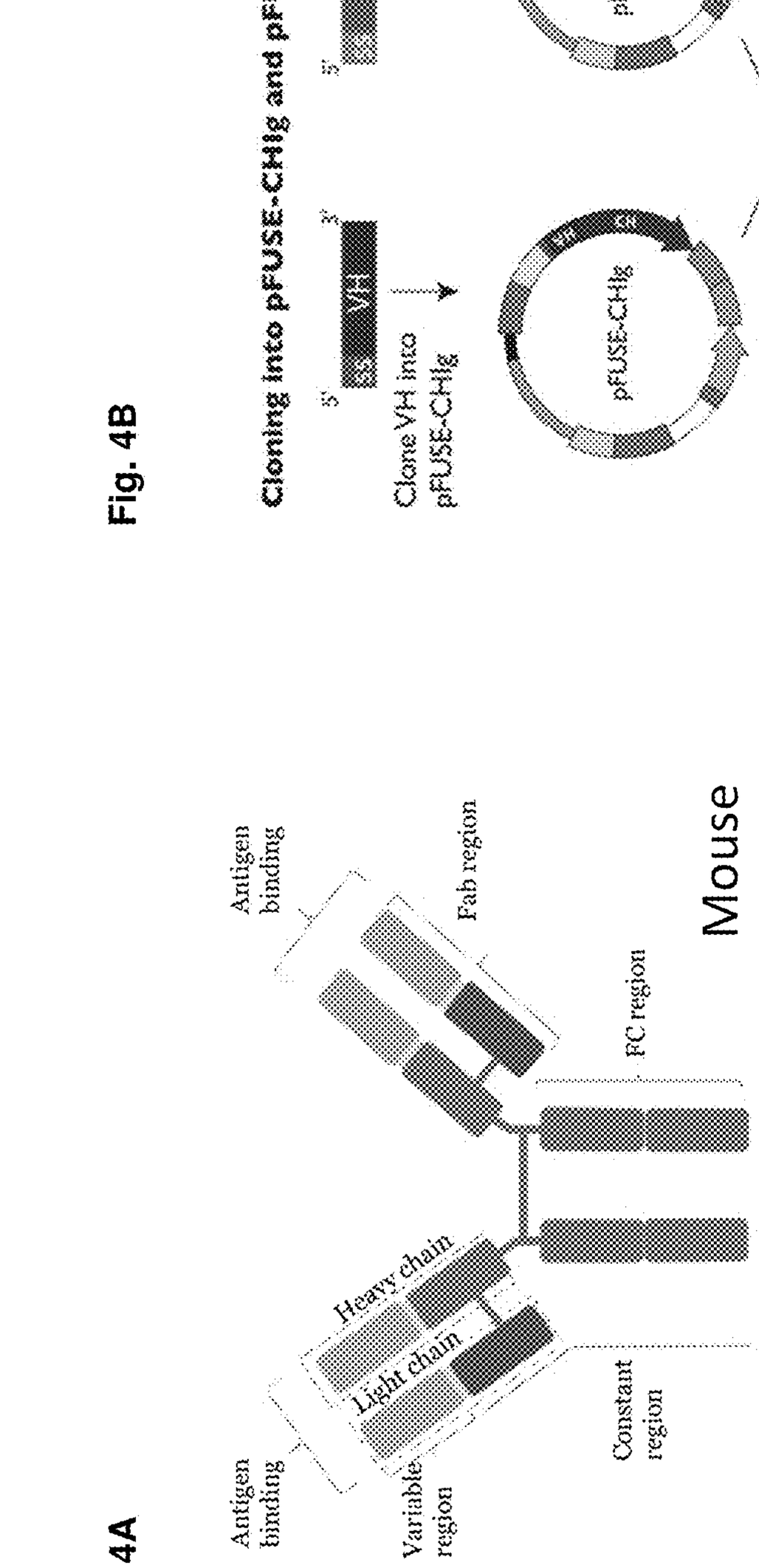


Fig. 4B

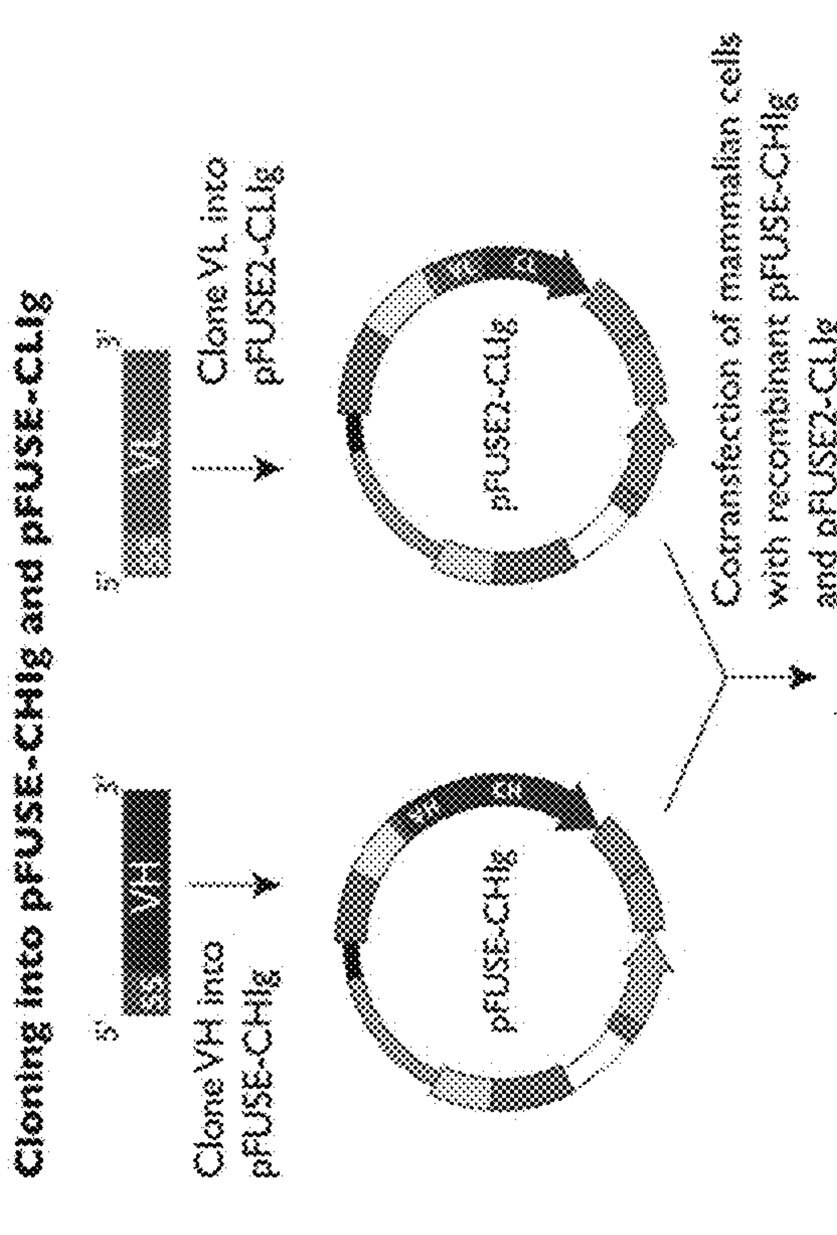


Fig. 4C

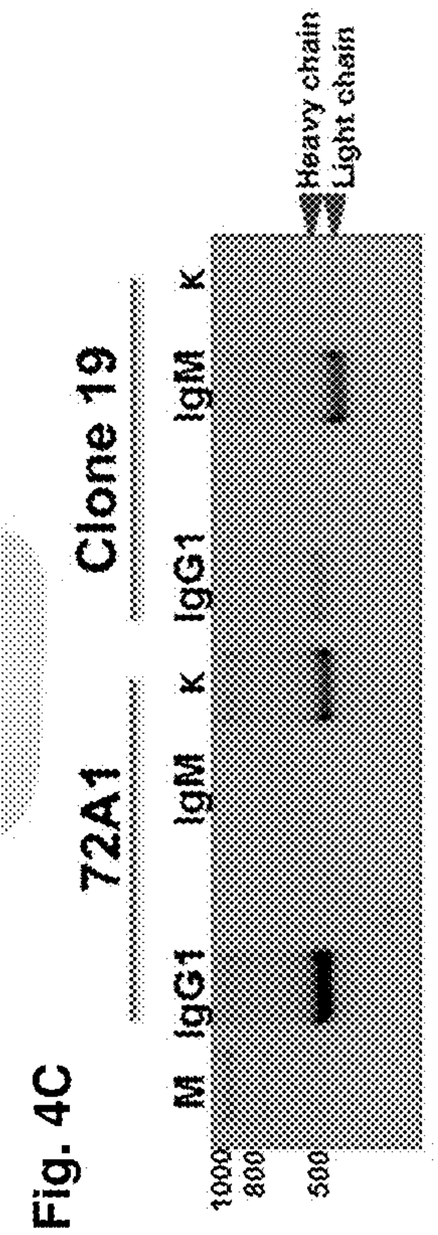
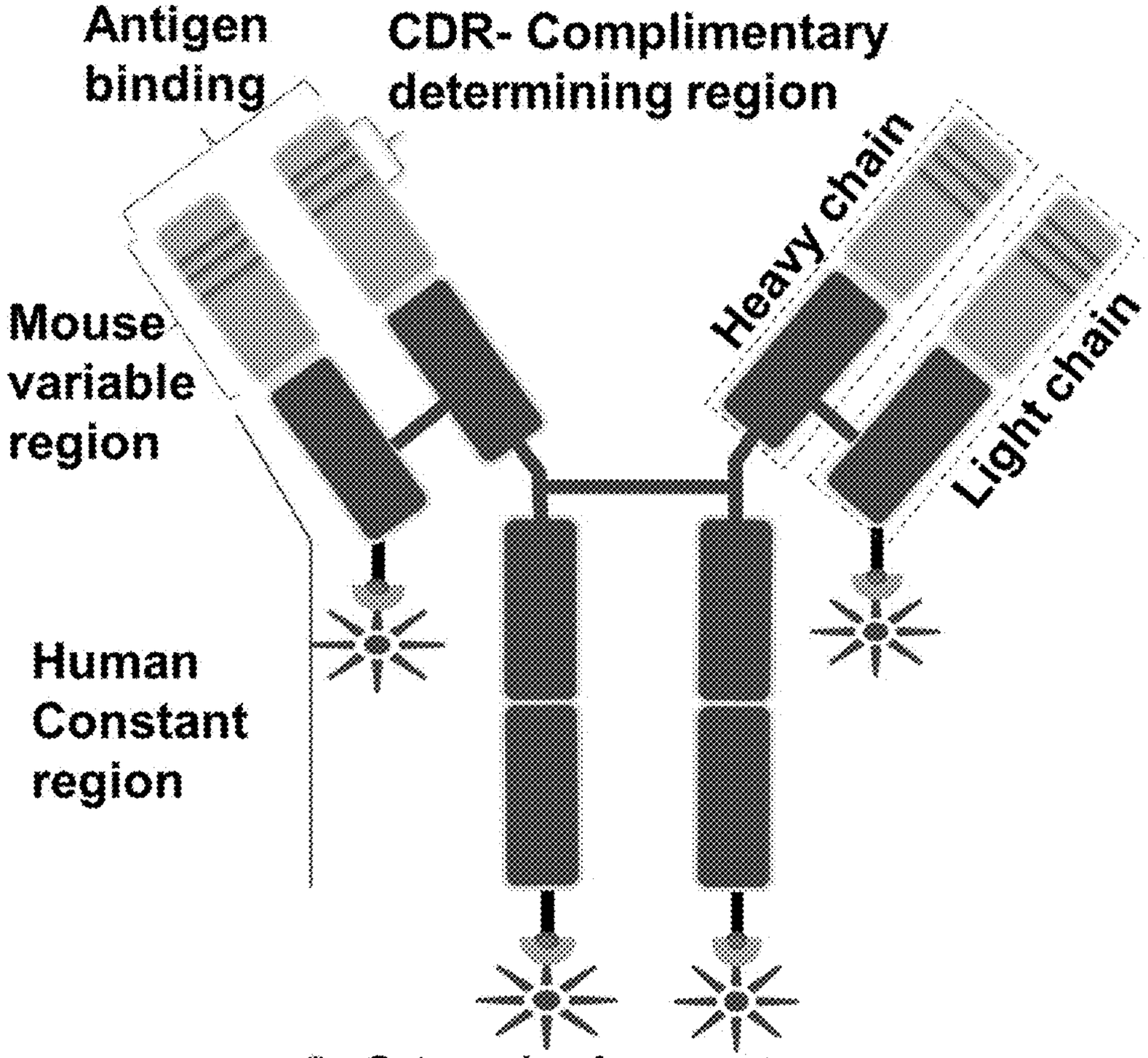


Fig. 5


Antigen binding
CDR- Complimentary determining region
Heavy chain
Light chain
Mouse variable region
Human Constant region
C-terminal acceptor group
Reactive donor group
Small molecule (L_2P_4)

EPSTEIN-BARR VIRUS ANTIBODIES AND USES THEREOF

PRIORITY CLAIM

[0001] The present invention is a continuation of U.S. patent application Ser. No. 16/609,078, filed Oct. 28, 2019, which is a 371 national phase of International Patent Application No. PCT/US2018/030030, filed Apr. 27, 2018, which claims the benefit of U.S. Provisional Patent Application No. 62/491,945, filed Apr. 28, 2017, the content of which are incorporated herein by reference in their entirety.

