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(54) **Title**: EPSTEIN-BARR VIRUS LMP2 SPECIFIC ANTIBODY AND USES THEREOF

(57) **Abstract**: Compositions and methods related to monoclonal antibodies that target Epstein-Barr virus (EBV) associated tumor cells due to binding to HLA/LMP2 peptide complexes for the detection and targeting of EBV infected tumor cells are provided.

EPSTEIN-BARR VIRUS LMP2 SPECIFIC ANTIBODY AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 62/016,536, filed June 24, 2014, entitled "EPSTEIN-BARR VIRUS LMP2 SPECIFIC ANTIBODY AND USES THEREOF," the entire disclosure of which is hereby incorporated herein by reference for all purposes.

FIELD

[0002] The disclosure relates to monoclonal antibodies that target Epstein-Barr virus (EBV) associated tumor cells by binding to HLA/LMP2 peptide complexes.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 18, 2015, is named 97520-948339_SL.txt and is 12,876 bytes in size.

BACKGROUND

[0004] Epstein-Barr Virus (EBV) is a ubiquitous human herpesvirus, which is found as a predominantly asymptomatic infection in all human communities. EBV has been linked to numerous human tumours of diverse tissue origin. These include nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), gastric carcinoma, T-cell lymphomas, leiomyosarcoma, and breast cancer [6]. Although the association of EBV with cancers such as gastric carcinoma (~15%), Hodgkin's lymphoma (20-40%), and sporadic Burkitt's lymphoma (~30%) is not complete, assignment of a causal role to EBV has been based in part on the observation that these diseases are clonal expansions of a single EBV-infected cell[7,8]. At the protein level, the EBV gene products consistently observed in NPC biopsies and other EBV malignancies include BRLF1, EBNA1, LMP1 and LMP2A[9,10].

[0005] Given the prevalence of EBV infection and its causative role in various cancers, it would be useful to have reagents that are able to detect and target EBV infected tumor cells based on the intracellular expression of EBV proteins. The present disclosure satisfies these and other needs.

SUMMARY

[0006] Described herein are compositions and methods related to monoclonal antibodies that target Epstein-Barr virus (EBV) associated tumor cells by binding to HLA/LMP2 peptide complexes for the detection and targeting of EBV infected tumor cells.

[0007] In particular, as disclosed herein, we have exploited the fact that a defined EBV epitope from LMP2 is expressed on the surface of EBV infected cells in the context of MHC class I [10, 11] to develop a novel monoclonal antibody, based on a technical innovation of standard hybridoma technology, that binds to the HLA-0201/ LMP2 complexes on the surface of EBV infected cells and targets them for immune mediated lysis. We have shown that this type of monoclonal antibody has a high affinity for HLA-A0201/LMP2 peptide complexes, can recognize all clinically observed variants of the epitope and can bind to EBV-infected human tumour biopsy.

[0008] Accordingly, in one aspect, the present disclosure provides a recombinant antibody or fragment thereof that binds to a HLA/peptide complex.

[0009] In some embodiments, the peptide is derived from Epstein-Barr virus Latent Membrane Protein 2 (LMP2). In some embodiments, the peptide has the sequence CLGGLLTMV (SEQ ID NO: 1). In some embodiments, the HLA is HLA-A0201.

[0010] In some embodiments, the recombinant antibody or fragment thereof is capable of competitively inhibiting specific binding to the peptide of SEQ ID NO: 1, or competitively inhibiting specific binding by a monoclonal antibody produced by an anti-HLA-A0201/LMP2 hybridoma described herein. In some embodiments, the recombinant antibody or fragment thereof is capable of competitively inhibiting specific binding by a monoclonal antibody produced by an anti-HLA-A0201/LMP2 hybridoma clone #243.

[0011] In some embodiments, the antibody or fragment thereof is selected from the group consisting of: (a) a whole immunoglobulin molecule; (b) an scFv; (c) a Fab fragment; (d) an F(ab')2; and (e) a disulfide linked Fv.

[0012] In some embodiments, the antibody or fragment thereof comprises a heavy chain immunoglobulin constant domain selected from the group consisting of: (a) a human IgM constant domain; (b) a human IgG1 constant domain; (c) a human IgG2 constant domain; (d) a human IgG3 constant domain; (e) a human IgG4 constant domain; and (f) a human IgA1/2 constant domain.

[0013] In some embodiments, the antibody or fragment thereof comprises a light chain immunoglobulin constant domain selected from the group consisting of: (a) a human Ig kappa constant domain; and (b) a human Ig lambda constant domain.

[0014] In some embodiments, the antibody or fragment thereof comprises a heavy chain comprising at least one CDR having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 31-33.

[0015] In some embodiments, the antibody or fragment thereof comprises a light chain comprising at least one CDR having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36-38.

[0016] In some embodiments, the antibody or fragment thereof comprises a heavy chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 31-33, or a sequence having at least 95% identity thereto.

[0017] In some embodiments, the antibody or fragment thereof comprises a light chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 36-38, or a sequence having at least 95% identity thereto.

[0018] In some embodiments, the antibody or fragment thereof comprises a heavy chain sequence at least 95% identical to SEQ ID NO: 30.

[0019] In some embodiments, the antibody or fragment thereof comprises a light chain sequence at least 95% identical to SEQ ID NO: 35.

[0020] In some embodiments, the antibody or fragment thereof binds to an antigen with an affinity constant (K_D) of less than 1×10^{-8} M. In some embodiments, the antibody or fragment thereof binds to an antigen with an affinity constant (K_D) of less than 1×10^{-9} M.

[0021] In a further aspect, the present disclosure provides a pharmaceutical composition comprising the antibody or fragment thereof according any of the aspects and embodiments above and a pharmaceutically acceptable carrier effective to reduce EBV infected tumor cells in a subject.

[0022] In a further aspect, the present disclosure provides a method of passive immunization comprising administration to a subject an effective amount of the antibody or fragment thereof according to any of the aspects and embodiments above.

[0023] In a further aspect, the present disclosure provides a method of treatment of EBV virus infection comprising administration to a subject in need thereof an amount of antibody or fragment thereof according to any of the aspects and embodiments above, effective to reduce or prevent the disease.

[0024] In some embodiments, the antibody is administered intravenously (IV), subcutaneously (SC), intramuscularly (IM), transdermally, or orally.

[0025] In some embodiments, the antibody is administered in an amount in the range of 1 to 100 milligrams per kilogram of the subject's body weight.

[0026] In a further aspect, the present disclosure provides a method of generating a recombinant antibody or fragment thereof that binds to a HLA/peptide complex, the method comprising the steps of: (a) generating a complex of HLA associated with a peptide; (b) immunizing an animal with the complex of step (a); (c) isolating B cells specific to the complex; and (d) immortalizing the B-cells from (c).

[0027] In some embodiments, the isolation in step (c) is by immuno-magnetic selection.

[0028] In some embodiments, the peptide is derived from Epstein-Barr virus Latent Membrane Protein 2 (LMP2).

[0029] In some embodiments, the peptide has the sequence CLGGLLTMV (SEQ ID NO: 1). In some embodiments, the HLA is HLA-A0201.

[0030] In a further aspect, the present disclosure provides an isolated nucleic acid encoding the antibody or fragment thereof according to any of the aspects and embodiments above.

[0031] In some embodiments, an expression vector comprising the above nucleic acid is provided. In some embodiments, a host cell comprising the expression vector above is provided. In some embodiments, the host cell is a bacterial cell, a eukaryotic cell, or a mammalian cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] Figure 1: Schematic illustrating the experimental procedures used for the production of TCR-like monoclonal antibodies.

[0033] Figure 2: Expression and purification of HLA heavy and light chains.

[0034] Figure 3: Pre-selection of B-lymphocytes from immunised mice using immunomagenetic selection.

[0035] Figure 4: Screening B cell hybridomas for TCR-like HLA-A0201/LMP2 specificity. Figure 4 discloses SEQ ID NOS 1, 2, 10, 3, 9, 6, 39, 8, 4, 5 and 11, respectively, in order of appearance.

[0036] Figure 5: Pre-selection of B cells for required specificity significantly enhances the percentage of A0201/LMP2 specific hybridomas versus unselected spenocytes.

[0037] Figure 6: Immunoglobulin isotype test for anti-HLA-A0201/LMP2 specific monoclonal.

[0038] Figure 7: Affinity determination of TCR-like monoclonals HLA-A0201/LMP2 #243 against increasing concentrations of antigen using Biacore™.

[0039] Figure 8: Fine mapping of interaction between HLA-A0201/LMP2 complex with #243 TCR-like monoclonal antibody by alanine walking. Figure 8B discloses SEQ ID NOS 1, 17-25 and 11, respectively, in order of appearance.

[0040] Figure 9: Variants of HLA-A0201 restricted LMP2 peptide (SEQ ID NO: 1) of interest. Figure 9 table discloses SEQ ID NOS 1 and 26-28, respectively, in order of appearance.

[0041] Figure 10: Surface binding of TCR-like monoclonal to T2 cells pulsed with peptides with various clinically observed peptides variant to CLGGLLTMV (SEQ ID NO: 1).

[0042] Figure 11: Anti-HLA-A02/LMP1 recognises LMP2 epitope presented on HLA-A0201, HLA-A0206 and HLA-A0207.

[0043] Figure 12: Deoxyribonucleic acid (top) and corresponding translated amino acid sequences (bottom) (heavy (SEQ ID NOS 29 and 30, respectively, in order of appearance with bolded CDR peptides disclosed as SEQ ID NOS 31-33, respectively, in order of appearance) and light chains (SEQ ID NOS 34 and 35, respectively, in order of appearance with bolded CDR peptides disclosed as SEQ ID NOS 36-38, respectively, in order of appearance) variable regions) of #243 derived from murine hybridoma single cell clones with specificity for HLA-A0201/LMP2 (peptide CLGGLLTMV (SEQ ID NO: 1)).

[0044] Figure 13: Immunological staining of EBV infected-HLA-A0201 positive nasopharyngeal carcinoma (NPC) biopsy with TCR-like monoclonal antibodies.

DETAILED DESCRIPTION

[0045] Provided herein are monoclonal antibodies that can target Epstein-Barr virus (EBV) associated tumour cells based on their surface expression of EBV derived peptides in association with human HLA-A0201. This represents a significant technical breakthrough that will underlie the development of a new family of monoclonal antibodies with the potential to target infected cells and tumour cells based on their intracellular expression of signature antigens. This is based on the fact that all endogenous cellular proteins (host or pathogen) are processed into short peptides for display at the cell surface in association with HLA molecules[1].