STATEMENT OF GOVERNMENT INTEREST

[0002] The present invention was made with government support under Grant No. R21CA205106, awarded by U.S. Public Health Service. The Government has certain rights in the invention.

BACKGROUND

[0003] Epstein-Barr virus (EBV) infection is the causal agent of acute infectious mononucleosis (62, 63). Persistent EBV infection in immunodeficient individuals is associated with numerous epithelial and lymphoid malignancies, such as nasopharyngeal carcinoma, gastric carcinoma, Burkitt lymphoma, Hodgkin lymphoma, and post-transplant lymphoproliferative diseases (PTLD) (1). Transplantation is the treatment of choice for a variety of patients with end-stage organ failure or hematologic malignancies, or in need of reconstructive transplantation (1). Transplantation success depends entirely on potent immunosuppressive drugs to prevent stem cell/organ rejection. However, these drugs impose several serious side effects, including an increased risk of infection with or reactivation of Epstein-Barr virus (EBV), and the resultant development of PTLDs, which are aggressive, life-threatening complications (2, 3). Through the early 2000s, PTLD patients who had been EBV-naive prior to transplantation showed mortality rates of 50-90% for stem cell and solid organ transplants; while recent data suggest outcomes have improved, challenges remain. PTLDs usually develop in EBV-naive patients, particularly pediatric patients, who receive organs from EBV+ donors. A variety of non-standardized, non-specific treatments are used to treat EBV+ PTLD cases (4-9). Initial clinical management typically involves reduction of immunosuppression; however, this can lead to graft-versus-host disease. Other treatments including radiation/chemotherapy and excision of PTLD lesions all have undesirable side effects. Second-line treatment often includes antibodies (Abs) against the B cell antigen, CD20; however, this also targets healthy B cells, further weakening the immune system and exposing patients to other opportunistic infections.

[0004] In over 50 years of EBV vaccine research, few candidates have demonstrated partial clinical efficacy, and none have been efficacious enough to elicit sterilizing immunity and be licensed (24). Antibodies, whether elicited in the host naturally or via passive immunization, provide an effective first-line of defense against viral infection.

[0005] Thus, there is an urgent need for a novel EBV-specific therapy that targets EBV+ cells to neutralize EBV infection and prevent subsequent PTLD development in EBV-naive patients.

SUMMARY

[0006] In one aspect, this disclosure relates to an Epstein-Barr virus (EBV) antibody or an immunogenic fragment thereof. In some embodiments, the EBV antibody or an immunogenic fragment thereof specifically binds to EBV glycoprotein 350/220. In some embodiments, the EBV antibody or an immunogenic fragment thereof specifically binds to EBV glycoprotein 350 or one or more epitopes represented by SEQ ID NOs: 1-3. In some embodiments, the EBV antibody comprises a VH region comprising CDR-1, CDR-2, and CDR-3 represented by SEQ ID NOs: 4-19, 20-35, and 36-51, respectively. In some embodiments, the EBV antibody comprises a VL region comprising CDR-1, CDR-2, and CDR-3 represented by SEQ ID NOs: 52-67, 68-83, and 84-99, respectively. In some embodiments, the antibody is not antibody clone 72A1 or an antibody comprising the CDRs of antibody clone 72A1. In some embodiments, the EBV antibody is a monoclonal antibody. In some embodiments, the EBV antibody is a chimeric antibody, a human antibody, or a humanized antibody.

[0007] In another aspect, this disclosure relates to an immunogenic peptide comprising one or more gp350 epitopes having an amino acid sequence identical to or sharing at least 60% similarity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In some embodiments, the immunogenic peptide comprises a first domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO:1, a second domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 2, and a third domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 3. In some embodiments, the immunogenic peptide comprises a first domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, and a second domain comprising a known immunogenic peptide such as keyhole limpet hemocyanin (KLH) peptide.

[0008] In another aspect, this disclosure relates to an EBV antibody-small molecule conjugate. The EBV antibodies disclosed herein can be conjugated to small molecules having activities against EBV-transformed cells. For example, the small molecules have anti-proliferative activities against EBV-transformed B lymphoma cells. In some embodiments, the small molecules are growth inhibitors of EBV infected B cells. In some embodiments, the small molecule is L_2P_4, 2-butynediamide, or a derivative thereof. In some embodiments, the small molecule is conjugated to the antibody via a linker or an adaptor. In some embodiments, the small molecule is conjugated to the constant region of the heavy chain or the light chain of the antibody.

[0009] In a related aspect, this disclosure relates to a pharmaceutical composition comprising the EBV antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, or the EBV antibody-small molecule conjugate disclosed herein. The pharmaceutical composition can further comprise one or more pharmaceutically acceptable excipients. The pharmaceutical composition can be formulated into any suitable formulation depending on the administration route.

[0010] In another aspect, this disclosure relates to a method of neutralizing EBV infection. The method includes administering to a subject infected with EBV a therapeutically effective amount of the EBV antibody disclosed herein

or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, the EBV antibody-small molecule conjugate, or the pharmaceutical composition described above. In some embodiments, the subject is human.

[0011] In another aspect, this disclosure relates to a method of preventing EBV infection. The method includes administering to a subject at an elevated risk of EBV infection a therapeutically effective amount of the EBV antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, the EBV antibody-small molecule conjugate, or the pharmaceutical composition described above. In some embodiments, the subject is human.

[0012] In another aspect, this disclosure relates to a method of preventing a post-transplant lymphoproliferative disease (PTLD). PTLD is associated with EBV infection of B cells, either as a consequence of reactivation of the virus post transplantation or from primary EBV infection. The method includes administering to a subject who is a transplant recipient a therapeutically effective amount of the EBV antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, the EBV antibody-small molecule conjugate, or the pharmaceutical composition described above. The administration can be before, during, and/or after the transplant. In some embodiments, the subject is a pediatric transplant recipient who is EBV naïve. In some embodiments, the subject is an adult transplant recipient. In some embodiments, the subject is human.

[0013] In another aspect, this disclosure relates to a method of treating an EBV-associated cancer. The method includes administering to a subject suffering from an EBV-associated cancer a therapeutically effective amount of the EBV antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, the EBV antibody-small molecule conjugate, or the pharmaceutical composition described above. In some embodiments, the examples of EBV-associated cancer include but are not limited to Hodgkin lymphoma, Burkitt lymphoma, gastric cancer, and nasopharyngeal carcinoma. In some embodiments, the subject is human.

[0014] In another aspect, this disclosure relates to a method of immunizing or vaccinating a subject against an EBV infection. The method includes administering to a subject suffering from an EBV infection a therapeutically effective amount of the EBV antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, or the pharmaceutical composition thereof as described above. In some embodiments, the subject is human.

[0015] In another aspect, this disclosure relates to a method of inducing the production of neutralizing antibodies against a EBV in a subject. The method includes administering to a subject an effective amount of a gp350 epitope represented by SEQ ID NO: 3 or an immunogenic peptide comprising the gp350 epitope represented by SEQ ID NO: 3. In some embodiments, the subject is human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIGS. **1**A-**1**D show characterization of novel anti-gp350 mAbs. FIG. **1**A: ELISA screening of hybridoma (HB) supernatants for anti-gp350-specific antibodies. Soluble EBV gp350 protein was used as the target antigen at 0.5 μg/ml. nAb-72A1 at 10 μg/ml and 1× phosphate buffered saline (PBS) were used as positive and negative (not shown) controls, respectively. Bound antibodies were detected using HRP-conjugated anti-mouse IgG (1:2,000). Twenty-three HB clones with ELISA signals two times greater than those of PBS control were considered as positive hybridomas. FIG. **1**B: Determining specificity of anti-gp350 producing hybridoma supernatant by immunoblotting with gp350-transfected stable CHO lysate. Western blot analysis was conducted on untransfected and pCAGGS-gp350 transfected CHO lysate. Anti-gp350 mAb 72A1 was used as a positive control (1:100) and anti-gp350 hybridoma clone supernatants were used at 1:50. Anti-mouse secondary antibody was used at 1:2000. FIG. **1**C: Flow cytometric analysis of surface expression of gp350 protein on gp350 expressing CHO cells for all 23 HB clones. 10^6 CHO cells were transfected with 1 μg of pCAGGS-gp350. gp350 expressing cells were resuspended in PBS, stained with anti-gp350 mAb (1:250), which detects the gp350 ED, followed by secondary goat anti-mouse conjugated to AF488. FIG. **1**D: Flow cytometric analysis of HB5, HB17 and HB19.

[0017] FIGS. **2**A and **2**B show PROMALS3D multiple sequence alignment of VH (FIG. **2**A) and VL (FIG. **2**B) regions of 15 mAbs and nAb-72A1 (SEQ ID NOS:100-131). The highly variable complementarity determining regions (CDR) 1-3, indicated by black boxes, define the antigen binding specificity. The conserved framework regions (FR) 1-4 flank the CDRs. Consensus amino acid (aa) are in bold and upper case. Consensus predicted secondary structure (ss) symbols: alpha-helix: h and beta-strand: e.

[0018] FIGS. **3**A and **3**B show neutralization activity of novel anti-gp350 mAbs against EBV-eGFP in Raji cells. EBV-eGFP was pre-incubated with 15 anti-gp350 mAbs at 10 μg/ml (FIG. **3**A) and 50 μg/ml (FIG. **3**B), followed by incubation with Raji cells for 48 h. EBV-eGFP+ cells were enumerated using flow cytometry. Anti-gp350 (nAb-72A1) and anti-gH/gL (E1D1) mAbs served as positive controls and non-neutralizing anti-gp350 (2L10) mAb served as negative control.

[0019] FIGS. **4**A-**4**C show construction of chimeric gp350 nAbs. FIG. **4**A: Construction of chimeric Ab. FIG. **4**B: Cloning strategy of heavy chain and light chain variable regions into expression vectors. FIG. **4**C: PCR amplification of heavy chain and light chain variable regions. 72A1 and clone 19 were used as examples of PCR amplification.

[0020] FIG. **5** illustrates an antibody-L2P4 conjugate.

DETAILED DESCRIPTION

[0021] Disclosed herein are EBV antibodies or immunogenic fragments thereof that specifically bind to gp350/gp220 or an epitope of gp350, immunogenic peptides comprising one or more gp350 epitopes, or one or more amino acid sequences having at least 60% similarity to one or more gp350 epitopes, EBV antibody-small molecule conjugates for treating or preventing EBV infection, in particular, in subjects receiving a transplant. In some embodiments, chimeric (human/mouse) anti-gp350 nAbs are conjugated to L2P4 to obtain an EBV-specific ADC that improves the

therapeutic efficacy of treating EBV-associated PTLDs. L2P4 described by Jiang et al., *Nature Biomedical Engineering* 1: 0042 (2017), is an example of the small molecules encompassed by this disclosure.

[0022] The term "antibody" as used herein refers to an immunoglobulin molecule or an immunologically active portion thereof that specifically binds to, or is immunologically reactive with a particular antigen, for example, EBV gp350/gp220, or a particular domain or fragment of gp350/gp220. In some embodiments, the antibody disclosed herein specifically binds to one or more epitopes on EBV gp350. The epitopes can be neutralizing epitopes or immunodominant epitopes. In some embodiments, the epitope has an amino acid sequence selected from $_{253}$TPIPGTGYAYSLRLTPRPVSRFL$_{275}$ (SEQ ID NO: 1), $_{875}$LLLLVMADCAFRRNLSTSHTYTTPPY$_{899}$ (SEQ ID NO: 2), and $_{381}$GAFASNRTFDIT$_{392}$ (SEQ ID NO: 3).

[0023] In a related aspect, this disclosure relates to a method of producing an EBV antibody using the epitopes disclosed herein according to any known technology. The method entails immunizing an animal such as a mouse or a rabbit with an immunogenic peptide disclosed herein or the epitope represented by SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO:3 alone, and screening for and isolating a hybridoma producing an EBV antibody. The EBV antibody produced from such hybridomas can be used for treating or preventing EBV infections.

[0024] In certain embodiments an antibody for use in the present methods or compositions is a full-length immunoglobulin molecule, which comprises two heavy chains and two light chains, with each heavy and light chain containing three complementary determining regions (CDRs). The CDRs of various antibodies are identified and listed in Table 1 below.

TABLE 1

	CDR Sequences					
Anti-	VH			VL		
bodies	CDR-1	CDR-2	CDR-3	CDR-1	CDR-2	CDR-3
72A1	GSSFTDY (SEQ ID NO: 4)	INPYNGG (SEQ ID NO: 20)	GGLRRVNWFAYW (SEQ ID NO: 36)	TGAVTTSNY (SEQ ID NO: 52)	GTN (SEQ ID NO: 68)	VLWHSNHWV (SEQ ID NO: 84)
HB-1	GFLLTTY (SEQ ID NO: 5)	IWAGGS (SEQ ID NO: 21)	RDRGYGYLYAMDYW (SEQ ID NO: 37)	QNVGTN (SEQ ID NO: 53)	STD (SEQ ID NO: 69)	QQYNTYPYT (SEQ ID NO: 85)
HB-2	GYTFTAY (SEQ ID NO: 6)	INYKTGE (SEQ ID NO: 22)	PYGYALDYW (SEQ ID NO: 38)	SSVNY* (SEQ ID NO: 54)	ATS* (SEQ ID NO: 70)	QQWSSNPPT* (SEQ ID NO: 86)
HB-3	GYTFASY (SEQ ID NO: 7)	INPNNGH* (SEQ ID NO: 23)	RNLYYYGRPDYW* (SEQ ID NO: 39)	QDIGNY* (SEQ ID NO: 55)	YTS* (SEQ ID NO: 71)	QQGNTLPPT* (SEQ ID NO: 87)
HB-5	GYTFTNH (SEQ ID NO: 8)	INPYNDY (SEQ ID NO: 24)	RSEGWLRRGAWFAY (SEQ ID NO: 40)	QSIGTS (SEQ ID NO: 56)	YAS (SEQ ID NO: 72)	QQSNSWPMLT (SEQ ID NO: 88)
HB-6	GYTFTDY* (SEQ ID NO: 9)	INTRTGE (SEQ ID NO: 25)	PYGYALDYW (SEQ ID NO: 41)	SSVNY* (SEQ ID NO: 57)	ATS* (SEQ ID NO: 73)	QQWSSNPPT* (SEQ ID NO: 89)
HB-7	GYTFTDY* (SEQ ID NO: 10)	ISPGRSG (SEQ ID NO: 26)	RYGHPSYLDVW (SEQ ID NO: 42)	QSVGNA (SEQ ID NO: 58)	SAS (SEQ ID NO: 74)	QQYSSYPLT (SEQ ID NO: 90)
HB-8	GYSFTNY* (SEQ ID NO: 11)	INTYTGE (SEQ ID NO: 27)	RYYYGSVYSAWFAYW (SEQ ID NO: 43)	QSIVHSNGNTY* (SEQ ID NO: 59)	KVS* (SEQ ID NO: 75)	FQGSHVPYT* (SEQ ID NO: 91)
HB-9	GFTFSSY (SEQ ID NO: 12)	ISSGGSY (SEQ ID NO: 28)	REDFYYGSSYGFFDVW (SEQ ID NO: 44)	QSIVHSNGNTY* (SEQ ID NO: 60)	KVS* (SEQ ID NO: 76)	FQGSHVPYT* (SEQ ID NO: 92)
HB-10	GYTFTSY* (SEQ ID NO: 13)	INPSNGH (SEQ ID NO: 29)	RNLYYYGRPDYW (SEQ ID NO: 45)	QDIGNY* (SEQ ID NO: 61)	YTS* (SEQ ID NO: 77)	QQNTLPPT (SEQ ID NO: 93)
HB-11	GDSITSG (SEQ ID NO: 14)	ISYSGS (SEQ ID NO: 30)	RGNGGNYDWYFDVW (SEQ ID NO: 46)	SSVNF (SEQ ID NO: 62)	YIS (SEQ ID NO: 78)	QQFTSSPSWT (SEQ ID NO: 94)
HB-12	GYTFTNY* (SEQ ID NO: 15)	INPNNGH* (SEQ ID NO: 31)	RNLYYYGRPDYW* (SEQ ID NO: 47)	QSLVHSNGNTY (SEQ ID NO: 63)	KVS* (SEQ ID NO: 79)	SQSTHVPLT (SEQ ID NO: 95)
HB-14	GYTFTDY* (SEQ ID NO: 16)	IHPRRGG (SEQ ID NO: 32)	RYGYPWYFDVW (SEQ ID NO: 48)	QSIVHDNGNTY (SEQ ID NO: 64)	KVS* (SEQ ID NO: 80)	FQGSHVPPT (SEQ ID NO: 96)
HB-17	GYTFTSY* (SEQ ID NO: 17)	INPNNGH* (SEQ ID NO: 33)	RNLFYYSRPDYW (SEQ ID NO: 49)	QDIGNY* (SEQ ID NO: 65)	YTS* (SEQ ID NO: 81)	QQGNTLPPT* (SEQ ID NO: 97)
HB-20	GYTFTSY* (SEQ ID NO: 18)	INPTNGH (SEQ ID NO: 34)	RNLYYYGRPDYW* (SEQ ID NO: 50)	QDIGNY* (SEQ ID NO: 66)	YTS* (SEQ ID NO: 82)	QQGNALPPT (SEQ ID NO: 98)
HB-22	GFSLTNY (SEQ ID NO: 19)	IWSDGS (SEQ ID NO: 35)	RNYYGNSYPAWFAYW (SEQ ID NO: 51)	QSIVHSNGNTY (SEQ ID NO: 67)	KVS* (SEQ ID NO: 83)	FQGSHVPWT (SEQ ID NO: 99)

[0025] The term "antibody," in addition to natural antibodies, also includes genetically engineered or otherwise modified forms of immunoglobulins, such as synthetic antibodies, intrabodies, chimeric antibodies, fully human antibodies, humanized antibodies, peptibodies and heteroconjugate antibodies (e.g., bispecific antibodies, multispecific antibodies, dual-specific antibodies, anti-idiotypic antibodies, diabodies, triabodies, and tetrabodies). The antibodies disclosed herein can be monoclonal antibodies or polyclonal antibodies. In those embodiments wherein an antibody is an immunologically active portion of an immunoglobulin molecule, the antibody may be, for example, a Fab, Fab', Fv, Fab' $F(ab')_2$, disulfide-linked Fv, single chain Fv antibody (scFv), single domain antibody (dAb), or diabody. The antibodies disclosed herein, including those that are immunologically active portion of an immunoglobulin molecule, retain the ability to bind a specific antigen, for example, EBV gp350 or one or more epitopes thereof, EBV gp220 or one or more epitopes thereof, or to bind a specific fragment of gp350/gp220.

[0026] In some embodiments, the EBV antibodies disclosed herein have undergone post-translational modifications such as phosphorylation, methylation, acetylation, ubiquitination, nitrosylation, glycosylation, or lipidation associated with expression in a mammalian cell line, including a human or a non-human host cell. Techniques for producing recombinant antibodies and for in vitro and in vivo modifications of recombinant antibodies are known in the art.

[0027] Provided in certain embodiments herein are chimeric, and/or humanized EBV antibodies. Various techniques are known in the art for humanizing antibodies from non-human species such that the antibodies are modified to increase their similarity to antibodies naturally occurring in humans. Six CDRs are present in each antigen binding domain of a natural antibody. These CDRs are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration. CDR sequences of certain antibodies identified herein are shown in Table 1. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability and form a scaffold to allow correct positioning of the CDRs. This disclosure also relates to antibodies comprising VH and VL regions comprising the CDRs shown in Table 1.

[0028] "Treating" or "treatment" of a disease or a condition may refer to preventing the disease or condition, slowing the onset or rate of development of the disease or condition, reducing the risk of developing the disease or condition, preventing or delaying the development of symptoms associated with the disease or condition, reducing or ending symptoms associated with the disease or condition, generating a complete or partial regression of the disease or condition, or some combinations thereof.

[0029] As used herein, the term "subject" refers to mammalian subject, preferably a human. The phrases "subject" and "patient" are used interchangeably herein.

[0030] The method for treating a condition or a viral infection includes administering a therapeutically effective amount of a therapeutic agent or a pharmaceutical composition. An "effective amount," "therapeutically effective amount" or "effective dose" is an amount of a composition (e.g., a therapeutic agent or a pharmaceutical composition) that produces a desired therapeutic effect in a subject, such as preventing or treating a target disease or condition or alleviating symptoms associated with the disease or condition. The precise therapeutically effective amount is an amount of the composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to the characteristics of the therapeutic agent (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), the nature of the pharmaceutically acceptable carrier or carriers in the formulation, and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation, namely by monitoring a subject's response to administration of a compound and adjusting the dosage accordingly. For additional guidance, see Remington: The Science and Practice of Pharmacy 21^{st} Edition, Univ. of Sciences in Philadelphia (USIP), Lippincott Williams & Wilkins, Philadelphia, Pa., 2005.

[0031] The pharmaceutical composition may include, among other things, an EBV antibody disclosed herein or an immunogenic fragment thereof, one or more gp350 epitopes represented by SEQ ID NOs: 1-3, one or more immunogenic peptides disclosed herein, or an EBV antibody-small molecule conjugate disclosed herein.

[0032] The pharmaceutical composition may also include one or more pharmaceutically acceptable carriers. A "pharmaceutically acceptable carrier" refers to a pharmaceutically acceptable material, composition, or vehicle that is involved in carrying or transporting a compound of interest from one tissue, organ, or portion of the body to another tissue, organ, or portion of the body. For example, the carrier may be a liquid or solid filler, diluent, excipient, solvent, or encapsulating material, or some combination thereof. Each component of the carrier must be "pharmaceutically acceptable" in that it must be compatible with the other ingredients of the formulation. It also must be suitable for contact with any tissue, organ, or portion of the body that it may encounter, meaning that it must not carry a risk of toxicity, irritation, allergic response, immunogenicity, or any other complication that excessively outweighs its therapeutic benefits.

[0033] The pharmaceutical compositions described herein may be administered by any suitable route of administration. A route of administration may refer to any administration pathway known in the art, including but not limited to aerosol, enteral, nasal, ophthalmic, oral, parenteral, rectal, transdermal (e.g., topical cream or ointment, patch), or vaginal. "Transdermal" administration may be accomplished using a topical cream or ointment or by means of a transdermal patch. "Parenteral" refers to a route of administration that is generally associated with injection, including infraorbital, infusion, intraarterial, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. In some embodiments, the therapeutic compositions described herein are administered by intravenous injection or intraperitoneal injection.