[0046] The advent of Major Histocompatability Complex (MHC) based tetramer technology has enabled the detection and analysis of rare populations of antigen-specific T lymphocytes[2]. However, existing methodologies cannot be utilized to determine the distribution of T cell receptor (TCR) ligands on tumor cells or to visualize antigen-presenting cells (APC) in tissues. In our laboratory, we have made a significant technological breakthrough that allows for the development of TCR-like anti-HLA/peptide specific monoclonal antibodies using an adapted hybridoma approach, with a higher degree of reliability than has been previously possible. As proof of principle, we have developed a monoclonal antibody specific for a peptide (amino acid sequence CLGGLLTMV (SEQ ID NO: 1)) derived from the tumour virus antigen, Latent Membrane Protein 2 (LMP2)[3] of Epstein-Barr Virus (EBV) that is expressed on the surface of EBV infected cells in association with MHC class I (specifically on a form termed Human Leukocyte Antigen-HLA-A0201). This epitope is also more immunogenic than other HLA-0201 restricted LMP2 epitopes in terms of donor responses and the number of specific CTL clones isolated [4]. Hence, this monoclonal represents a unique new reagent that can be utilized to detect and target EBV infected tumor cells based on their intracellular expression of LMP2. Given that Epstein-Barr virus infection has been linked to the development of several important forms of human cancer including nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), gastric carcinoma, T-cell lymphomas, leiomyosarcoma, and breast cancer[5] this antibody can be utilized as a targeted delivery system for chemotherapeutic drugs, cytokines, pro-inflammatory mediators and toxins that will target the tumors based on their infection with EBV.

DEFINITIONS

[0047] It is to be understood that this disclosure is not limited to particular methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise.

[0048] The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, or ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0049] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. Any methods and materials similar or equivalent to those described herein can be used in the practice of the present disclosure.

[0050] "Vertebrate," "mammal," "subject," "mammalian subject," or "patient" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as experimental animals such as rabbits, rats, and mice, cows, horses, goats, and other animals. Animals include all vertebrates, *e.g.*, mammals and non-mammals, such as mice, sheep, dogs, cows, avian species, ducks, geese, pigs, chickens, amphibians, and reptiles.

[0051] "Treating" or "treatment" refers generally to either (i) the prevention of infection or reinfection, *e.g.*, prophylaxis, or (ii) the reduction or elimination of symptoms of a disease of interest, *e.g.*, therapy. Treating a subject with the compositions described herein can prevent or reduce the risk of infection from Epstein-Barr virus. Treatment can be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease.

[0052] "Preventing" or "prevention" refers to prophylactic administration with compositions described herein.

[0053] "Therapeutically-effective amount" or "an amount effective to reduce or eliminate infection" or "an effective amount" refers to an amount of an antibody composition that is sufficient to prevent Epstein-Barr virus infection or to alleviate (*e.g.*, mitigate, decrease, reduce) at least one of the symptoms associated with such an infection. It is not necessary

that the administration of the composition eliminate the symptoms of Epstein-Barr virus infection, as long as the benefits of administration of the composition outweigh the detriments. Likewise, the terms "treat" and "treating" in reference to Epstein-Barr virus infection, as used herein, are not intended to mean that the subject is necessarily cured of infection or that all clinical signs thereof are eliminated, only that some alleviation or improvement in the condition of the subject is effected by administration of the composition.

[0054] "Passive immunity" refers generally to the transfer of active humoral immunity in the form of pre-made antibodies from one individual to another. Thus, passive immunity is a form of short-term immunization that can be achieved by the transfer of antibodies, which can be administered in several possible forms, for example, as human or animal blood plasma or serum, as pooled animal or human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, as high-titer animal or human IVIG or IG from immunized subjects or from donors recovering from a disease, and as monoclonal antibodies. Passive transfer can be used prophylactically for the prevention of disease onset, as well as, in the treatment of several types of acute infection. Typically, immunity derived from passive immunization lasts for only a short period of time, and provides immediate protection, but the body does not develop memory, therefore the patient is at risk of being infected by the same pathogen later.

ANTIBODIES

[0055] As used herein, the term "antibody" refers to any immunoglobulin or intact molecule as well as to fragments thereof that bind to a specific epitope. Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, humanized, single chain, Fab, Fab', F(ab)' fragments and/or F(v) portions of the whole antibody and variants thereof. All isotypes are encompassed by this term, including IgA, IgD, IgE, IgG, and IgM.

[0056] As used herein, the term "antibody fragment" refers specifically to an incomplete or isolated portion of the full sequence of the antibody which retains the antigen binding function of the parent antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0057] An intact "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH_1, CH_2 and CH_3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a

light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system. The term antibody includes antigen-binding portions of an intact antibody that retain capacity to bind. Examples of binding include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, Nature, 341:544-546 (1989)), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR).

[0058] In some embodiments, the antibody (e.g., a mouse monoclonal antibody) comprises CDR sequences that were generated by affinity maturation, and thus would not naturally be found in the organism from which the immune response was generated. For example, if a mouse was immunized with an antigen or immunogen described herein, one of skill in the art would understand that, after affinity maturation, the CDR sequences of the antibody produced by a hybridoma described herein would differ from the germline sequences of the immunized mouse, and therefore would not be found in nature.

[0059] As used herein, the term "single chain antibodies" or "single chain Fv (scFv)" refers to an antibody fusion molecule of the two domains of the Fv fragment, V_L and V_H. Although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see, *e.g.*, Bird *et al.*, Science, 242:423-426 (1988); and Huston *et al.*, Proc Natl Acad Sci USA, 85:5879-5883 (1988)). Such single chain antibodies are included by reference to the term "antibody" fragments and can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

[0060] As used herein, the term "human sequence antibody" includes antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies described herein can include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). Such antibodies can be generated in non-human transgenic animals, *e.g.*, as described in PCT App. Pub. Nos. WO 01/14424 and WO 00/37504. However, the term "human sequence antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (*e.g.*, humanized antibodies).

[0061] Also, recombinant immunoglobulins can be produced. See, Cabilly, U.S. Patent No. 4,816,567, incorporated herein by reference in its entirety and for all purposes; and Queen *et al.*, Proc Natl Acad Sci USA, 86:10029-10033 (1989).

[0062] As used herein, the term "monoclonal antibody" refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions (if present) derived from human germline immunoglobulin sequences. In certain aspects, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

[0063] As used herein, the term "antigen" refers to a substance that prompts the generation of antibodies and can cause an immune response. It can be used interchangeably in the present disclosure with the term "immunogen". In the strict sense, immunogens are those substances that elicit a response from the immune system, whereas antigens are defined as substances that bind to specific antibodies. An antigen or fragment thereof can be a molecule (*i.e.*, an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein can induce the production of antibodies (*i.e.*, elicit the immune response), which bind specifically to the antigen (given regions or three-dimensional structures on the protein).

[0064] As used herein, the term "humanized antibody," refers to at least one antibody molecule in which the amino acid sequence in the non-antigen binding regions and/or the antigen-binding regions has been altered so that the antibody more closely resembles a

human antibody, and still retains its original binding ability. Humanized antibodies include those antibodies that, while initially starting off containing antibody amino acid sequences that are not human, have had at least some of these nonhuman antibody amino acid sequences replaced with human antibody sequences. This is in contrast with human antibodies, in which the antibody is encoded (or capable of being encoded) by genes possessed a human.

[0065] In addition, techniques developed for the production of "chimeric antibodies" (Morrison, *et al.*, Proc Natl Acad Sci, 81:6851-6855 (1984), incorporated herein by reference in their entirety) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. For example, the genes from a mouse antibody molecule specific for an autoinducer can be spliced together with genes from a human antibody molecule of appropriate biological activity. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

[0066] In addition, techniques have been developed for the production of humanized antibodies (see, *e.g.*, U.S. Patent No. 5,585,089 and U.S. Patent No. 5,225,539, which are incorporated herein by reference in their entirety). An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, referred to as complementarity determining regions (CDRs). Briefly, humanized antibodies are antibody molecules from non-human species having one or more CDRs from the non-human species and a framework region from a human immunoglobulin molecule.

[0067] Alternatively, techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies against an immunogenic conjugate of the present disclosure. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Fab and F(ab')2 portions of antibody molecules can be prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See *e.g.*, U.S. Patent No. 4,342,566. Fab' antibody molecule portions are also well-known and are produced from F(ab')2 portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide.

[0068] An "antigen binding protein" ("ABP") as used herein means any protein that binds a specified target antigen (e.g., a peptide derived from LMP2). "Antigen binding protein"

includes but is not limited to antibodies and binding parts thereof, such as immunologically functional fragments.

[0069] Peptibodies are another example of antigen binding proteins. The term "immunologically functional fragment" (or simply "fragment") of an antibody or immunoglobulin chain (heavy or light chain) antigen binding protein, as used herein, is a species of antigen binding protein comprising a portion (regardless of how that portion is obtained or synthesized) of an antibody that lacks at least some of the amino acids present in a full-length chain but which is still capable of specifically binding to an antigen. Such fragments are biologically active in that they bind to the target antigen and can compete with other antigen binding proteins, including intact antibodies, for binding to a given epitope. In some embodiments, the fragments are neutralizing fragments. These biologically active fragments can be produced by recombinant DNA techniques, or can be produced by enzymatic or chemical cleavage of antigen binding proteins, including intact antibodies. Immunologically functional immunoglobulin fragments include, but are not limited to, Fab, a diabody (heavy chain variable domain on the same polypeptide as a light chain variable domain, connected via a short peptide linker that is too short to permit pairing between the two domains on the same chain), Fab', F(ab')2, Fv, domain antibodies and single-chain antibodies, and can be derived from any mammalian source, including but not limited to human, mouse, rat, camelid or rabbit. It is further contemplated that a functional portion of the antigen binding proteins disclosed herein, for example, one or more CDRs, could be covalently bound to a second protein or to a small molecule to create a therapeutic agent directed to a particular target in the body, possessing bifunctional therapeutic properties, or having a prolonged serum half-life. As will be appreciated by one of skill in the art, an antigen binding protein can include nonprotein components.

[0070] The term "neutralizing antigen binding protein" or "neutralizing antibody" refers to an antigen binding protein or antibody, respectively, that binds to a ligand and prevents or reduces the biological effect of that ligand. This can be done, for example, by directly blocking a binding site on the ligand or by binding to the ligand and altering the ligand's ability to bind through indirect means (such as structural or energetic alterations in the ligand). In some embodiments, the term can also denote an antigen binding protein that prevents the protein to which it is bound from performing a biological function. In assessing the binding and/or specificity of an antigen binding protein, e.g., an antibody or immunologically functional fragment thereof, an antibody or fragment can substantially inhibit binding of a ligand to its binding partner when an excess of antibody reduces the

quantity of binding partner bound to the ligand by at least about 1-20, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-85%, 85-90%, 90-95%, 95-97%, 97-98%, 98-99% or more (as measured in an in vitro competitive binding assay). In some embodiments, the neutralizing ability is characterized and/or described via a competition assay. In some embodiments, the neutralizing ability is described in terms of an IC50 or EC50 value.