[0034] The epitopes disclosed herein have various uses. For example, the epitopes can be used to produce immuno-

genic peptides. Such an immunogenic peptide comprises one or more gp350 epitopes having an amino acid sequence identical to or sharing at least 60% similarity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3. In some embodiments, the immunogenic peptide comprises a first domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO:1, a second domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 2, and a third domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 3. In some embodiments, the immunogenic peptide comprises a first domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, and a second domain comprising a known immunogenic peptide such as keyhole limpet hemocyanin (KLH) peptide.

[0035] Additionally, the epitopes or immunogenic peptides disclosed herein can be used for producing anti-gp350 antibodies using any existing technology.

[0036] In certain embodiments, disclosed herein is a method of treating or preventing EBV infection in a subject, comprising administering a therapeutically effective amount of an anti-gp350 antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, an immunogenic peptide described herein, an EBV antibody-drug conjugate described herein, or a pharmaceutical composition comprising the anti-gp350 antibody or an immunogenic fragment thereof, one or more epitopes, one or more immunogenic peptides, or the EBV antibody-drug conjugate.

[0037] In certain embodiments, disclosed herein is a method of treating or preventing EBV infection in a subject, comprising administering a therapeutically effective amount of an anti-gp350 antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes represented by SEQ ID NOs: 1-3, an immunogenic peptide described herein, an EBV antibody-drug conjugate described herein, or a pharmaceutical composition comprising the anti-gp350 antibody or an immunogenic fragment thereof, one or more epitopes, one or more immunogenic peptides, or the EBV antibody-drug conjugate.

[0038] In certain embodiments, disclosed herein is a method of treating or preventing EBV-associated PTLD in a subject, comprising administering a therapeutically effective amount of an anti-gp350 antibody disclosed herein or an immunogenic fragment thereof, one or more epitopes described herein, one or more immunogenic peptides described herein, an EBV antibody-drug conjugate described herein, or a pharmaceutical composition comprising the anti-gp350 antibody or an immunogenic fragment thereof, one or more epitopes, one or more immunogenic peptides, or the EBV antibody-drug conjugate, before or after the transplant in the subject.

[0039] As shown in the working examples, 15 novel EBV gp350-specific mAbs were generated, their binding to gp350 was characterized, their neutralization activity against EBV infection in vitro was determined, their cognate epitopes were mapped, and the exact aa residues they recognize on gp350 were defined. Six of the eight previously described epitopes responsible for generating neutralizing and non-nAbs were confirmed and the exact aa residues that they bind were defined. This study also confirmed that the binding epitopes on gp350 that elicit nAbs are between aa 4-443 (59). An additional neutralizing epitope and two new non-neutralizing epitopes, with one located downstream of the gp350 ectodomain (aa 1-841) were identified. Importantly, the newly developed mAbs have many uses in vaccine development, diagnosis of viral infection, and therapeutic/prophylactic management of post-transplant lymphoproliferative diseases, either individually, in combination with nAb-72A1, or with other mAbs such as anti-gH/gL (E1D1).

[0040] To overcome the existing challenges facing PTLD treatment, novel EBV antibodies and EBV antibody-drug conjugates (ADCs) are developed. The EBV neutralizing antibodies (nAbs) that specifically block new or reactivated EBV infection are conjugated with small molecules that specifically target latent viral protein, EBV nuclear antigen 1 expressed in all EBV+ malignancies. The recent identification and isolation of nAbs against the highly variable viruses HIV-1 (10, 11), influenza (12-14), and respiratory syncytial virus (15) has direct implications for successful EBV protection. Indeed, in 2012, an international, multidisciplinary expert panel recommended use of intravenous (IV) anti-viral nAbs for preventing or treating EBV+ PTLD (16). EBV uses multiple surface glycoproteins (gps), including the major gp350, to infect host cells (17, 18). These gps are expressed on EBV virions and in EBV+ cells (19, 20), and stimulate immune responses in humans and in animal models (21-23), making them attractive targets for an EBV vaccine (24). Multiple lines of evidence suggest that use of anti-gp350 nAbs to protect against EBV-PTLDs is feasible (16): (A) Maternal Abs protect against EBV infection in neonates (25, 26); (B) gp350-expressing EBV+ cells activate complement (27) and mediate Ab-dependent cellular cytotoxicity (28); (C) gp350 vaccines reduce EBV load and protect against EBV+ lymphomas in marmosets (29-32) and protect EBV-naive adults from EBV-induced mononucleosis (32-34); (D) Compared to control mice, SCID mice injected with peripheral blood mononuclear cells from EBV-naive donors and immunized with anti-gp350 (72A1) mouse nAb are completely protected against EBV and development of EBV+ tumors or PTLD-like lesions (35); and (E) 72A1 also conferred short-term protection against acquiring EBV after transplantation in 3 out of 4 pediatric patients in a small phase 1 clinical trial (35). However, there was a major drawback: all 4 patients who received 72A1 developed human anti-mouse Abs (HAMA), which can cause side effects and limit treatment efficacy, with one developing a hypersensitivity reaction. This suggests that 72A1 in its native form is not a safe treatment for humans (35). Thus, chimeric (human/mouse), humanized, or human nAbs, which are safe and effective in the treatment of various cancers, are needed (7, 36-38).

[0041] Pre-existing antibodies provide the primary defense against viral infection. Prophylactic prevention of EBV primary infection has mainly focused on blocking the first step of viral entry by generating neutralizing antibodies (nAbs) that target EBV envelope glycoproteins. Five glycoproteins, in particular, gp350/220 (gp350), gp42, gH, gL, and gB, are required for efficient infection of permissible host cells and have emerged as potential prophylactic targets (23, 24, 61, 64).

[0042] Several studies have indicated that the EBV gp350 as the major immunodominant glycoprotein is an ideal target for EBV nAbs production. Although the ectodomain of EBV gp350 (aa 1-841) has been shown to contains at least eight unique CD21 binding epitopes, only one of these epitopes

(aa 142-161) is capable of eliciting nAbs (57-58). The aa residues that constitute the other epitopes and their role in generating nAb has not been elucidated, as this information would be valuable in the precise design of effective EBV peptide vaccine. To date, nAb-72A1 remains the only EBV antibody with proven clinical prophylactic efficacy, as its been shown to confer short-term protection by reducing and delaying EBV infection onset in immunized pediatric transplant patients (35).

[0043] EBV predominantly infects epithelial cells and B cells, reflecting the viral tropism and the cellular ontogeny for EBV-associated malignancies (17). There are two schools of thought on how the initial EBV transmission into the human host occurs. In the first infection model, the incoming virus engages with ephrin receptor A2 via heterodimeric gH/gL, which triggers gB fusion with the epithelial cell membrane and entry of the virus into the cytoplasm (17). This interaction is thought to occur in the oral mucosa, where the virus undergoes lytic replication to release virions that subsequently infect B cells. In the alternative model, the incoming virus binds to the host cell via complement receptor type 1 (CR1)/CD35 (45) and/or CR2/CD21 through its major immunodominant glycoprotein, gp350 (65). The interaction between gp350 and CD35 and/or CD21 triggers viral adsorption, capping, and endocytosis into the B cell (66), which subsequently leads to interaction between heterotrimeric viral glycoproteins complex, gp42/gH/gL, binding to HLA class II molecules to activate gB membrane fusion and entry. Because these two models are not necessarily mutually exclusive, and given that both gp350 and gH/gL complex are important in initiating the first viral contact with host cells, use of nAbs that target either gp350 or gH/gL complex or both may potently block incoming virus at the oral mucosa.

[0044] Nearly all EBV-infected individuals develop nAbs directed to the ectodomains of these glycoproteins (52, 67). These antibodies can prevent neonatal infection, and can protect against acute infectious mononucleosis in adolescents and several human lymphoid and epithelial malignancies associated with EBV infection (30, 32-34). Although numerous monoclonal antibodies (mAbs) have been generated against EBV gp350 (53, 68, 69), only two murine mAbs, the non-neutralizing 2L10 and the neutralizing 72A1, have been extensively characterized and made commercially available (68, 69). Importantly, nAb-72A1 conferred short-term clinical protection against EBV transmission after transplantation in pediatric patients in a small phase I clinical trial (35).

[0045] EBV gp350 is the most immunogenic envelope glycoproteins on the virions. It is a type 1 membrane protein that encodes for 907 amino acid (aa) residues. A single splice of the primary transcript deletes 197 codons and joins gp350 codons 501 and 699, in frame, to generate the gp220 messenger RNA. Both gp350 and gp220 are comprised of the same 18-aa residue at the C terminus that is located within the viral membrane, a 25-aa residue at the transmembrane-spanning domain, and a large highly glycosylated N-terminal ectodomain, aa 1-841 (57). The first 470 aa of gp350 are sufficient for binding CD21 in B cells, as demonstrated by a truncated gp350 (aa 1-470) blocking the binding of EBV to B cells and reducing viral infectivity (17). The gp350-binding domain on CD21 is mapped to N-terminal short consensus repeats (SCRs) 1 and 2, which also bind to a bioactive fragment of complement protein 3 (C3d) (34, 68). A soluble truncated EBV gp350 fragment (aa 1-470) and soluble CD21 SCR1 and SCR2 can block EBV infection and immortalization of primary B cells (57). However, gp350 binding to CD35 is not restricted to N-terminal SCRs; it binds long homologous repeat regions as well as SCRs 29-30 (57).

[0046] The gp350 ectodomain is heavily glycosylated, with both N- and O-linked sugars, which accounts for over half of the molecular mass of the protein. Currently there is only one crystal structure available for gp350, comprised of a truncated structure between 4-443 aa, with at least 14 glycosylated arginines coating the protein with sugars, with the exception of a single glycan-free patch (59). Mutational studies of several residues in the glycan-free patch resulted in the loss of CD21 binding (59), suggesting that binding of CD35 and CD21 by gp350 is mediated within this region.

[0047] There are at least eight unique CD21 binding epitopes located at the N-terminus of the gp350 ectodomain (58); at least one of these epitopes (aa 142-161) is capable of eliciting nAbs (57, 58). The aa residues 142-161 are also the binding site for nAb-72A1 (59, 68). Using gp350 synthetic peptides binding to CD21 on the surface of a B cell line, additional gp350 epitope was identified in the C-terminal region of gp350 (aa 822-841), suggesting it is involved in EBV invasion of B cells (58). The role of other epitopes in eliciting nAbs has not been fully investigated. Furthermore, the exact aa residues that comprise the core binding sites for epitope capable of eliciting neutralization and non-neutralization antibodies have not been determined. Mapping the EBV gp350 protein residues defining immunodominant epitopes, identifying the critical aa residues of the eight epitopes, and defining their roles in generating neutralizing and non-nAbs will guide rational design and construction of an efficacious EBV gp350-based vaccine that would focus the B-cell responses to the protective epitopes.

[0048] As demonstrated in the working examples, 23 hybridomas producing antibodies against EBV gp350 were generated. To assess their clinical potential and utility in informing future prophylactic and therapeutic vaccine design: (1) the ability of the antibodies produced by the new hybridomas to detect gp350 protein was tested by enzyme-linked immunosorbent assay (ELISA), flow cytometry, and immunoblot; (2) the unique CDRs of the heavy and light chains of all 23 hybridomas were sequenced to identify novel mAbs; (3) the efficacy of each mAb to neutralize EBV infection in vitro was measured; and (4) RepliTope peptide microarrays were used to identify gp350 core aa residues recognized by neutralizing and non-neutralizing mAbs. Using the newly generated antibodies, a new epitope bound preferentially by nAbs was identified, distinct from the canonical neutralizing epitope bound by nAb-72A1, as well as two immunodominant epitopes bound by both neutralizing and non-nAbs.

[0049] FIG. **1** demonstrates characterization of the 23 gp350-specific antibodies produced by hybridomas and their ability to bind gp350 protein. FIG. **2** demonstrates that out of 23 hybridomas, 15 hybridomas were determined to be monoclonal and novel based on the VH and VL CDR sequences compared to those of the reported nAb-72A1 (57).

[0050] Following confirmation that the new mAbs recognized EBV gp350 antigen and had unique VH-VL sequences, further characterization revealed that mAbs

HB9, HB10, HB11, HB17, and HB20 inhibited EBV infection in a dose-dependent manner, with HB17 and HB20 being the best neutralizers (FIG. **3**). Thus, provided herein are new nAbs and non-nAbs against EBV infection for therapeutic and prophylactic uses.

[0051] Various methods, including lectin/ricin immune-affinity assay (69), purified mAbs (53, 70), purified soluble gp350 mutants (57), synthetic peptides (51, 55, 54), cell binding assays (58), and crystal structure of partial gp350 protein (4-443) (59), have been used to identify the critical gp350 epitopes responsible for its interaction with the CD35 and CD21 cellular receptors (for a detailed summary review see Table 2).

TABLE 2

Summary of gp350 Epitope Mapping over Time Using Different Methodologies

Method	mAbs/protein/peptides	Number of epitopes	Reference
Competitive binding assay Tagged mAb vs untagged mAbs	Newly generated mAbs from purified virus or whole cell perp after induction	8 epitopes- Sequence not defined	Qualtiere et al., 1987 (53)
Binding studies- determine the effects of anti-gp350 mAbs on gp350 binding to CR2	Newly generated mAbs (61)	2 possible regions identified by sequence alignment to C3d sequence. 1. aa 21-28 2. aa 372-378	Nemerow et al 1987 (54)
Binding studies	Peptide and protein	1. aa 21-28 2. N-terminus of gp350	Nemerow et al 1989 (55)
Dot Blot immunoasaay Purified truncated protein incubated with mAbs	Protein - 8 clones overlapping N and C terminus portions of protein mAbs from Qualtiere et al., 1987	3 sequences defined 1. aa 310-325 2. aa 326-439 3. aa 733-841	Zhang et al., 1991 (56)
Peptide digest and immunoprecipitation	Truncated and mutant protein mAbs (72A1 and BOS-1)	Narrowed down to the first 470 residues	Tanner et al., 1998 (57)
Peptide cell binding assay- to 2 CR2 positive (Raji and Ramos) and 1 negative (P3HR-1) cell line	Synthesized peptides covering gp350 (907aa)	7 regions 3 identified 1. aa 142-161 2. aa 282-301 3. aa 822-841	Urquiza et al 2005 (58)
Crystal structure and binding studies	Mutant proteins, mAbs 72a1	3 epitopes (based on 72A1 binding and gp350 4-443) 1. aa 16-29 2. aa 142-161 3. aa 282-301	Szakonyi et al., 2006 (59)
Structural docking studied and antigenicity mapping	Gp350 and CR2 crystal structure alignment/docking	Single epitope (based on gp350 (1-470)) 1. aa147-165	Sitompul et al., 2012 (60)
Structural alignment (computer modeling of gp350 and 72A1)/docking studies	Peptides (used in immunization) and mAb (72A1)	3 epitopes 1. aa 14-20 2. aa 144-161 3. aa 194-211	Tanner et al., 2015 (51)

[0052] The epitope mapping assay identified a total of nine epitopes including two new epitopes, one bound by all mAbs, including nAb-72A1 and 2L10, regardless of the mAb neutralizing capability suggesting it is an immunodominant epitope on the gp350 protein. Portions of six of the previously described epitopes, including the only currently recognized neutralizing epitope were also identified and their exact aa residues were defined. However, two previously reported epitopes located at aa 282-301 and aa 194-211, which have been reported to be involved in the binding of nAb-72A1 or CD21, respectively (51, 58, 59), were not identified.

[0053] As demonstrated in the working examples, the comparative epitope mapping analysis results identified a novel neutralizing epitope, $_{381}$GAFASNRTFDIT$_{392}$ (SEQ ID NO; 3), which was bound preferentially by the nAbs HB20 and nAb-72A1, but not the non-neutralizing mAb HB5. The new neutralizing epitope is distinct from the reported canonical nAb-72A1 binding epitope aa 142-161, on gp350, suggesting that epitope 381-392 is a novel epitope on gp350 capable of eliciting nAbs using existing technology and protocols.

[0054] Virus-specific treatments are less likely to target basic metabolic mechanisms of healthy cells, making them more likely to efficiently kill virus-infected cells with fewer side effects. Until recently, few drug regimens have specifically targeted EBV+ lymphomas. However, in 2015, a few small molecules showed activity against EBV-transformed cells (39). Furthermore, in 2017, Jiang et al. described a novel small molecule (L2P4) that shows discriminating anti-proliferative activities against EBV-transformed B lymphoma cells (40).

[0055] Having described the invention with reference to the embodiments and illustrative examples, those in the art may appreciate modifications to the invention as described and illustrated that do not depart from the spirit and scope of the invention as disclosed in the specification. The examples are set forth to aid in understanding the invention but are not intended to, and should not be construed to limit its scope in any way. The examples do not include detailed descriptions of conventional methods. Such methods are well known to

those of ordinary skill in the art and are described in numerous publications. Further, all references cited above and in the examples below are hereby incorporated by reference in their entirety, as if fully set forth herein.

EXAMPLES

Example 1: Materials and Methods

[0056] Cells and viruses. EBV-AGS, a human gastric carcinoma cell line infected with a recombinant Akata virus expressing enhanced fluorescent green protein (eGFP) was a kind gift of Dr. Lisa Selin (University of Massachusetts Medical School). Anti-EBV gH/gL (E1D1) hybridoma cell line was a kind gift of Dr. Lindsey Hutt-Fletcher (Louisiana State University Health Sciences Center). Chinese hamster ovary cells (CHO); human embryonic kidney cells expressing SV-40 T antigen (HEK-293T); HEK-293 6E suspension cells; EBV-positive Burkitt lymphoma cells (Raji); myeloma cells (P3X63Ag8.653); and anti-EBV gp350 nAb-72A1 hybridoma cells (HB168) were purchased from American Type Culture Collection (ATCC). EBV-AGS cells were maintained in Ham's F-12 media supplemented with 500 μg/ml neomycin (G418, Gibco). Raji, P3X63Ag8.653, and HB168 hybridoma cells were maintained in RPMI 1640. CHO and HEK-293T cells were maintained in DMEM. HEK-293 6E cells were maintained in FreeStyle F17 Expression Medium supplemented with 0.1% Pluronic F-68. All culture media were supplemented with 10% fetal bovine serum (FBS), 2% penicillin-streptomycin, and 1% I-glutamine, with the exception of Freestyle F17 expression medium.

[0057] Antibodies and plasmids. Primary antibodies: EBV anti-gp350 nAb (72A1) and anti-gH/gL (E1D1) were purified from the supernatant of HB168 and E1D1 hybridoma cell lines, respectively, using Capturem™ Protein A Maxiprep spin columns (Takara). Anti-gp350/220 mAb (2L10) was purchased from Millipore Sigma.

[0058] Secondary antibodies: Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG for immunoblot or ELISA were purchased from Bio-Rad. Alexa Fluor (AF) 488-conjugated goat anti-mouse IgG (H+L) for flow cytometry was purchased from Life Sciences Tech. Goat anti-mouse IgG (H+L) secondary antibody and DyLight 650 for epitope mapping were purchased from Thermo Fisher Scientific.

[0059] The construction of the pCI-puro vector and pCAGGS-gp350/220-F has been described (23, 45).

[0060] Virus production and purification. eGFP-tagged EBV was produced from the EBV-infected AGS cell line as described (46). Briefly, EBV-AGS cells were seeded to 90% confluency in T-75 flasks in Ham's F-12 medium containing G418 antibiotic. After the cells reached confluency, G418 media was replaced with Ham's F-12 medium containing 33 ng/ml 12-O-tetradecanoylphorbol-13-acetate (TPA) and 3 mM sodium butyrate (NaB) to induce lytic replication of the virus. Twenty-four h post-induction, the media was replaced with complete Ham's F-12 media without G418, TPA, or NaB and cells were incubated for 4 days at 37° C. The cell supernatant was collected, centrifuged, and filtered using 0.8 μm filter to remove cell debris. The filtered supernatant was ultra-centrifuged using a Beckman-Coulter type 19 rotor for 70 min at 10,000 rpm to pellet the virus. EBV-eGFP virus was titrated in both HEK-293T cells and Raji cells, and stocks were stored at −80° C. for subsequent experiments.

[0061] Generation and purification of gp350 virus-like particles. To generate gp350 VLPs, equal amounts (8 μg/plasmid) of the relevant plasmids (pCAGGS-Newcastle disease virus (NDV) M, —NP, and gp350 ectodomain fused to fusion protein cytoplasmic and transmembrane domains) were co-transfected into 80% confluent CHO cells seeded in T-175 cm2 flasks; supernatant from transfected cells was collected and VLPs were purified and composition characterized as previously described (47).

[0062] Production of hybridoma cell lines. Seven days prior to immunization, two eight-week-old BALB/c mice were bled for collection of pre-immune serum. The mice were immunized with purified UV-inactivated EBV three times (Day 0, 21, and 35), and boosted every 7 days three times (Day 42, 49, and 56) with VLPs incorporating gp350 on the surface after Day 35. The mice were sacrificed, and their splenocytes were isolated, purified, and used to fuse with P3X63Ag8.653 myeloma cells at a ratio of 3:1 in the presence of polyethylene glycol (PEG, Sigma). Hybridoma cells were seeded in flat-bottom 96-well plates and selected in specialized hybridoma growth media with HAT (Sigma) and 10% FBS.

[0063] Indirect ELISA. Hybridoma cell culture supernatant from wells that had colony-forming cells were tested for antibody production by indirect ELISA. Briefly, immunoplates (Costar 3590; Corning Incorporated) were coated with 50 μl of 0.5 μg/ml recombinant EBV gp350/220 (Millipore) protein diluted in phosphate buffered saline (PBS, pH 7.4) and incubated overnight at 4° C. After washing three times with PBS containing 0.05% (v/v) Tween 20 (washing buffer), plates were blocked with 100 μl washing buffer containing 2% (w/v) bovine serum albumin (BSA) then incubated for 1 h at room temperature and washed as above. 100 μl of hybridoma supernatant was added to each well (in triplicate) and incubated for 2 h at room temperature. PBS and nAb-72A1 were added as negative and positive controls, respectively. The plates were washed as described above, followed by incubation with goat anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (1:2,000 diluted in PBS) at room temperature for 1 h. The plates were washed again and the chromogenic substrate 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, Life Science Technologies) was added. The reaction was stopped using ABTS peroxidase stop solution containing 5% sodium dodecyl sulfate (SDS) in water. The absorbance was read at an optical density of 405 nm using an ELISA reader (Molecular Devices). Three independent were performed.

[0064] Antibody purification, quantification, and isotyping. Hybridoma cells from selected individual positive clones were expanded stepwise from 96-well plates to T-75 flasks. At confluence in T-75 flasks, supernatant from individual clones was collected, clarified by centrifugation (4,000 g, 10 min, 4° C.), and filtered through a 0.22-μm-membrane filter (Millipore). Antibodies were further purified by Capturem™ Protein A Maxiprep (Takara) and stored in PBS (pH 7.4) at 4° C. Antibodies were analyzed by SDS-PAGE to determine purity. Bicinchoninic acid assay (BCA assay; Thermo Fisher Scientific) was conducted to determine the concentration of purified antibodies. Isotype identification was performed with the Rapid ELISA mouse mAb isotyping kit (Thermo Fisher Scientific).