[0071] The term "compete" when used in the context of antigen binding proteins (e.g., antigen binding proteins or antibodies) that compete for the same epitope means competition between antigen binding proteins as determined by an assay in which the antigen binding protein (e.g., antibody or immunologically functional fragment thereof) being tested prevents or inhibits (e.g., reduces) specific binding of a reference antigen binding protein (e.g., a ligand, or a reference antibody) to a common antigen (e.g., a peptide derived from LMP2). Numerous types of competitive binding assays can be used to determine if one antigen binding protein competes with another, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see, e.g., Stahli et al., 1983, Methods in Enzymology 9:242-253); solid phase direct biotin-avidin EIA (see, e.g., Kirkland et al., 1986, J. Immunol. 137:3614-3619) solid phase direct labeled assay, solid phase direct labeled sandwich assay (see, e.g., Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using 1-125 label (see, e.g., Morel et al., 1988, Molec. Immunol. 25:7-15); solid phase direct biotin-avidin EIA (see, e.g., Cheung, et al., 1990, Virology 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, Scand. J. Immunol. 32:77-82). Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabelled test antigen binding protein and a labeled reference antigen binding protein. Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually the test antigen binding protein is present in excess. Antigen binding proteins identified by competition assay (competing antigen binding proteins) include antigen binding proteins binding to the same epitope as the reference antigen binding proteins and antigen binding proteins binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur. Additional details regarding methods for determining competitive binding are provided in the examples herein. Usually, when a competing antigen binding protein is present in excess, it will inhibit (e.g., reduce) specific binding of a reference antigen binding protein to a common antigen by at least 40-45%, 45-50%, 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or 75% or more. In

some instances, binding is inhibited by at least 80-85%, 85-90%, 90-95%, 95-97%, or 97% or more. In some instances, binding is inhibited by at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 95%, 97%, 98%, 99% or more.

Antigen Binding Protein Variants

[0072] Other antibodies that are provided are variants of the ABPs described in this application and comprise variable light and/or variable heavy chains that each have at least 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%, 99%, or above 99% identity to the amino acid sequences of ABPs described herein (either the entire sequence or a subpart of the sequence, e.g., one or more CDR). In some instances, such antibodies include at least one heavy chain and one light chain; whereas in other instances the variant forms contain two identical light chains and two identical heavy chains (or subparts thereof). For example, by comparing similar sequences, one can identify those sections (e.g., particular amino acids) that can be modified and how they can be modified while still retaining (or improving) the functionality of the ABP.

[0073] In some aspects, an ABP can comprise a polypeptide comprising an amino acid sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to an amino acid sequence described herein. In an aspect, an ABP can comprise a polypeptide comprising an amino acid sequence having at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, or 500 (or any integer within these numbers) contiguous amino acids of the amino acid sequences described herein.

[0074] In some aspects, an ABP can comprise a polypeptide encoded by a nucleotide sequence at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a nucleotide sequence described herein. In an aspect, an ABP can comprise a polypeptide encoded by a nucleotide sequence having at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, or 500 (or any integer within these numbers) contiguous nucleotides of the nucleotide sequences described herein.

[0075] In light of the present disclosure, a skilled artisan will be able to determine suitable variants of the ABPs as set forth herein using well-known techniques. In certain embodiments, one skilled in the art can identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that can be

important for biological activity or for structure can be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

[0076] In certain embodiments, conservative modifications to the heavy and light chains of antibodies will produce antibodies having functional and chemical characteristics similar to those of the antibodies from the cell lines described herein. In some embodiments, amino acid substitutions are selected such that the side chains have similar size, charge, hydrophobicity and/or polar groups and would be expected to produce antibodies having functional and chemical characteristics similar to those of the antibodies described herein. In contrast, in certain embodiments, substantial modifications in the functional and/or chemical characteristics of antibodies can be accomplished by selecting substitutions in the amino acid sequence of the heavy and light chains that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

[0077] For example, a "conservative amino acid substitution" can involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide can also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis." Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. In certain embodiments, amino acid substitutions can be used to identify important residues of antibodies, or to increase or decrease the affinity of the antibodies described herein.

[0078] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar proteins. One skilled in the art can opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[0079] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar ABPs. In view of such information, one skilled in the art can predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art can choose

not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues can be involved in important interactions with other molecules.

[0080] Moreover, one skilled in the art can generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants can be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change can be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[0081] A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4):422-427 (1996), Chou et al., Biochemistry, 13(2):222-245 (1974); Chou et al., Biochemistry, 113(2):211-222 (1974); Chou et al., Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148 (1978); Chou et al., Ann. Rev. Biochem., 47:251-276 and Chou et al., Biophys. J., 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

[0082] Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-19 (1996)), "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci. USA, 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Holm, supra (1999), and Brenner, supra (1997)).

[0083] In certain embodiments, antigen binding protein variants include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of a parent polypeptide. In certain embodiments, protein variants

comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X can be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the parent amino acid sequence. Cysteine variants can be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

[0084] According to certain embodiments, amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (4) confer or modify other physiocochemical or functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) can be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In certain embodiments, a conservative amino acid substitution typically may not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden & J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., Nature, 354:105 (1991), which are each incorporated herein by reference.

[0085] In some embodiments, the variants are variants of the nucleic acid sequences of the ABPs disclosed herein. One of skill in the art will appreciate that the above discussion can be used for identifying, evaluating, and/creating ABP protein variants and also for

nucleic acid sequences that can encode for those protein variants. Thus, nucleic acid sequences encoding for those protein variants are contemplated. For example, an ABP variant can have at least 80, 80-85, 85-90, 90-95, 95-97, 97-99 or greater percent identity (or at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity) to at least one nucleic acid sequence described herein or at least one to six (and various combinations thereof) of the CDR(s) encoded by the nucleic acid sequences described herein.

[0086] In some embodiments, the antibody (or nucleic acid sequence encoding it) is a variant if the nucleic acid sequence that encodes the particular ABP (or the nucleic acid sequence itself) can selectively hybridize to any of the nucleic acid sequences that encode the proteins described herein under stringent conditions. In one embodiment, suitable moderately stringent conditions include prewashing in a solution of 5.times.SSC; 0.5% SDS, 1.0 mM EDTA (pH 8:0); hybridizing at 50 C, -65 C, 5.times.SSC, overnight or, in the event of cross-species homology, at 45 C with 0.5.times.SSC; followed by washing twice at 65 C for 20 minutes with each of 2.times., 0.5.times., and 0.2.times.SSC containing 0.1% SDS. Such hybridizing DNA sequences are also within the scope of this disclosure, as are nucleotide sequences that, due to code degeneracy, encode an antibody polypeptide that is encoded by a hybridizing DNA sequence and the amino acid sequences that are encoded by these nucleic acid sequences. In some embodiments, variants of CDRs include nucleic acid sequences and the amino acid sequences encoded by those sequences, that hybridize to one or more of the CDRs within the sequences noted above. The phrase "selectively hybridize" referred to in this context means to detectably and selectively bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the disclosure selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the disclosure and a nucleic acid sequence of interest will be at least 80%, and more typically at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching, for example gap lengths of 5 or less or 2 or less. Alternatively, two protein sequences (or polypeptide sequences derived

from them) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, M. O., in Atlas of Protein Sequence and Structure, pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

ANTIBODY ASSAYS

[0087] A number of screening assays are known in the art for assaying antibodies of interest to confirm their specificity and affinity and to determine whether those antibodies cross-react with other proteins.

[0088] The terms “specific binding” or “specifically binding” refer to the interaction between the antigen and their corresponding antibodies. The interaction is dependent upon the presence of a particular structure of the protein recognized by the binding molecule (*i.e.,* the antigen or epitope). In order for binding to be specific, it should involve antibody binding of the epitope(s) of interest and not background antigens.

[0089] Once antibodies are produced, they are assayed to confirm that they are specific for the antigen of interest and to determine whether they exhibit any cross reactivity with other antigens. One method of conducting such assays is a sera screen assay as described in U.S. App. Pub. No. 2004/0126829, the contents of which are hereby expressly incorporated herein by reference. However, other methods of assaying for quality control are within the skill of a person of ordinary skill in the art and therefore are also within the scope of the present disclosure.

[0090] Antibodies, or antigen-binding fragments, variants or derivatives thereof of the present disclosure can also be described or specified in terms of their binding affinity to an antigen. The affinity of an antibody for an antigen can be determined experimentally using

any suitable method. (See, *e.g.,* Berzofsky *et al.*, "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W. H. Freeman and Company: New York, N.Y. (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (*e.g.,* salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (*e.g.,* K_D, K_a, K_d) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

[0091] The affinity binding constant (K_{aff}) can be determined using the following formula:

$$K_{aff} = \frac{(n-1)}{2\left(n\left[mAb'\right]_t - \left[mAb\right]_t\right)}$$

in which:

$$n = \frac{[mAg]_t}{[mAg']_t}$$

[0092] [*mAb*] is the concentration of free antigen sites, and [*mAg*] is the concentration of free monoclonal binding sites as determined at two different antigen concentrations (*i.e.,* $[mAg]_t$ and $[mAg']_t$) (Beatty *et al.*, J Imm Meth, 100:173-179 (1987)).

[0093] As disclosed herein, surface plasmon resonance (SPR) can be used for detection and measurement of antibody-antigen affinity and kinetics. (See, *e.g.*, Hearty, S., *et al.*, Methods Mol. Biol., 907:411-42 (2012); Malmqvist, M., Current Opinion in Immunology, 5: 282-286 (1993); Chatellier, J, *et al.*, J. Molecular Recognition, 9: 39-51 (1996); Margulies, D. H., *et al.*, Current Opinion in Immunology, 8: 262-270 (1996); Forbes, B.E., *et al.*, Eur. J. Biochem., 269:961-968 (2002).)

[0094] The term "high affinity" for an antibody refers to an equilibrium association constant (K_{aff}) of at least about 1×10^7 liters/mole, or at least about 1×10^8 liters/mole, or at least about 1×10^9 liters/mole, or at least about 1×10^{10} liters/mole, or at least about 1×10^{11} liters/mole, or at least about 1×10^{12} liters/mole, or at least about 1×10^{13} liters/mole, or at least about 1×10^{14} liters/mole or greater. "High affinity" binding can vary for antibody isotypes. K_D, the equilibrium dissociation constant, is a term that is also used to describe antibody affinity and is the inverse of K_{aff}.