[0065] RNA extraction, cDNA synthesis, and sequencing of the variable region of the mAbs. Total RNA was extracted from 1×10^6 hybridoma cells using the RNeasy Mini Kit

(Qiagen). Each hybridoma clone cDNA was synthesized in a total volume of 20 μl using Tetro Reverse Transcriptase (200 u), RiboSafe RNase Inhibitor, Oligo(dT)18 primer, dNTP mix (10 mM each nucleotide), and 100-200 ng RNA. Reverse transcription was performed at 45° C. for 30 min, and terminated at 85° C. for 5 min. The cDNA was stored at −20° C. Immunoglobulin (Ig) VH and VL were amplified using the mouse Ig specific primer set purchased from Novagen (48). The VH and VL genes were amplified in separate reactions and PCR products were visualized on 1% agarose gel.

[0066] The VH and VL amplicons were sequenced using an Illumina MiSeq platform: duplicate 50 μl PCR reactions were performed, each containing 50 ng of purified cDNA, 0.2 mM dNTPs, 1.5 mM MgCl2, 1.25 U Platinum Taq DNA polymerase, 2.5 μl of 10×PCR buffer, and 0.5 μM of each primer designed to amplify the VH and VL. The amplicons were purified using AxyPrep Mag PCR Clean-up kit (Thermo Fisher Scientific). The Illumina primer PCR PE1.0 and index primers were used to allow multiplexing of samples. The library was quantified using ViiA™ 7 Real-Time PCR System (Life Technologies) and visualized for size validation on an Agilent 2100 Bioanalyzer (Agilent Technologies) using a high-sensitivity cDNA assay. The sequencing library pool was diluted to 4 nM and run on a MiSeq desktop sequencer (Illumina). The 600-cycle MiSeq Reagent Kit (Illumina) was used to run the 6 μM library with 20% PhiX (Illumina), and FASTQ files were used for data analysis.

[0067] Chimeric mAb construct generation. To generate chimeric mAbs, the VH and VL sequences were cloned into the dual-vector system pFUSE CHIg/pFUSE CLIg (InvivoGen), which express the constant region of the heavy and light chains of human immunoglobulins, respectively (Genewiz). The constructs were transiently transfected into HEK-293 6E cells. The supernatants were collected at 72 h post-transfection and IgG was purified using protein A/G affinity chromatography.

[0068] Immunoblot analysis. CHO cells were cultured and stably co-transfected with pCAGGS-gp350/220 F and pCI-puro vector containing a puromycin resistance gene. Forty-eight h post-transfection, DMEM media containing 10 μg/ml of puromycin was added to enrich for cells expressing gp350 protein. Puromycin-resistant clones were expanded, followed by flow cytometry sorting using nAb-72A1 to a purity >90%. EBV gp350-positive CHO cells were harvested and lysed in radioimmunoprecipitation assay buffer (RIPA) followed by centrifugation at 15,000 g for 15 min on a benchtop centrifuge. The supernatants were collected and heated at 95° C. for 10 min in SDS sample buffer containing β-mercaptoethanol, then separated using SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane using an iBlot™ Transfer System (Thermo Fisher Scientific) followed by incubation in blocking buffer (1% BSA; 20 mM Tris-HCl, pH 7.5; 137 mM NaCl; and 0.1% Tween-20 [TBST]) for 1 h. The blots were incubated in TBST containing purified anti-gp350 antibodies (1:50) overnight at 4° C. After three washes with TBST, the blots were incubated with a goat anti-mouse conjugated to horseradish peroxidase (1:2000) in TBST for 1 h. After three washes, the antibody-protein complexes were detected using the Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare). All experiments were independently repeated three times.

[0069] Flow cytometry. To assess the ability of purified anti-gp350 mAb to detect surface expression of EBV gp350 protein by flow cytometry, CHO cells that stably express EBV gp350 were harvested and stained with purified anti-gp350 (10 μg/ml), followed by Alexa Fluor® 488 goat anti-mouse IgG secondary antibody. Flow cytometric analysis was performed on a C-6 FC (BD Biosciences) and data was analyzed using FlowJo Cytometry Analysis software (FlowJo, LLC) as described (47). All the experiments were independently repeated three times.

[0070] EBV neutralization assay. Purified individual anti-gp350 mAbs were incubated with purified AGS-EBV-eGFP (titer calculated to infect at least 20% of HEK293 cells seeded in 100 μl of serum-free DMEM) for 2 h at 37° C. To represent EBV infection of B cells, the pre-incubated anti-gp350 mAbs/AGS-EBV-eGFP were used to infect 5×105 Raji cells seeded in a 96-well plate. Anti-gp350 neutralizing 72A1 and non-neutralizing 2L10 mAbs served as positive and negative controls, respectively. Plates were incubated at 37° C. and the number of eGFP+ cells was determined using flow cytometry 48 h post-infection. All dilutions were performed in quintuplicate and the assays repeated three times for Raji cells. Antibody EBV neutralization activity was calculated as: % neutralization=(EBValone−EBVmAb)/(EBValone)×100.

[0071] Epitope mapping. Anti-gp350 mAbs were incubated with a multi-well EBV GP350/GP340 RepliTope (JPT) peptide microarray displaying 224 peptides (15mers with 11 aa overlap) in 3×7 subarrays. Briefly, anti-gp350 mAbs were diluted in blocking solution (TBS-T and 2% BSA) to a final concentration of 10 μg/ml and incubated with the microarray slide for 1 h at 30° C. with shaking. Slides was washed 5 times with wash buffer (TBS-T), followed by incubation with 1 μg/ml secondary antibody Dylight 650 (Thermo Fisher Scientific) for 1 h at 30° C. After washing 5 times with wash buffer and 2 times with distilled water, microarray slides were dried by centrifugation. Detection was performed using the Agilent DNA microarray scanner.

Example 2: Development and Characterization of Monoclonal Antibodies Against EBV Gp350

[0072] New EBV gp350-specific mAbs were generated and biochemically characterized, and their ability to neutralize EBV infection was evaluated. In addition, the antibodies were used to map immunodominant epitopes on the EBV gp350 protein. 23 novel monoclonal antibodies specific against EBV gp350 were developed. To generate hybridomas, BALB/c mice were immunized with purified UV-inactivated EBV and boosted with virus-like particles (VLPs) that incorporate EBV gp350 ectodomain on the surface to enrich for production of anti-gp350 antibodies, then splenocytes were isolated from the immunized mice and fused with myeloma cells. Specifically, five eight-week-old BALB/c mice were immunized with virus-like particles incorporating gp350/220 on the surface, four times (day 0, 14, 28, and 56) via intraperitoneal injection without adjuvants. At day 64, immunized mice were boosted once intravenously. Animals were sacrificed at Day 70 to harvest splenocytes for fusion with the mouse myeloma P3X63Ag8 cell line.

[0073] Indirect ELISA was used to screen supernatants from the hybridomas for specificity against purified EBV gp350 ectodomain protein (aa 4-863) and 23 hybridomas producing gp350 specific antibodies were identified. These

novel antibodies were analyzed by flow cytometric analysis for surface expression of gp350 protein on 10^6 CHO cells transfected with 1 μg of pCAGGS-gp350. gp350 expressing cells were resuspended in PBS, stained with anti-gp350 mAb, which detects the gp350 ED, followed by secondary Ab goat anti-mouse conjugated to AF488. Additionally, western blot analysis was conducted on untransfected and pCAGGS-gp350 transfected CHO lysate. Anti-gp350 mAb 72A1 was used as a positive control (1:100) and anti-gp350 hybridoma clone supernatants were used at 1:50, and anti-mouse secondary antibody was used at 1:2000.

[0074] The isotypes of the new antibodies were determined to be IgG1 (n=14), IgG2a (n=5), IgG2b (n=1), a mixture of IgG1 and IgG2b (n=1), and a mixture of IgG1 and IgM (n=2). It was found that all 23 hybridoma producing antibodies, designated HB1-23, recognized the gp350 antigen in an initial ELISA screening using unfractionated and unpurified hybridoma supernatants (data not shown). Affinity purification with protein A followed by SDS-PAGE was used to confirm the purity of the antibodies. When quantified amount of the purified antibodies (10 μg/ml) was re-evaluated using indirect ELISA, all of the 23 antibodies had ELISA signals two times greater than those of phosphate buffered saline (negative control), and were considered as positive or specific to gp350. Of these, five (HB4, HB5, HB7, HB13, and HB14) demonstrated binding affinity equal to or greater than that of the positive control, nAb-72A1 (FIG. **1**A). This difference in binding of the 23 antibodies could be due to differential exposure of cognate epitopes on gp350 in the assay performed.

[0075] Determining the nature of the binding between an antibody and its target antigen is an important consideration for the performance and specificity of an antibody, as it can involve the recognition of a linear or conformational epitope (49). The antibodies were characterized using immunoblot analysis of denatured gp350 antigen expressed from Chinese hamster ovary (CHO) cells, and 16 of the antibodies reacted to both the 350 kDa and the 220 kDa splice variant. In contrast, HB2, HB3, HB6, HB7, HB13, HB20, and HB21 failed to recognize either of the denatured isoforms of gp350 (FIG. **1**B). The antibodies were further characterized by flow cytometric analysis of CHO cells stably expressing gp350 on the cell surface, and HB1, HB2, HB3, HB5, HB6, HB9, HB11, HB12, HB15, HB17, HB19, HB20, and HB21 antibodies readily recognized gp350 (FIGS. **1**C, **1**D). Given that HB2, HB3, HB20, and HB21 detected gp350 by flow cytometry, but not by immunoblot, suggests that these four antibodies recognized conformational epitopes on gp350, whereas HB5, HB9, HB11, HB15, HB17, and HB19 recognized both linear and conformational epitopes (FIGS. **1**B-**1**D). The observation that all 23 anti-gp350 antibodies recognized the gp350 antigen either by indirect ELISA, flow cytometry, or immunoblot assay suggests that antibodies that are specific to EBV gp350 protein were successfully produced.

Example 3: Analysis of the Variable Heavy and Variable Light Chain Sequences

[0076] The complementarity determining regions (CDRs) of the anti-gp350 mAbs were sequenced by amplifying mRNA representing unique heavy/light variable chain sequences using RT-PCR. RT-PCR products were sequenced and analyzed. The sequences of the heavy and light chain variable region genes (VH and VL, respectively) of the 23 new anti-gp350 antibodies, as well as the nAb-72A1, were determined and compared the sequences to published nAb-72A1 sequences (50, 51). The sequence of the CDR of this antibody was recently determined and published, revealing two unique IgG1 heavy chains and two unique light chains, one kappa and one lambda (50, 51). PCR was used to amplify the genes encoding the VH and VL chain regions in cDNA generated from the 23 hybridoma cells as well as from HB168 (nAb-72A1). The PCR products presented distinct bands at approximately 350-400 bp and 450-500 bp for VH and VL, respectively (data not shown). The purified fragments were sequenced using Illumina MiSeq, followed by in silico analysis and CDRs for both VH and VL were identified (FIGS. **2**A, **2**B). Two VH and VL sequences of nAb-72A1 were identified as >94% identical to the previously published sequences (50), suggesting that nAb-72A1 exists as a mixed antibody, instead of the reported mAb (51). Similar to nAb-72A1, HB4, HB13, HB15, and HB23 hybridomas each produced a mixture of two antibodies, with unique sequences of the VH chain showing at >5% frequencies, suggesting that they are not mAbs (Table 3). Coding sequences for VL chains for HB7, HB9, and HB17 were unable to be identified, unless the frequencies were lowered to >1% (Table 3); in this case, the identified coding VL chain sequences were identical.

TABLE 3

Summary of Illumina Dual Demultiplex of V_H and V_L Regions

Sample	Chain	Starting Pairs	PEAR Merged Reads	Length Filtered Reads	Primer Matched Reads	3x Reads	>5% Unique Coding	>5% Unique Non-Coding	
HB1	HEAVY	51,641	51,210	46,655	32,482	22,725	1	0	
	LIGHT	280,048	279,012	100,725	68,041	58,570	1	1	
HB2	HEAVY	22,793	22,621	16,475	11,415	7,429	1	0	
	LIGHT	167,230	166,496	161,764	132,752	115,886	1	1	
HB3	HEAVY	26,382	26,162	25,542	16,910	11,709	1	0	
	LIGHT	12,681	12,609	11,753	9,809	8,023	1	1	
HB4	HEAVY	38,811	38,238	17,151	11,957	7,217	2	0	§
	LIGHT	179,249	129,752	111,996	78,392	66,419	1	1	
HB.5	HEAVY	42,951	42,173	35,793	25,842	17,173	1	0	
	LIGHT	176,073	175,267	168,806	141,712	127,045	1	1	
HB6	HEAVY	26,142	25,981	22,245	15,658	10,453	1	0	
	LIGHT	171,996	171,370	167,730	138,348	122,397	1	1	

TABLE 3-continued

Summary of Illumina Dual Demultiplex of V_H and V_L Regions

Sample	Chain	Starting Pairs	PEAR Merged Reads	Length Filtered Reads	Primer Matched Reads	3x Reads	>5% Unique Coding	>5% Unique Non-Coding	
HB7	HEAVY	32,443	32,094	25,449	17,615	11,836	1	0	
	LIGHT	67,031	63,924	37,344	26,271	22,378	1	1	*
HB8	HEAVY	140,091	103,349	92,744	58,292	31,583	1	0	
	LIGHT	151,244	115,527	102,439	82,154	70,803	1	1	
HB9	HEAVY	37,057	36,473	19,544	11,585	7,358	1	0	
	LIGHT	409,432	310,529	136,074	106,820	90,063	1	2	*
HB10	HEAVY	38,181	37,981	26,043	17,104	11,391	1	0	
	LIGHT	114,255	112,498	106,914	84,368	75,370	1	1	
HB11	HEAVY	22,225	21,841	6,956	4,408	2,465	1	0	
	LIGHT	106,465	102,278	65,332	50,232	44,527	1	0	
HB12	HEAVY	83,044	82,355	46,350	30,276	20,886	1	0	
	LIGHT	53,098	47,336	15,823	7,560	5,845	1	1	
HB13	HEAVY	81,451	80,372	47,995	32,216	20,139	2	0	§
	LIGHT	27,314	24,774	8,987	5,457	4,104	2	1	
HB14	HEAVY	76,299	75,357	28,309	19,104	12,939	1	0	
	LIGHT	153,011	149,264	48,474	29,710	25,133	1	1	
HB.15	HEAVY	26,551	26,410	16,387	11,434	7,002	2	0	§
	LIGHT	78,525	77,778	43,509	29,504	24,731	1	1	
HB16	HEAVY	54,249	53,943	9,517	7,128	4,179	1	0	
	LIGHT	42,048	40,351	30,602	22,758	18,251	2	1	§
HB17	HEAVY	111,614	110,882	81,428	50,844	35,949	1	0	
	LIGHT	102,490	100,488	83,925	65,925	57,727	1	1	*
HB18	HEAVY	211,215	155,410	146,256	91,009	50,308	1	0	
	LIGHT	212,261	161,879	155,235	123,096	105,959	1	1	
HB19	HEAVY	109,692	82,221	20,546	12,587	7,274	1	1	
	LIGHT	70,828	69,744	62,572	48,354	42,051	1	1	
HB20	HEAVY	15,781	15,632	12,789	7,757	4,852	1	0	
	LIGHT	135,527	133,208	118,513	90,717	78,701	1	1	
HB21	HEAVY	15,312	15,202	8,577	5,645	3,420	1	0	
	LIGHT	102,450	100,171	89,059	68,552	60,500	1	1	
HB22	HEAVY	217,959	156,488	154,008	95,755	50,245	1	0	
	LIGHT	205,334	156,986	143,386	108,728	85,136	1	0	
HB23	HEAVY	196,390	143,929	123,028	71,076	39,358	2	0	§
	LIGHT	158,594	120,140	115,476	90,004	78,787	2	0	
72A1	HEAVY	213,480	158,199	156,215	107,395	68,486	2	0	§
	LIGHT	187,216	140,964	132,945	105,783	91,208	1	1	

* Hybridoma with V_L chain sequences identified with >1% frequency, § Hybridoma with more than one unique, plausible-coding V_H chain sequence with >5% frequency.
The term "unique" refers to unique sequence counts (so, identical sequences found in a substantial frequency of merged reads, not necessarily unique compared to other samples).

[0077] The analysis and comparison of the VH and VL chain gene sequences of the 23 hybridomas compared to HB168 (nAb-72A1) showed unique sequences within the CDR 1-3 region. Only HB8 and HB18 had identical VH and VL chain gene sequences, suggesting that the two are the same clone isolated separately; therefore, HB18 was excluded from subsequent experiments. One of the two HB15 antibodies had identical VH and VL gene sequences to that of HB10, however based on the previous characterization, the presence of the additional antibody in HB15 was sufficient to confer subtle differences in biochemical characterizations for gp350 between the two antibodies. Thus, based on the sequence analysis (FIG. **2**), 15 unique anti-gp350 mAbs were generated, with distinct biochemical and sequence identity from commercially available nAb-72A1.

Example 4: Neutralization Assay

[0078] The ability of the 15 mAbs (10 μg/ml or 50 μg/ml) to neutralize purified eGFP-tagged AGS-EBV infection of the Raji B cell line in vitro was evaluated following standardized procedures (52) and determined the percentage of eGFP+ cells using flow cytometry as described (45, 47). The nAbs 72A1 and E1D1 were used as positive controls, whereas the non-neutralizing mAb 2L10 was used as a negative control. Because HB4, HB7, HB13, HB15, HB16, HB19, HB21, and HB23 were confirmed to be mixtures based on isotyping or sequence data, they were eliminated from further consideration in the neutralization assay. An antibody was considered a neutralizer if it inhibited EBV infection >20% at 10 μg/ml and >60% at 50 μg/ml. Several mAbs inhibited EBV infection in a dose-dependent manner. HB20 and HB17 were the most effective at preventing EBV infection of Raji cells in vitro, whereby they reduced infection by 40% and >60%, and >30% and 80% at 10 μg/ml and 50 μg/ml, respectively (FIGS. **3**A and **3**B). The HB9 and HB10 antibodies prevented EBV infection of Raji cells by ~25% at 10 μg/ml and ~60% at 50 μg/ml. The HB11 antibody neutralized <20% at 10 μg/ml, but showed a dose-dependent increase at 50 μg/ml by neutralizing EBV infection by 60%. By the set neutralization parameters, HB1-3, HB5-8, HB12, HB14, and HB22 did not neutralize EBV infection of Raji cells. In comparison, both nAb-72A1 and nAb-E1D1 neutralized EBV infection by >70% and

40%, respectively, at 10 μg/ml. The nAb-72A1 neutralized EBV infection by >95% at 50 μg/ml, whereas nAb-E1D1 neutralized infection by >60% at 50 μg/ml. As expected, the negative control, mAb-2L10, did not neutralize viral infection at either concentration. Based on the neutralization assay results, the remaining 15 mAbs were organized into two distinct groups, neutralizers (+) and non-neutralizers (−) as summarized in Table 4.

TABLE 4

Summarized Biochemical and Functional Characterizations of Anti-gp350 Antibodies

Antibody	IgG sub-class	Light chain	ELISA binding to purified EBV gp350/220	FACS (CHO Cells)	Western blot	Neutralization activity
HB1	IgG1	κ	+	+	+	−
HB2	IgG2a	κ	+	+	−	−
HB3	IgG2a	κ	−	+	−	−
HB4	IgG1	κ	+	−	+	ND
HB5	IgG2a	κ	+	+	+	−
HB6	IgG1	κ	−	+	−	−
HB7	IgG1	κ	+	−	−	−
HB8	IgG1	κ	+	−	+	−
HB9	IgG2a	κ	−	+	+	+
HB10	IgG1	κ	−	−	+	+
HB11	IgG1	κ	+	+	+	+
HB12	IgG1	κ	−	+	+	−
HB13	IgG1	κ	+	−	−	ND
HB14	IgG1	κ	+	−	+	−
HB15	IgG1	κ	+	+	+	ND
HB16	IgG/IgM	κ	+	−	+	ND
HB17	IgG2b	κ	+	+	+	+
HB19	IgG/IgM	κ	+	+	+	ND
HB20	IgG2a	κ	−	+	−	+
HB21	IgG1/IgG2b	κ	−	+	−	ND
HB22	IgG1	κ	−	−	+	−
HB23	IgG1	κ	−	+	+	ND
72A1	IgG1	κ	+	+	+	+
E1D1 (anti gH/gL)	IgG1	κ	−	−	−	+

ND = Not determined, + = positive, − = negative, ELISA = enzyme-linked immunosorbent assay, κ = Kappa.

Example 5: Epitope Mapping

[0079] The 15 new anti-gp350 mAbs (neutralizers vs. non-neutralizers) were used to identify most, if not all, of the relevant immunodominant aa residues targeted by both the nAbs and non-nAbs. RepliTope approach was used, in which overlapping peptides [15-mer with 11 aa overlap] that cover the complete sequence of gp350 (aa 1-907) were immobilized on microarray slides and probed with the purified anti-gp350 antibodies in an ELISA format. nAb-72A1 was used as positive control, because the cognate epitopes bound by the antibody have previously been reported.

[0080] Two epitopes, $_{253}$TPIPGTGYAYSLRLTPRPVSRFL$_{275}$ and $_{393}$VSGLGTAPKTLIITRTATNATTT$_{415}$, were both bound by all 15 mAbs, as well as nAb-72A1 and 2L10, regardless of their neutralizing or non-neutralizing capabilities. This consensus suggests that these epitopes are immunodominant. Several of the 15 mAbs (HB2, HB3, HB8, HB11, HB12, HB14, HB17, and HB22), as well as nAb-72A1, bound to $_{341}$ANSPNVTVTAFWAWPNNTE$_{359}$. Two epitopes, aa 341-359 and aa 393-415, were found within the previously identified single epitope II, which is encoded by nucleotides between 3,186 bp and 3,528 bp, corresponding to aa 326-439 of gp350 (56). Two mAbs, HB1 and HB10, bound $_{605}$TTPTPNATGPTVGETSPQA$_{623}$, an epitope located within the gp350 (501-699) splice region that is involved in generation of the 220 kDa splice variant. A total of eight mAbs (HB1-3, HB8, HB10-12, and HB22) also bound to the region between $_{1}$MEAALLVCQYTIQSLIHLTGEDPG$_{24}$, which includes a region homologous to C3d, another molecule known to interact with CD21 (51, 55). Two epitopes common between most nAbs and non-nAbs, $_{821}$PPSTSSKLRPRWTFTSPPV$_{839}$ and $_{875}$LLLLVMADCAFRRNLSTSHTYTTPPY$_{899}$, were located upstream and downstream, respectively, of the transmembrane domain on the C-terminus of gp350. Epitope aa 821-839 is located within the previously identified epitope I, which is located between aa 733-841 (56). Furthermore, epitope aa 821-839 is potentially involved in EBV infection of B cells (58). However, the study could not identify two epitopes located at aa 282-301 and aa 194-211, which were previously shown to be involved in the binding of nAb-72A1 and CD21, respectively (51, 58, 59). It was shown that nAb-72A1 bound $_{145}$EMQNPVYLIPETVPYIKWDN$_{164}$, one of the neutralizing epitopes on gp350 (51, 58, 59). Nine gp350 epitopes identified by 15 neutralizing and non-neutralizing mAbs are shown in the sequence below and summarized in Table 5). Residues in bold represent the gp220 splice variant region. Residues in boxes represent RepliTope-identified epitopes and exact residues. Italic residues represent canonical neutralizing epitope, underlined residues represent epitope bound by all assayed mAb.