[0095] K_D, the equilibrium dissociation constant, is a term that is also used to describe antibody affinity and is the inverse of K_{aff}. If K_D is used, the term "high affinity" for an antibody refers to an equilibrium dissociation constant (K_D) of less than about 1×10^{-7} mole/liters, or less than about 1×10^{-8} mole/liters, or less than about 1×10^{-9} mole/liters, or

less than about 1 x 10^{-10} mole/liters, or less than about 1 x 10^{-11} mole/liters, or less than about 1 x 10^{-12} mole/liters, or less than about 1 x 10^{-13} mole/liters, or less than about 1 x 10^{-14} mole/liters or lower.

[0096] The production of antibodies according to the present disclosure provides for antibodies with the characteristics of those produced in the course of a physiological human immune response, i.e. antibody specificities that can only be selected by the human immune system. In the present case, this includes a response to the human pathogen Epstein-Barr virus. In some embodiments, antibodies of the present disclosure possess the characteristics of those produced in the course of a response to infection by Epstein-Barr virus. These antibodies can be used as prophylactic or therapeutic agents upon appropriate formulation.

[0097] In relation to a particular pathogen, a "neutralizing antibody", "broadly neutralizing antibody", or "neutralizing monoclonal antibody", all of which are used interchangeably herein, is one that can neutralize the ability of that pathogen to initiate and/or perpetuate an infection in a host. In some embodiments, monoclonal antibodies produced in accordance with the present disclosure have neutralizing activity, where the antibody can neutralize at a concentration of 10^{-9}M or lower (e.g. 10^{-10}M, 10^{-11}M, 10^{-12}M or lower).

[0098] The immunoglobulin molecules of the present disclosure can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or subclass of immunoglobulin molecule. In some embodiments, the antibodies are antigen-binding antibody fragments (e.g., human) and include, but are not limited to, Fab, Fab' and $F(ab')_2$, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding antibody fragments, including single-chain antibodies, can comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the present disclosure are antigen-binding fragments comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains.

RECOMBINANT EXPRESSION

[0099] The methods of the present disclosure also provide for obtaining and/or sequencing a nucleic acid for an antibody from a selected B cell clone; and utilizing the nucleic acid to generate a host cell that can express the antibody of interest.

[00100] In some embodiments, the nucleotide sequence encoding a desired antibody can be sequenced and thereafter employed in a heterologous expression system, e.g. 293 cells or

CHO cells. In some embodiments, an antibody can be recombinantly expressed by obtaining one or more nucleic acids (e.g. heavy and/or light chain genes) from the a B cell clone that encodes the antibody of interest and inserting the nucleic acid into a host cell in order to permit expression of the antibody of interest in that host.

[00101] Production of antibodies using recombinant DNA methods is described, for example, in U.S. Pat. No. 4,816,567. For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding a monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Vectors that can be used generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Examples of such expression system components are disclosed in, for example, U.S. Pat. No. 5,739,277. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells (see, e.g., U.S. Pat. No. 5,739,277).

PHARMACEUTICAL COMPOSITIONS

[00102] The presently disclosed subject matter provides pharmaceutical compositions comprising the antibodies produced in accordance with the present disclosure. In some embodiments, a pharmaceutical composition can comprise one or more monoclonal antibodies produced in using the methods disclosed herein. In some embodiments, a panel of monoclonal antibodies produced according to the present disclosure can be included in a pharmaceutical composition. In some embodiments, the monoclonal antibodies produced according to the present disclosure can be included with one or more additional agents, for example, antiviral or anticancer drugs or analgesics.

[00103] In some embodiments a pharmaceutical composition can also contain a pharmaceutically acceptable carrier or adjuvant for administration of the antibody. In some embodiments, the carrier is pharmaceutically acceptable for use in humans. The carrier or adjuvant should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers can be large, slowly metabolized macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, ammo acid copolymers and inactive virus particles.

[00104] Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonate and benzoates.

[00105] Pharmaceutically acceptable carriers in therapeutic compositions can additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, can be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

[00106] The compositions of the presently disclosed subject matter can further comprise a carrier to facilitate composition preparation and administration. Any suitable delivery vehicle or carrier can be used, including but not limited to a microcapsule, for example a microsphere or a nanosphere (Manome et al. (1994) Cancer Res 54:5408-5413; Saltzman & Fung (1997) Adv Drug Deliv Rev 26:209-230), a glycosaminoglycan (U.S. Pat. No. 6,106,866), a fatty acid (U.S. Pat. No. 5,994,392), a fatty emulsion (U.S. Pat. No. 5,651,991), a lipid or lipid derivative (U.S. Pat. No. 5,786,387), collagen (U.S. Pat. No. 5,922,356), a polysaccharide or derivative thereof (U.S. Pat. No. 5,688,931), a nanosuspension (U.S. Pat. No. 5,858,410), a polymeric micelle or conjugate (Goldman et al. (1997) Cancer Res 57:1447-1451 and U.S. Pat. Nos. 4,551,482, 5,714,166, 5,510,103, 5,490,840, and 5,855,900), and a polysome (U.S. Pat. No. 5,922,545).

[00107] Antibody sequences can be coupled to active agents or carriers using methods known in the art, including but not limited to carbodiimide conjugation, esterification, sodium periodate oxidation followed by reductive alkylation, and glutaraldehyde crosslinking (Goldman et al. (1997) Cancer Res. 57:1447-1451; Cheng (1996) Hum. Gene Ther. 7:275-282; Neri et al. (1997) Nat. Biotechnol. 15:1271-1275; Nabel (1997) Vectors for Gene Therapy. In Current Protocols in Human Genetics, John Wiley & Sons, New York; Park et al. (1997) Adv. Pharmacol. 40:399-435; Pasqualini et al. (1997) Nat. Biotechnol. 15:542-546; Bauminger & Wilchek (1980) Meth. Enzymol. 70:151-159; U.S. Pat. No. 6,071,890; and European Patent No. 0 439 095).

[00108] A therapeutic composition of the present disclosure comprises in some embodiments a pharmaceutical composition that includes a pharmaceutically acceptable carrier. Suitable formulations include aqueous and non-aqueous sterile injection solutions which can contain anti-oxidants, buffers, bacteriostats, bactericidal antibiotics and solutes which render the formulation isotonic with the bodily fluids of the intended recipient; and

aqueous and non-aqueous sterile suspensions which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a frozen or freeze-dried (lyophilized) condition requiring only the addition of sterile liquid carrier, for example water for injections, immediately prior to use. Some exemplary ingredients are SDS in the range of in some embodiments 0.1 to 10 mg/ml, in some embodiments about 2.0 mg/ml; and/or mannitol or another sugar in the range of in some embodiments 10 to 100 mg/ml, in some embodiments about 30 mg/ml; and/or phosphate-buffered saline (PBS). Any other agents conventional in the art having regard to the type of formulation in question can be used. In some embodiments, the carrier is pharmaceutically acceptable. In some embodiments the carrier is pharmaceutically acceptable for use in humans.

[00109] Pharmaceutical compositions of the present disclosure can have a pH between 5.5 and 8.5, between 6 and 8, or about 7. The pH can be maintained by the use of a buffer. The composition can be sterile and/or pyrogen free. The composition can be isotonic with respect to humans. Pharmaceutical compositions of the presently disclosed subject matter can be supplied in hermetically-sealed containers.

[00110] Pharmaceutical compositions can include an effective amount of one or more antibodies as described herein. In some embodiments, a pharmaceutical composition can comprise an amount that is sufficient to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic effect. Therapeutic effects also include reduction in physical symptoms. The precise effective amount for any particular subject will depend upon their size and health, the nature and extent of the condition, and therapeutics or combination of therapeutics selected for administration. The effective amount for a given situation is determined by routine experimentation as practiced by one of ordinary skill in the art.

TREATMENT REGIMENS: PHARMACOKINETICS

[00111] The pharmaceutical compositions of the disclosure can be administered in a variety of unit dosage forms depending upon the method of administration. Dosages for typical antibody pharmaceutical compositions are well known to those of skill in the art. Such dosages are typically advisory in nature and are adjusted depending on the particular therapeutic context or patient tolerance. The amount antibody adequate to accomplish this is defined as a "therapeutically effective dose." The dosage schedule and amounts effective for this use, *i.e.*, the "dosing regimen," will depend upon a variety of factors, including the stage

of the disease or condition, the severity of the disease or condition, the general state of the patient's health, the patient's physical status, age, pharmaceutical formulation and concentration of active agent, and the like. In calculating the dosage regimen for a patient, the mode of administration also is taken into consideration. The dosage regimen must also take into consideration the pharmacokinetics, *i.e.*, the pharmaceutical composition's rate of absorption, bioavailability, metabolism, clearance, and the like. See, *e.g.*, the latest Remington's; Egleton, *Peptides* 18: 1431-1439, 1997; Langer, *Science* 249: 1527-1533, 1990.

[00112] For purposes of the present disclosure, a therapeutically effective amount of a composition comprising an antibody, contains about 0.05 to 1500 µg protein about 10 to 1000 µg protein, about 30 to 500 µg or about 40 to 300 pg, or any integer between these values. For example, antibodies described herein can be administered to a subject at a dose of about 0.1 µg to about 200 mg, e.g., from about 0.1 µg to about 5 µg, from about 5 µg to about 10 µg, from about 10 µg to about 25 µg, from about 25 µg to about 50 µg, from about 50 µg to about 100 µg, from about 100 µg to about 500 µg, from about 500 µg to about 1 mg, from about 1 mg to about 2 mg, with optional boosters given at, for example, 1 week, 2 weeks, 3 weeks, 4 weeks, two months, three months, 6 months and/or a year later. It is understood that the specific dose level for any particular patient depends upon a variety of factors including the activity of the specific antibody employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

[00113] Routes of administration include, but are not limited to, oral, topical, subcutaneous, intramuscular, intravenous, subcutaneous, intradermal, transdermal and subdermal. Depending on the route of administration, the volume per dose is about 0.001 to 10 ml, about 0.01 to 5 ml, or about 0.1 to 3 ml. Compositions can be administered in a single dose treatment or in multiple dose treatments on a schedule and over a time period appropriate to the age, weight and condition of the subject, the particular antibody formulation used, and the route of administration.

KITS

[00114] Also provided are kits comprising antibodies produced in accordance with the present disclosure which can be used, for instance, for therapeutic applications described above. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers can be formed from a

variety of materials such as glass or plastic. The container holds a composition which includes an active agent that is effective for therapeutic applications, such as described above. The active agent in the composition can comprise the antibody. The label on the container indicates that the composition is used for a particular therapy or non-therapeutic application, and can also indicate directions for either *in vivo* or *in vitro* use, such as those described above.