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  1 MEAALLVCQY TIQSLIHLTG EDPGFFNVEI PEFPFYPTCN VCTADVNVTI NFDVGGKKHQ
 61 LDLDFGQLTP HTKAVYQPRG AFGGSENATN LFLLELLGAG ELALTMRSKK LPINVTTGEE
121 QQVSLESVDV YFQDVFGTMW CHHAEMQNPV YLIPETVPYI KWDNCNSTNI TAVVRAQGLD
181 VTLPLSLPTS AQDSNFSVKT EMLGNEIDIE CIMEDGEISQ VLPGDNKFNI TCSGYESHVP
241 SGGILTSTSP VATPIPGTGY AYSLRLTPRP VSRFLGNNSI LYVFYSGNGP KASGGDYCIQ
301 SNIVFSDEIP ASQDMPTNTT DITYVGDNAT YSVPMVTSED ANSPNVTVTA FWAWPNNTET
361 DFKCKWTLTS GTPSGCENIS GAFASNRTFD IT/VSGLGTAP KTLIITRTAT NATTTTHKVI
421 FSKAPESTTT SPTLNTTGFA DPNTTTGLPS STHVPTNLTA PASTGPTVST ADVTSPTPAG
481 TTSGASPVTP SPSPWDNGTE SKAPDMTSST SPVTTPTPNA TSPTPAVTTP TPNATSPTPA
541 VTTPTPNATS PTLGKTSPTS AVTTPTPNAT SPTLGKTSPT SAVTTPTPNA TSPTLGKTSP
601 TSAVTTPTPN ATGPTVGETS PQANATNHTL GGTSPTPVVT SQPKNATSAV TTGQHNITSS
661 STSSMSLRPS SNPETLSPST SDNSTSHMPL LTSAHPTGGE NITQVTPASI STHHVSTSSP
721 APRPGTTSQA SGPGNSSTST KPGEVNVTKG TPPQNATSPQ APSGQKTAVP TVTSTGGKAN
781 STTGGKHTTG HGARTSTEPT TDYGGDSTTP RPRYNATTYL PPSTSSKLRP RWTFTSPPVT
841 TAQATVPVPP TSPRRFSNLS MLVLQSASLA VLTLLLLLVM ADCAFRRNLS TSHTYTTPPY
901 DDAETYV
```

TABLE 5

Antibody Binding gp350 Epitopes

Epitope	Amino acid sequence	Epitope Properties
1	$_{1}$MEAALLVCQYTIQSLIHLTGEDPG$_{24}$	
2	$_{145}$EMQNPVYLIPETVPYIKWDN$_{164}$	Neutralizing
3	$_{253}$TPIPGTGYAYSLRLTPRPVSRF L$_{275}$	Immunodominant/ novel
4	$_{341}$ANSPNVTVTAFWAWPNNTE$_{359}$	
5	$_{381}$GAFASNRTFDIT$_{392}$	Neutralizing/ novel
6	$_{393}$VSGLGTAPKTLIITRTATNATT T$_{415}$	Immunodominant
7	$_{605}$TTPTPNATGPTVGETSPQA$_{623}$	
8	$_{821}$PPSTSSKLRPRWTFTSPPV$_{839}$	
9	$_{875}$LLLLVMADCAFRRNLSTSHTYTTP PY$_{899}$	novel

Example 6: Construction of Chimeric Gp350 nAbs

[0081] Chimeric gp350 nAbs were constructed according to the diagrams of FIG. **4**. First, mouse antibodies against human gp350 were developed, and then the mouse antibody variable region is fused to human constant region, for example, to human IgM. The heavy chain and light chain variable regions of the mouse antibody were cloned into pFUSE-CHIg and pFUSE2-CLIg vectors, respectively, followed by co-transfection of mammalian cells with recombinant pFUSE-CHIg and pFUSE2-CLIg vectors. The expression vectors were obtained from InvivoGen, and the expression was conducted in CHO cells or HEK293 cells, available from ATCC. The construction schemes and expression of heavy and light chains of clone 19 are shown in FIG. **4**.

[0082] Analysis of VH-VL sequence from the HB168 (nAb-72A1) hybridoma revealed that the hybridoma produced two antibodies: one that is gp350-specific and another that recognizes mineral oil-induced plasmacytoma (MOPC) (57). To further investigate gp350 for additional neutralizing epitopes, the gp350-specific nAb-72A1 VH-VL sequence was used to generate chimeric (mouse/human) recombinant antibodies. Similarly, the VH-VL sequence for the HB20 antibody, which the neutralization analysis above showed to be one of the best nAb, was used to generate chimeric antibody. A negative control chimeric recombinant antibody was generated using VH-VL sequences from the gp350-specific but non-neutralizing HB5 antibody mentioned above (FIG. **3**).

[0083] Comparative analysis of the epitope binding pattern was performed using the chimeric recombinant antibodies and a novel epitope was revealed, $_{381}$GAFASNRTFDIT$_{392}$, which was bound by HB20 and nAb-72A1, but not by HB5, and is distinct from the $_{145}$EMQNPVYLIPETVPYIKWDN$_{164}$ epitope, which is bound only by the nAb-72A1. The sequence below shows the novel gp350-neutralizing epitope identified by epitope mapping of neutralizing (nAb-72A1 and HB20) vs non-neutralizing (HB5) anti-gp350 mAbs. Epitopes bound by nAb-72A1 (double underlined), HB20 (wavy underlined), and HB5 (boxed) are indicated. Residues 501-700 represent splice variant region (bold), single underlined residues represent epitopes bound by nAb-72A1, HB20 and HB5, and italic residues represent epitopes bound by nAbs.

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  1 MEAALLVCQY TIQSLIHLTG EDPGFFNVEI PEFPFYPTCN VCTADVNVTI NFDVGGKKHQ
 61 LDLDFGQLTP HTKAVYQPRG AFGGSENATN LFLLELLGAG ELALTMRSKK LPINVTTGEE
121 QQVSLESVDV YFQDVFGTMW CHHAEMONPV YLIPETVPYI KWDNCNSTNI TAVVRAQGLD
181 VTLPLSLPTS AQDSNFSVKT EMLGNEIDIE CIMEDGEISQ VLPGDNKFNI TCSGYESHVP
241 SGGILTSTSP VATPIPGTGY AYSLRLTPRP VSRFLGNNSI LYVFYSGNGP KASGGDYCIQ
301 SNIVFSDEIP ASQDMPTNTT DITYVGDNAT YSVPMVTSED ANSPNVTVTA FWAWPNNTET
361 DFKCKWTLTS GTPSGCENIS GAFASNRTFLD IT VSGLGTAP KTLIITRTAT NATTTTHKVI
421 FSKAPESTTT SPTLNTTGFA DPNTTTGLPS STHVPTNLTA PASTGPTVST ADVTSPTPAG
481 TTSGASPVTP SPSPWDNGTE SKAPDMTSST SPVTTPTPNA TSPTPAVTTP TPNATSPTPA
541 VTTPTPNATS PTLGKTSPTS AVTTPTPNAT SPTLGKTSPT SAVTTPTPNA TSPTLGKTSP
601 TSAVTTPTPN ATGPTVGETS PQANATNHTL GGTSPTPVVT SQPKNATSAV TTGQHNITSS
661 STSSMSLRPS SNPETLSPST SDNSTSHMPL LTSAHPTGGE NITQVTPASI STHHVSTSSP
721 APRPGTTSQA SGPGNSSTST KPGEVNVTKG TPPQNATSPQ APSGQKTAVP TVTSTGGKAN
781 STTGGKHTTG HGARTSTEPT TDYGGDSTTP RPRYNATTYL PPSTSSKLRP RWTFTSPPVT
841 TAQATVPVPP TSQPRFSNLS MLVLQWASLA VLTLLLLLVM ADCAFRRNLS TSHTYTTPPY
901 DDAETYV
```

Example 7: Development of Antibody-Small Molecule Conjugates (ADCs)

[0084] Using small molecule L2P4 as an example, antibody-small molecule conjugates can be developed as illustrated in FIG. **5**. One or more small molecules can be conjugated to the antibody heavy chain or light chain via a reactive donor group and a C-terminal acceptor group. Small molecule L2P4 was disclosed in the publication by Jiang et al.[40] After validating the function of the purified chimeric gp350 nAbs using ELISA, flow cytometry (FC), and surface plasmon resonance, the nAbs can be conjugated to L2P4 in a site-specific manner via a Val-Cit dipeptide linker, which releases the active agent upon internalization of the ADC.[41, 42]

Example 8: Efficacy Test of ADCs

[0085] To screen for optimal dose and identify the best nAb clone for pre-clinical studies, the ability of the ADC to neutralize EBV in vitro and to protect against PTLD in vivo is tested using a humanized mouse, as described[43,44]. In brief, purified chimeric ADC (or controls: PBS, or isotype-matched non-nAbs) is injected by I.V. into humanized mice, followed by EBV-B95-8-eGFP challenge. Mice are monitored regularly and euthanized upon signs of illness or after a preset limit of 100 days. Routine histology and necropsy are conducted to assess the efficacy of the ADC to protect against EBV infection and PTLD development.

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[0086] The references listed below, and all references cited in the specification are hereby incorporated by reference in their entireties, as if fully set forth herein.

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1-4. (canceled)

5. The antibody of claim **30**, wherein the antibody is a monoclonal antibody.

6. The antibody of claim **30**, wherein the antibody is a chimeric antibody, a humanized antibody or a human antibody.

7. An immunogenic peptide comprising one or more gp350 epitopes, wherein each epitope has an amino acid sequence identical to or sharing at least 60% similarity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

8. The immunogenic peptide of claim **7**, comprising a first domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO:1, a second domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 2, and a third domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 3.

9. The immunogenic peptide of claim **7**, comprising a first domain comprising an amino acid sequence having at least 60% similarity to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3, and a second domain comprising a known immunogenic peptide such as keyhole limpet hemocyanin (KLH) peptide.

10. An antibody-small molecule conjugate comprising:

a V_H region comprising CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-19, CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 20-35, and CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 36-51;

a V_L region comprising CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 52-67, CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 68-83, and CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 84-99; and

a small molecule having an anti-proliferative activity against EBV-transformed cells,

wherein the small molecule is conjugated to the antibody.

11. The conjugate of claim **10**, wherein the antibody is a monoclonal antibody.

12. The conjugate of claim **10**, wherein the antibody is a chimeric antibody, a humanized antibody or a human antibody.

13. The conjugate of claim **10**, wherein the small molecule is a growth inhibitor of EBV infected B cells.

14. The conjugate of claim **10**, wherein the small molecule is L2P4, 2-butynediamide, or a derivative thereof.

15. The conjugate of claim **10**, wherein the small molecule is conjugated to the antibody via a linker or an adaptor.

16. The conjugate of claim **10**, wherein the small molecule is conjugated to the constant region of the heavy chain or the light chain of the antibody.

17. A pharmaceutical composition comprising the immunogenic peptide of claim **7**.

18. A method of neutralizing EBV infection comprising administering to a subject infected with EBV a therapeutically effective amount an antibody or an immunogenic fragment thereof comprising:

a V_H region comprising CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-19, CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 20-35, and CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 36-51; and

a V_L region comprising CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 52-67, CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 68-83, and CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 84-99.

19. A method of preventing EBV infection comprising administering to a subject at an elevated risk of EBV infection a therapeutically effective amount of the antibody or the immunogenic fragment thereof of claim **1**.

20. A method of preventing a post-transplant lymphoproliferative disease (PTLD) comprising administering to a subject who is a transplant recipient a therapeutically effective amount of the antibody or the immunogenic fragment thereof of claim **1**.

21-22. (canceled)

23. A method of treating an EBV-associated cancer comprising administering to a subject suffering from an EBV-associated cancer a therapeutically effective amount of the antibody or the immunogenic fragment thereof of claim **1**.

24. (canceled)

25. A method of immunizing or vaccinating a subject against EBV infection comprising administering to the subject a therapeutically effective amount of the immunogenic peptide of claim **7**, or a pharmaceutical composition thereof.

26. A method of inducing the production of neutralizing antibodies against a EBV in a subject, comprising administering an effective amount of a gp350 epitope represented by SEQ ID NO: 3 or an immunogenic peptide comprising SEQ ID NO: 3.

27-29. (canceled)

30. An antibody or an immunogenic fragment thereof comprising:

a V_H region comprising CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4-19, CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 20-35, and CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 36-51; and

a V_L region comprising CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 52-67, CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 68-83, and CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 84-99.

* * * * *