[00115] The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present disclosure in any way.

EXAMPLES

Example 1: Overview of Experimental Procedures used for the Production of TCR-like Monoclonal antibodies.

[00116] As shown in Figure 1, human MHC class-I heavy chains (HC) and light chains (LC) are expressed as recombinant proteins and refolded into HLA monomers in the presence of an antigenic peptide CLGGLLTMV (SEQ ID NO: 1) from LMP2. The monomers are purified by FPLC and used as immunogens in Balb/C mice. Splenic B lymphocytes with desired antigenic specificities are purified using immuno-magnetic beads prior to polyethylene glycol (PEG) mediated fusion with the Myeloma cell line NS1. The resulting hybridoma clones are screened by flow cytometry using peptide pulsed HLA expressing human cell lines.

Example 2: Expression and purification of HLA heavy and light chains.

[00117] As shown in Figure 2, recombinant HLA-A0201 heavy and light chains were expressed in BL21 *E.coli*. The heavy and light chains were isolated as inclusion bodies and dissolved in 8M urea. Protein content of heavy chain (HC) and light chain (LC) inclusion bodies was analysed using SDS-Page (A). Purification and analysis of folded monomers. Heavy chain, light chain and the LMP2 peptide (CLGGLLTMV (SEQ ID NO: 1)) were refolded into complete HLA-complexes *in vitro*. Anion-exchange chromatography was used to purify the monomers. (B) FPLC profiles (i) followed by SDS-PAGE analysis (ii). Peak A at 19mins contained light chain (β2m) only whereas peak B at 36-37mins contained both heavy chain (44kD) and light chain (12kD). Fractions collected from peak B were pooled and analyzed and further purified using a size-exclusion column. The peak was collected from the chromatography (Ci) before being analysed on a non-denaturing native gel (Cii), lane 1. The gel was immunoblotted with the anti-HLA conformation specific monoclonal

w6/32 and compared with two previously successfully folded monomers (lane 2 and 3). This confirmed that the purified monomer was correctly folded.

[00118] To perform Examples 1 and 2, the following methods were used.

[00119] A single inoculant of the successfully transformed cell containing the pET30a-HLA-A0201 plasmid was inoculated into 15 ml of LB media containing 50 μg/ml of kanamycin selective antibiotics. This inoculant was incubated at 37^{0}C for up to 16 hrs with shaking to serve as a starter culture.

[00120] 10 ml of starter culture was inoculated into 1 L of LB media containing 50 μg/ml of kanamycin selective antibiotics and incubated at 37 ^{0}C for 1.5 hr with shaking. Subsequently, the OD_{600} was measured every half an hour to determine the mid-log phase of the culture (OD_{600} between 0.6 to 0.8) and protein expression was induced with 1 mM of isopropyl β-D-I-thiogalactopyranoside (IPTG) (Sigma-Aldrich). The culture was further incubated in the dark at 37 ^{0}C for 4 hrs with shaking. Thereafter, the culture was centrifuged at 4 ^{0}C, 5000 rpm for 10 mins to pellet down the cells. The pellet was resuspended in 10ml of resuspension buffer with 10 mM dithiothreitol (DTT) (Sigma-Aldrich), 0.2 mM phenylsulphonylfluoride (PMSF) (Sigma-Aldrich), and 5 μl 1 mg/ml pepstatin A (Sigma-Aldrich). The resuspended pellet was stored at -80 ^{0}C overnight.

[00121] The inclusion bodies were thawed at room temperature. For every 10 ml of resuspended pellet, 25 ml of lysis buffer with 1 ml 1 mg/ml DNase I (Sigma Aldrich), 10 mM DTT and 5 mM MgCl2 was added. The mixture was shaken vigorously on ice for 20 mins followed by addition of 10 mM sodium ethylenediaminetetraacetate (NaEDTA, pH8.0). Sonication on the cells was carried out for 5 cycles, each consisting of 30 s pulsing with 30 s breaks. After sonication, the mixture was pelleted down at 10 000 rpm, 4 ^{0}C for 15 mins followed by resuspending it in 30 ml of wash buffer with 1 mM DTT, 0.2 mM PMSF and 30 μl 1 mg/ml pepstatin A. The mixture was further homogenized for 30 s and centrifuged down at 10 000 rpm, 4 ^{0}C for 15 mins. This step was repeated twice. The pellet was further resuspended in 20 ml of wash buffer with 1 mM DTT, 0.2 mM PMSF and 20 μl 1 mg/ml pepstatin A. The mixture was further homogenized for 30 s and centrifuged down at 10 000 rpm, 4 ^{0}C for 15 mins. The pellet was resuspended with 400 μl of water to form a white paste. 20 ml of 8 M urea buffer supplemented with 0.1 mM DTT, 0.2 mM PMSF and 20 μl 1 mg/ml pepstatin A was added to the white paste. The mixture were shaken for 1 hr at room temperature and centrifuged down at 10 000 rpm, 4 ^{0}C for 1 hr. The supernatant containing either the HLA-A0201 or beta 2 microglobulin constructs were collected into 50 ml falcon

tube and 0.2 mM PMSF and 10 µl 1mg/ml pepstatin A were added into each tube. These construct where then aliquot into 1.5 ml eppendorf tubes and stored at -80 ^{0}C.

FPLC purification

Anion exchange chromatography

[00122] Pumps A and B were pre-washed with RO water at flow rate of 1 ml/min and the HiPrep 16/10 DEAE FF column (AKTA, GE Healthcare) was attached onto the AKTA FPLC System (GE Healthcare). The column was equilibrated with 5 column volumes (CV) of RO water followed by 5 CV of buffer A, 5 CV of buffer B and 5 CV of buffer A. Buffer A is a solution of 20 mM Tris (pH 8.0) while buffer B is 20 mM Tris/ 1 M NaCl (pH 8.0). The dialyzed solution was loaded into the equilibrated column at a flow rate of 5 ml/min and run according to a stepwise gradient program. Protein was eluted in an increasing salt concentrations using buffer B at a flow rate of 5 ml/min and fractions were collected in 1 ml samples. The refolded HLA monomers were eluted with approximately 15 % of buffer B and the selected samples were subsequently analyzed on a SDS-PAGE gel. Fractions that contain both the heavy chain (35 kDa) and beta-2 microglobulin (12 kDa) were pooled together and concentrated to approximately 500 µl using the Centricon Centrifugal Filter Unit with Ultracel YM-30 membrane (Millipore Corporation). The purified fraction was used for further purification.

Superdex (size-exclusion column)

[00123] The Superdex HR 10/30 75 column (GE Healthcare) was equilibrated with 5 CV of RO water and 5 CV of buffer A before use. Approximately 200 µl of the anion exchanged purified HLA monomers were injected into the sample loop using a 1 ml syringe with no introduction of air bubbles. The monomers would be subsequently loaded into the column and eluted using an isocratic gradient with a flow rate of 0.8 ml/min in 20 mM Tris (pH 8.0). Fractions were collected in 0.5 ml aliquots and analyzed on SDS-PAGE gel. Fractions that contain both the heavy chain (35 kDa) and beta-2 microglobulin (12 kDa) were pooled together and further concentrated to 500 µl using the Centricon YM-10 (Millipore Corporation). The purified monomers were quantitated and stored at -80 0C for further use.

Biotinylation of HLA monomers

[00124] Approximately 70 µl of purified monomer was biotinylated with 10 µl of biomix A, 10 µl of biomix B and 10 µl of supplemental biotin, 0.2 µl of 3 mg/ml BirA enzyme (Avidity LLC), 0.2 µl of 0.1 M PMSF and 0.1 µl of 1 mg/ml pepstatin A at 28^{0}C for 18 hrs. 1 ml of 10 mM Tris was added to the biotinylated mixture, transferred to a Centricon YM-30,

and centrifuged at 3500 rpm for 10 mins to wash out the mixture to leave behind the biotinylated monomers.

[00125] The amount of biotinylated product was quantitated by immunoprecipitation using streptavidin beads (Sigma-Aldrich Inc.). Approximately 10 µl of beads were washed with 1 ml of 10 mM Tris, 150 mM NaCl, 0.1% Triton-X100 and pulsed down at not more than 8000 rpm. The supernatant was discarded and 20 µg of previously biotinylated monomers were added with 500 µl of 10 mM Tris, 2 µl of 0.1 M PMSF and 1 µl of 1 mg/ml of pepstatin A. The mixture was left to shake at 4 ^{0}C for 16 hrs. After incubation, the beads were washed similarly as stated above twice. The supernatant were subsequently discarded and the sample was ran on 15 % SDS-PAGE gel and visualized with Coomassie blue staining. The percentage of successfully biotinylated HLA monomers was determined by comparing against non-immunoprecipiated HLA monomers. A negative control of streptavidin beads only was used. The quantity of monomers was determined by visual comparison with known concentrations of bovine serum albumin (BSA) standards (Sigma-Aldrich) that were ran on the same SDS-PAGE gel. The biotinylated monomers were subsequently aliquot and stored at -80 ^{0}C for further use.

[00126] To enrich specific splenocytes, 50 µg of biotinylated monomer was incubated with half the splenocytes harvested at 4 ^{0}C for 20 mins. After which 5ml of cold RPMI was added and centrifuged at 1100 rpm for 5mins to wash out the unbound biotinylated monomers. The pelleted cells were incubated with 100 µl of anti-biotin microbeads (Miltenyi Biotec GmbH) and incubated at 4 ^{0}C for a further 30 mins. In the meantime, the LS columns (Miltenyi Biotec GmbH) were pre-wet with 1 ml of cold PBS followed by 1 ml of RPMI medium. After 30 mins, the unbound anti-biotin microbeads (Miltenyi Biotec GmbH) were washed out by centrifuging the mixture of pelleted cells and anti-biotin microbeads at 1100 rpm for 5 mins with 3 ml of cold RPMI medium. About 1 million of the pelleted cells were then added directly onto the each pre-wet LS column. After the appropriate cells were adsorbed onto the column, 3 x 1 ml of cold RPMI medium was used to wash out the unbound cells. After which, the LS column with the bound cells was removed from the magnetic board and cells were flushed out onto a 15 ml Falcon tube with cold 3 ml of RPMI medium. This process was repeated with another 3 ml of RPMI medium to elute out the remaining cells. The 15 ml Falcon tube now contains splenocytes enriched with specificity of interest.

[00127] The non-enriched and enriched splenocytes were subsequently treated similarly for hybridoma fusion. The splenocytes were added directly onto the NS1 myeloma cells and

centrifuged at 1100 rpm for 3 mins to pellet the cells into close proximity. The supernatant was discarded and 1 ml of warm polyethylene glycol 1500 (PEG) (Sigma-Aldrich Inc.) was added slowly over 1 min with a glass pipette. The suspension was incubated for 1 min at 37 ^{0}C for cell fusion between the splenocytes and the myeloma cells. Following incubation, 3 ml of RPMI medium was added at a rate of 1 ml/ min while consistently rotating the tube gently. Subsequently, the suspension was topped up with 5 ml of RPMI medium and centrifuging at 1100 rpm for 3 mins pelleted the cells down. The pellet was then resuspended with 20 ml of HAT medium prior to incubation at 37 ^{0}C in a CO_2 incubator for 2 hrs. After 2 hrs, the cell suspension was topped up with an appropriate volume with HAT medium and 100 µl of the suspension was aliquoted into each well of a 96-well plate that had been previously been incubated with similar volume of feeder layer. The plates were incubated at 37 ^{0}C in a CO_2 incubator and each well was further topped up with 100 µl of HAT medium on day 7. The plates were observed 8-10 days later macroscopically for visible colonies and on day 14, the medium was changed to HT media. The clones were scored from day 14 onwards and supernatant of scored clones were harvested for flow cytometry screening.

Example 3: Pre-selection of B-lymphocytes from Immunised Mice using Immunomagenetic Selection.

[00128] As shown in Figure 3, biotynlated HLA Monomers bind to specific B cell Receptors. Anti-biotin coated Miltenyi MACs beads used to isolate bound B cells on magnetic columns prior to myeloma fusion.

[00129] To perform Example 3, the following methods were used.

[00130] Biotinylation of HLA monomers and selection of antigen specific B cells

[00131] Approximately 70 µl of purified monomer was biotinylated with 10 µl of biomix A, 10 µl of biomix B and 10 µl of supplemental biotin, 0.2 µl of 3 mg/ml BirA enzyme (Avidity LLC), 0.2 µl of 0.1 M PMSF and 0.1 µl of 1 mg/ml pepstatin A at 28^{0}C for 18 hrs. 1 ml of 10 mM Tris was added to the biotinylated mixture, transferred to a Centricon YM-30, and centrifuged at 3500 rpm for 10 mins to wash out the mixture to leave behind the biotinylated monomers.

[00132] The amount of biotinylated product was quantitated by immunoprecipitation using streptavidin beads (Sigma-Aldrich Inc.). Approximately 10 µl of beads were washed with 1 ml of 10 mM Tris, 150 mM NaCl, 0.1% Triton-X100 and pulsed down at not more than 8000 rpm. The supernatant was discarded and 20 µg of previously biotinylated monomers were added with 500 µl of 10 mM Tris, 2 µl of 0.1 M PMSF and 1 µl of 1 mg/ml of pepstatin A.

The mixture was left to shake at 4 ^{0}C for 16 hrs. After incubation, the beads were washed similarly as stated above twice. The supernatant were subsequently discarded and the sample was ran on 15 % SDS-PAGE gel and visualized with Coomassie blue staining. The percentage of successfully biotinylated HLA monomers was determined by comparing against non-immunoprecipiated HLA monomers. A negative control of streptavidin beads only was used. The quantity of monomers was determined by visual comparison with known concentrations of bovine serum albumin (BSA) standards (Sigma-Aldrich) that were ran on the same SDS-PAGE gel. The biotinylated monomers were subsequently aliquot and stored at -80 ^{0}C for further use.

[00133] To enrich specific splenocytes, 50 µg of biotinylated monomer was incubated with half the splenocytes harvested at 4 ^{0}C for 20 mins. After which 5ml of cold RPMI was added and centrifuged at 1100 rpm for 5mins to wash out the unbound biotinylated monomers. The pelleted cells were incubated with 100 µl of anti-biotin microbeads (Miltenyi Biotec GmbH) and incubated at 4 ^{0}C for a further 30 mins. In the meantime, the LS columns (Miltenyi Biotec GmbH) were pre-wet with 1 ml of cold PBS followed by 1 ml of RPMI medium. After 30 mins, the unbound anti-biotin microbeads (Miltenyi Biotec GmbH) were washed out by centrifuging the mixture of pelleted cells and anti-biotin microbeads at 1100 rpm for 5 mins with 3 ml of cold RPMI medium. About 1 million of the pelleted cells were then added directly onto the each pre-wet LS column. After the appropriate cells were adsorbed onto the column, 3 x 1 ml of cold RPMI medium was used to wash out the unbound cells. After which, the LS column with the bound cells was removed from the magnetic board and cells were flushed out onto a 15 ml Falcon tube with cold 3 ml of RPMI medium. This process was repeated with another 3 ml of RPMI medium to elute out the remaining cells. The 15 ml Falcon tube now contains splenocytes enriched with specificity of interest.

Example 4: Screening B cell Hybridomas for TCR-like HLA-A0201/LMP2 specificity.

[00134] As shown in Figure 4: (A) The binding of antibodies in hybridomas clone supernatants to T2 cells pulsed with LMP2 peptide (Red Histogram) was compared by flow cytometry with T2 cells pulsed with the HLA-A0201 restricted Influenza A peptide GILGFVFTL (Blue Histogram) and cells stained with anti-mouse IgG antibody only (Green Histogram). The antibody of clone #243 were also screened against a panel of HLA-A0201 restricted peptides from Epstein-Barr virus--LMP1 (YLLEMLWRL (SEQ ID NO: 2)), LMP2 (CLGGLLTMV (SEQ ID NO: 1)), EBNA1 (FMVFLQTHI (SEQ ID NO: 3)), Cytomegalovirus--pp65 (NLVPMVATV (SEQ ID NO: 4)), pp65 (ILARNLVPM (SEQ ID

NO: 5)), IE1 (VLEETSVML (SEQ ID NO: 6)), IE1 (VLAELVKQI (SEQ ID NO: 7)), IE1 (YILGADPLRV (SEQ ID NO: 8)), Hepatitis B virus--core (FLPSDFFPS (SEQ ID NO: 9)), BCG--Ag85 (FIYAGSLSAL (SEQ ID NO: 10)) and Influenza matrix A--M1 (GILGFVFTL (SEQ ID NO: 11)). These peptides were pulsed onto T2 cells for staining. The legend of the histogram is stated on the figure 4B. The TCR-like anti-HLA-A0201/LMP2 #243 is exquisitely specific for only EBV-LMP2 (CLGGLLTMV (SEQ ID NO: 1)) associated with HLA-A0201 (Red Histogram).

Example 5: Pre-selection of B cells for required specificity significantly enhances the percentage of A0201/LMP2 specific Hybridomas versus Unselected spenocytes.

[00135] As shown in Figure 5, we compared unselected splenocytes with splenocytes selected on the basis of their binding to HLA-A0201/LMP2 monomers prior to fusion. Unselected splenocytes generated no HLA-A0201/LMP2 specific hybridomas compared to splenocytes selected for their binding capacity to the immunogen.

[00136] To perform Example 5, the following methods were used.

Screening of clones by flow cytometry

[00137] The supernatant of scored clones were screened by flow cytometry using T2 cells. 100 µl of the supernatant of scored clones was pipetted out of the well and transferred into two labeled flow cytometry tubes (BD Biosciences). Appropriate number of T2 cells were harvested and one set was incubated with HLA-A0201 restricted peptide (Mimotope) while the other set was incubated with our peptide of interest HLA-A0201 restricted peptide of Epstein-Barr virus $LMP2A_{426-434}$ (CLGGLLTMV (SEQ ID NO: 1)) (Mimotope) for 30 mins at 37 ^{0}C in a CO_2 incubator. After incubation, an equal amount of peptide-pulsed cells were aliquoted into tubes containing the supernatant. The tubes with the supernatant were subsequently incubated at 4 ^{0}C for 30 mins. To wash away the excess supernatant and unbound antibodies, 4 ml of cold PBS supplemented with 1 % FBS was added into each tube and centrifuged at 350 g for 5 mins. The supernatant was discarded and 10 µl of diluted goat anti-mouse IgG (H+L) RPE conjugated antibody (Dako) was added to the pelleted cells. The tubes were incubated in the dark at 4 ^{0}C for 30 mins. After incubation, 4 ml of previously described wash buffer was added and centrifuged at 350 g for 5 mins to wash out the unbound secondary antibodies. The cells were resuspended and fixed with 500 µl of 4 % Para formaldehyde (PFA) (Thermo Fisher Scientific) before being analyzed with CellQuest Pro Software on a BD FACsCalibur machine (BD Biosciences).

[00138] The exquisite specificity of the TCR-like mAbs were determined by exogenously pulsing T2 cells with 5 μM of each HLA-A0201 restricted peptides from various pathogens including their respective targeted peptide. As T2 cell lacks both transporter associated with antigen processing-TAP1 and TAP2, it is unable to express endogenously processed peptides on its MHC class I and will express peptides that are exogenously pulsed onto its MHC class I. The bindings of the various TCR-like mAbs were analyzed by flow cytometry after staining with anti-murine IgG (H+L)-conjugated with RPE (Dako).

[00139] The antibodies were screened against a panel of HLA-A0201 restricted peptides that include $EBNA1_{562-570}$ (FMVFLQTHI (SEQ ID NO: 3)) (red), $LMP1_{125-133}$ (YLLEMLWRL (SEQ ID NO: 2)) (purple), $LMP2A_{426-434}$ (CLGGLLTMV (SEQ ID NO: 1)) (brown), Influenza virus $M1_{58-66}$ (GILGFVFTL (SEQ ID NO: 11)) (dark green), *Mycobacterium tuberculosis* $Ag85B_{143-152}$ (FIYAGSLSAL (SEQ ID NO: 10)) (pink), Hepatitis B virus $sAg_{183-191}$ (FLLTRILTI (SEQ ID NO: 12)) (light blue), Human immunodeficiency virus $Pol_{476-484}$ (ILKEPVHGV (SEQ ID NO: 13)) (blue), $gp120_{120-128}$ (KLTPLCVTL (SEQ ID NO: 14)) (yellow), Gag_{77-85} (SLYNTIAVL (SEQ ID NO: 15)) (grey), CMV $IE1_{316-324}$ (VLEETSVML (SEQ ID NO: 6)) (orange),$IE1_{81-89}$ (VLAELVKQI (SEQ ID NO: 7)) (black), $pp65_{495-503}$ (NLPVMVATV (SEQ ID NO: 16)) (light brown) and cells stained with anti-murine IgG (H+L) antibody (light green). It was observed that theTCR-like mAb was exquisitely specific only for its respective peptide associated with HLA-A0201 and not the rest of HLA-A0201 peptide complexes.

[00140] 1 x 10^6 cells were pulsed with increasing concentrations of peptide, ranging from 1nM to 10 000nM (final concentration) and incubated with 0.5μg of TCR-like mAb at 4°C for 30 min, followed by detection with 0.5μl of 10μg/ml RPE goat anti-mouse IgG (Dako) and analysis by flow cytometry.

[00141] To determine the sensitivity of the TCR-like monoclonal antibody, a titration of the peptides was tested with T2 cells. T2 cells were pulsed with various concentrations of the peptides and negative control for the TCR-like monoclonal antibody, namely $EBNA1_{562-570}$ (FMVFLQTHI (SEQ ID NO: 3)). As seen from the plot, the monoclonal antibodies were used to detect their respective HLA-A0201 bound peptides. The TCR-like mAbs targeting HLA-A0201/EBNA1 was able to detect its targeted peptide pulsed down to 14pM.

Example 6: Immunoglobulin Isotype Test for anti-HLA-A0201/LMP2 specific monoclonal.

[00142] As shown in Figure 6, the isotype profile for clone LMP2#243 shown above is of IgG1 and kappa light chain. HC=Heavy Chains, LC=Light Chains.

[00143] To perform Example 6, the following methods were used.

Antibody isotyping

[00144] Supernatant of positively scored hybridoma was collected and placed into each development tube. The tube was incubated at room temperature for 30 sec and vortexed briefly to resuspend the colored latex. The isotyping strip of the Isostrip Mouse Monoclonal Isotyping kit (Roche) was placed into each tube and incubated until the distinctive blue bands appear. The bands indicate the class or subclass and light chain composition of the antibody found in the supernatant.

Example 7: Affinity determination of TCR-like monoclonals HLA-A0201/LMP2 #243 against increasing concentrations of antigen using Biacore™.

[00145] As shown in Figure 7, antigen concentrations ranged from 0 to 320nM. The dissociation constant K_D was determined by measuring the binding kinetics of the HLA-A0201/LMP2 monomers to anti-HLA-A2/LMP2 antibody. Serially diluted concentrations of the HLA-A0201/LMP2 monomers (320nM to 10nM) were flowed over the immobilized antibody to determine the K_{on} and K_{off} .The binding response were analyzed using a global fit algorithms (BIA evaluations 3.1) assuming 1:1 Langmuir binding. The K_D of antibody to HLA-A0201/LMP2 monomers was determined to be 6.98nM. The data points were fitted using four-parameter logistics. The affinity for the antibody produced by #243 is significantly higher than that generated by BB7.2 (K_D =162nM).

[00146] To perform Example 7, the following methods were used.

Surface Plasmon Resonance (SPR)

[00147] Kinetic studies were evaluated by surface plasmon resonance using a BIAcore 3000™ (GE Healthcare). The monoclonal antibodies were immobilized covalently onto the surface of sensor chip CM5 by amine coupling. To determine the dissociation constant (K_D) of the antibodies, the respective MHC class I/peptide were flowed at different concentrations ranging from 320 nM to 10 nM over the relevant flow cells at a rate of 30 μL min^{-1} at 25^0C. Responses were recorded and analyzed using BIAevaluation software 3.2.

[00148] The affinity of each of the antibodies to their respective HLA-A0201/peptide was determined using surface plasmon resonance (SPR) using the Biacore 3000 optical biosensor (GE Healthcare). The TCR-like monoclonal antibody was immobilized to a Biacore CM5 sensor chip via amine group linkage up to 1200 RU. The kinetic measurements of the binding of the antibody were determined by flowing various concentrations of HLA-A02/LMP-2A

peptide monomer over the surface of the respective antibody coated chip. Dilutions of 320 nM to 10 nM of monomers were injected at 30 µl/min and the binding response was calculated assuming 1:1 Langmuir binding. The data were analyzed using a global fit algorithm (BIA evaluation 3.1) to calculate association rate (K_{on}) and dissociation rate (K_{off}) values to determine the dissociation constant or affinity constant (K_D). The K_D of the TCR-like mAb against HLA-A02/LMP2A is 6.98 nM.

Example 8: Fine mapping of interaction between HLA-A0201/LMP2 complex with #243 TCR-like monoclonal antibody by alanine walking.

[00149] As shown in Figure 8, each of the peptides were mutated with alanine at the position indicated by the peptide's number allocation within the HLA-A0201 restricted LMP2 epitope sequence CLGGLLTMV (SEQ ID NO: 1). The antibody staining of each peptide pulsed on T2 cells were visualized using flow cytometry. The histogram showed the flow cytometry results of staining with #243 monoclonal antibody on various T2 cells pulsed with the peptide allocated as stated (A). The positions mutated to alanine at various positions are shown on the table B. The measured mean fluorescence index (MFI) for the readings from flow cytometry were plotted in a histogram (C). The interaction between #243 antibody and antigen (CLGGLLTMV (SEQ ID NO: 1)) is sensitive between positions 5 to 9 as any mutation in the amino acids resulted in a decrease in the ability of the antibody to recognize the antigen.

[00150] To perform Example 8, the following methods were used.

Alanine scanning

[00151] Alanine scanning mutagenesis of the peptide of interest is a method of systematic alanine substitution for the identification of sites or amino acid for proper conformation. This form of mutagenesis enables the fine mapping of antibody-epitope interaction with non-polar hydrophobic amino acid (alanine). With the substitution of alanine, all side chain atoms beyond the β-carbon are removed and the role of the side chain functional groups of the original amino acids can be inferred from these alanine mutations. Alanine with methyl side chain lacks the unusual backbone dihedral angel preferences. Although glycine with an H side chain similarly nullifies the side chain, it enables conformational flexibility due to other non-mutated amino acids within the peptide.

[00152] This method is significantly less laborious than saturation mutagenesis substitutes with all 20 naturally occurring amino acids and similar results are observed from both methodology. Using alanine substitution of each amino acid on the peptide, we can

interrogate the sites necessary for antibody recognition. These peptides are pulsed onto TAP-deficient T2 cells exogenously to associate with HLA-A0201 on the cell surface for antibody detection. With flow cytometry, the mean fluorescence index (MFI) of each antibody binding on each mutated peptide can be determined and the effect of positional amino acid can be examined.

Example 9: Variants of HLA-A0201 restricted LMP2 peptide of interest.

[00153] As shown in Figure 9, the sequence of these peptides were determined from sequencing of Epstein-Barr virus obtained from both healthy infected volunteers and patients with EBV-associated malignancies. The variants of the peptide are shown according to the position and amino acid mutations and accordingly allocated a designation for further studies. Adapted from Lee *et al.*,1997[4].

Example 10: Surface binding of TCR-like monoclonal to T2 cells pulsed with peptides with various clinically observed peptides variant to CLGGLLTMV (SEQ ID NO: 1).

[00154] As shown in Figure 10, the bar chart represents the mean fluorescent index of the various peptides pulsed T2 cells followed by detection with anti-HLA-A0201/LMP2 antibody #243. The flow cytometry histogram is a representative data from each of the T2 cells pulsed with the peptides indicated by the arrows before being stained with the TCR-like monoclonal antibody against HLA-A0201/LMP2 #243. The allocation of the peptides is based on Figure 9. As observed the antibody #243 binds to all clinical variants of the peptide, CLGGLLTMV (SEQ ID NO: 1).

Example 11: Anti-HLA-A02/LMP2 recognises LMP2 epitope presented on HLA-A0201, HLA-A0206 and HLA-A0207.

[00155] As shown in Figure 11, flow cytometry was used to analyse binding to 4 BCL cell lines pulsed with LMP2 peptide and clinically observed variants of the epitope. Each set of BLCLs were pulsed with B95-8 (Red Histogram), P1S (Yellow Histogram), P6I (Orange Histogram), P1SP9A (Purple Histogram) or unpulsed (Blue Histogram) as from figure 9. CM936 (HLA-A0201) showed a good degree of binding along with CM371 and CF1007 (HLA-A0206 and HLA-A0207 respectively). CM392 (HLA-A0203) did not exhibit any binding suggesting that these HLA-A02 polymorphism result in different epitope specificities or binding.

[00156] To perform Example 11, the following methods were used.

Application of TCR-like mAbs in the context of HLA-A02 haplotype polymorphism

[00157] The majority of HLA-A02 positive Caucasian consists of A0201 and A02 Southern Chinese population consist of four major groups namely A0201, A0203, A0206 and

A0207. The polymorphism of these 4 subfamilies is due to amino acid mutations in the α1 and α2 chains. The MHC class I diagram illustrates the anchor amino acids at positions 2 and 9 of a typical A0201 restricted peptide. Differential recognition of the TCR-like mAb against LMP-$2A_{426-434}$ epitope presented on HLA-A0201, -A0203, -A0206 and -A0207. Flow cytometry was used to analyze binding to 4 BLCL pulsed with the LMP-$2A_{426-434}$ peptides and its clinical variant and stained with the TCR-like mAb. This was in comparison of pulsed BLCLs stained with an isotype antibody.

Example 12: DNA and corresponding translated amino acid sequences of heavy and light chains variable regions of #243 derived from murine hybridoma single cell clones with specificity for HLA-A0201/LMP2 (peptide CLGGLLTMV (SEQ ID NO: 1)).

[00158] Shown in Figure 12 is the deoxyribonucleic acid (top) and corresponding translated amino acid sequences (bottom) (heavy and light chains variable regions) derived from murine hybridoma single cell clones with specificity for HLA-A0201/LMP2 (peptide CLGGLLTMV (SEQ ID NO: 1)).

Example 13: Immunological staining of EBV infected-HLA-A0201 positive nasopharyngeal carcinoma (NPC) biopsy with TCR-like monoclonal antibodies.

[00159] As shown in Figure 13, the control panel was stained with secondary antibodies only. The biopsy was also stained with BB7.2 antibody that is specific for HLA-A02 .The rest of the panels showed the staining of the biopsy with HLA-A0201/LMP2 and HLA-A0201/EBNA1 antibodies and the combination staining for the last panel is a combination of both TCR-like monoclonal antibodies. Both biopsies were observed to stain positive for HLA-A0201/LMP2 and HLA-A0201/EBNA1 complexes in a number of cells. Positive staining was seen by reddish brown colour.

[00160] To perform Example 13, the following methods were used.

Immunohistochemistry

[00161] Frozen NPC biopsies were sectioned (4 μm) and embedded onto slides. Slides were fixed and subsequently stained with TCR-like mAbs or BB7.2. Peroxidase block (Dako) was used prior to treatment with Dako labeled polymer. After further washing, immunoperoxidase staining was developed with DAB (Dako).

Biopsy and blood collection

[00162] Informed consent from healthy volunteers and patients were taken before biopsy or blood extraction. NPC patients at the National University Hospital were enrolled with

informed consent. The respective IRBs approved this study and conformed to the WMA Declaration of Helsinki and the NIH Belmont Report (IRB no: 07-043E).

Discussion

[00163] While existing tetramer technology can be utilized to study EBV-specific T-lymphocytes generated as a result of natural infection, there are no current methodologies that allow the visualization, quantification and analysis of the TCR ligands expressed on the surface of EBV infected cells and tumors. This severely impedes the ability of EBV researchers to directly address the impact of candidate immune evasion mechanisms on the processing and presentation of EBV antigens in infected cells and APC. This also impairs targeting of tumor cells *in situ* based on their association with EBV. In our laboratory, we have developed techniques that allow for the production of novel monoclonal antibodies with fine specificity for EBV epitopes expressed in the context of HLA on the surface of EBV infected cells (Figure 1-outline of new methodology). The EBV antigen that we have successfully targeted is Latent Membrane Protein 2 (LMP2: CLGGLLTMV (SEQ ID NO: 1)/HLA-A0201). These monoclonals represent unique immunological targeting reagents that will enable a thorough investigation of EBV-LMP2 antigen processing/presentation during latency and tumouragenesis and the targeting of LMP2 expressing EBV-associated tumour cells *in situ*.

[00164] As disclosed herein, we have designed, expressed and FPLC purified recombinant, membrane free, fully folded, heterotrimeric complexes of HLA-A0201 heavy and light chains plus an antigenic peptide derived from the Latent Membrane Protein 2 of Epstein Barr virus (amino acid sequence:CLGGLLTMV (SEQ ID NO: 1)). This complex was used as a source of antigen to stimulate antibody responses in immunized Balb/C mice (see Figure 1 and 2). Spleens were removed from the immunized mice after 45 days and used as a source of B-lymphocytes for myeloma fusion/hybridoma generation. Antigen specific antibody producing hybridomas were screened by flow cytometry using peptide pulsed human B-cell lines.

[00165] We have also developed a technical innovation of standard hybridoma approaches that addresses the technical challenge of generating monoclonal antibodies with fine specificity for peptide/HLA-A0201 complexes. A standard monoclonal antibody protocol involves immunizing mice with the antigen of choice then fusing immune splenocytes from the immunized mice with a myeloma fusion partner to generate immortalized antibody producing hybridomas. However, we found that this 'classical' approach does not work. No hybidomas generated using the classical approach was neither specific for human HLA-

A0201 or this human HLA plus the EBV derived LMP2 peptide. Hence, we developed a technical innovation of standard hybridoma technology that involves pre-purifying the antigen specific B cells prior to myeloma fusion from the immunized mice, using a biotinylated form of the monomer linked to an immunomagnetic bead (see Figure 3). This greatly enables the obtaining of hybridomas that have the correct specificity compared to standard hybridoma approaches (see Figure 5).

[00166] We have also designed a screening methodology to test for monoclonal hybridomas producing antibodies that are HLA-A0201/LMP2 specific. This involved using Flow Cytometry to compare the binding activities of the antibodies in multiple hybridoma cell line supernatants for an HLA-A0201 expressing human cell line (T2) that have been pulsed with the LMP2 peptide (CLGGLLTMV (SEQ ID NO: 1)) or with an HLA-A0201 restricted peptide from Influenza A (GILGFVFTL (SEQ ID NO: 11)). Hybridoma supernatants with clear specificity for the LMP2 peptide on HLA-A0201 can be identified using this methodology (see Figure 4).

[00167] We have also demonstrated the importance of our adaptation to standard hybridoma technology by comparing the numbers of specific hybridoma clones generated from the same splenocyte pool using immunomagnetic pre-selection of antigen specific cells versus no pre-selection prior to myeloma fusion and immortalization (see Figure 5).

[00168] We have also shown that the antibody secreted by the immortalized hybridoma clone #243, has a mouse IgG1 heavy chain and a Kappa light chain (see Figure 6).

[00169] We have also shown that high affinity antibodies can be made with specificity for HLA-A0201/LMP2 peptide complexes. We measured the affinity binding constant of our anti-HLA-A2/LMP2 monoclonal (#243) and can show that it has a higher binding affinity (KD = 6.98nM) for these complexes than a pan-HLA-A02 antibody (162nM) (BB7.2) (see Figure 7).

[00170] We have also analyzed the interaction of antigen-antibody between HLA-A0201/LMP2 (CLGGLLTMV (SEQ ID NO: 1)) and antibody #243. The fine interaction between the antibody and antigen was carried out using alanine walking, where each amino acid of the antigen was substituted with alanine. This enables the determination of the essential sites of binding between antibody #243 and the antigen (CLGGLLTMV (SEQ ID NO: 1)) (see Figure 8).

[00171] We have also analyzed the ability of our antibody #243 to detect all clinically observed variants of the peptide of interest (see Figure 10).

[00172] We have also analyzed the ability of our antibody #243 to detect HLA-A02/LMP2 complexes and its clinically observed variants in human cell lines expressing the Caucasian form of HLA-A02 (HLA-A0201) versus the Asian variants of HLA-A02, HLA-A0203, HLA-A0206 and HLA-A0207[12]. The changes in amino acid sequence expressed by the different variants of HLA-A02 plus their frequency in Caucasian and Asian populations are illustrated in Figure 11A. The ability of antibody #243 to recognize these complexes by Flow cytometry is shown in Figure 11B.

[00173] We have also sequenced the variable regions of the antibody heavy and light chains. These can now be combined with the known sequence of the conserved constant regions of murine IgG1 to yield a complete IgG sequence (see Figure 12 for sequenced variable regions).

[00174] We have also shown that antibody #243 is able to detect HLA-A0201/LMP2 complexes in biopsy of HLA-0201 and EBV-positive nasopharyngeal carcinoma in Figure 13.

[00175] To our knowledge, there are no antibodies that have been made previously with specificity for EBV derived peptides expressed in association with human HLA on the surface of EBV infected cells. Hence, this antibody represents the first antibody worldwide that has the potential to be used to target EBV infected cells based on their HLA expression profile.

[00176] Moreover, in order to make this very specialized monoclonal, we developed an improved method for making specific antibodies based on a prior selection of the antigen specific B cells that should be applicable to all known HLA/peptide combinations from tumor, virus, bacteria, and parasite derived protein antigens. Whilst antigen specific T lymphocyte cell lines can be made in 1-2 laboratories worldwide with a 'similar' specificity, these cell lines require a high degree of technical proficiency to make.

[00177] Other laboratories have successfully produced TCR-like monoclonal antibodies using a standard hybridoma approach or by employing phage antibody libraries. However, the numbers of this type of antibody remain extremely low and none of them have been designed for targeting EBV epitopes. The affinities of the antibodies made by phage display are predictably low (see appendix I). Hence, our methodology represents a way of making the generation of these antibodies easier. Also, the high affinity that we observe for #243 suggests that our methodology may also incorporate a qualitative improvement in the types of antibody that can be discovered.

[00178] Additional embodiments of the present disclosure include:

[00179] The monoclonal antibodies can be humanized and used to elicit an immune attack against cells and tissues infected with Epstein-Barr virus in human patients. The disease applications include EBV-linked lymphoproliferative disease, infectious mononucleosis, Nasopharyngeal Carcinoma, Burkitt's Lymphoma, Hodgkins lymphoma.

[00180] The monoclonal antibodies can be tagged with toxins and chemotherapeutic reagents to be used as an 'immunotoxin' that better targets these toxic agents to tumour cells.

[00181] These monoclonal antibodies can be tagged with radionuclides to improve targeting of EBV infected tumour cells *in vivo* in both a diagnostic and therapeutic capacity. For instance, detecting EBV infected nasopharyngeal carcinoma cells in disparate lymph nodes after the tumour has metastasized by PET would be one approach. This will enable otolaryngologist to better target the diseased lymph nodes for surgery and/or radiotherapy.

[00182] A possible major advantage of this type of antibody is the ability to employ it in combination with other similar antibodies targeting different EBV peptides in association with different HLA-types. Most human beings express up to 25 different peptide/HLA combinations from EBV proteins on their infected cells. Hence, this allows us to potentially employ up to 25 different antibodies simultaneously, all of which are EBV tumour specific. This could be hugely advantageous in ensuring that the tumour cells and viruses are given no opportunity to adapt to this form of therapy.

[00183] Humanized anti-HLA-A2/LMP2 antibody can be used as a diagnostic to directly quantify the numbers of infected cells *ex vivo* or *in vivo*.

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[00184] While specific aspects have been described and illustrated herein, such aspects should be considered illustrative only and not as limiting the disclosure as construed in accordance with the accompanying claims.

[00185] All publications,patent applications, and sequence accession numbers (e.g., Genbank accession numbers) cited in this specification are herein incorporated by reference in their entirety for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference for all purposes.

[00186] Although the foregoing disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this disclosure that certain changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

What is Claimed:

1. A recombinant antibody or fragment thereof that binds to a HLA/peptide complex, wherein the peptide has the sequence CLGGLLTMV (SEQ ID NO: 1).

2. The recombinant antibody or fragment thereof according to claim 1, wherein the peptide is derived from Epstein-Barr virus Latent Membrane Protein 2 (LMP2).

3. The recombinant antibody or fragment thereof according to claim 1, wherein the HLA is HLA-A0201.

4. The recombinant antibody or fragment thereof according to claim 1, wherein the the recombinant antibody or fragment thereof is capable of competitively inhibiting specific binding to the peptide of SEQ ID NO: 1, or binding by a monoclonal antibody produced by an anti-HLA-A0201/LMP2 hybridoma.

5. The recombinant antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof is selected from the group consisting of: (a) a whole immunoglobulin molecule; (b) an scFv; (c) a Fab fragment; (d) an F(ab')2; and (e) a disulfide linked Fv.

6. The antibody or fragment thereof according to claim 1, which comprises a heavy chain immunoglobulin constant domain selected from the group consisting of: (a) a human IgM constant domain; (b) a human IgG1 constant domain; (c) a human IgG2 constant domain; (d) a human IgG3 constant domain; (e) a human IgG4 constant domain; and (f) a human IgA1/2 constant domain.

7. The antibody or fragment thereof according to claim 1, which comprises a light chain immunoglobulin constant domain selected from the group consisting of: (a) a human Ig kappa constant domain; and (b) a human Ig lambda constant domain.

8. The antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof comprises a heavy chain comprising at least one CDR having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 31-33.

9. The antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof comprises a light chain comprising at least one CDR having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 36-38.

10. The antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof comprises a heavy chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 31-33, or a sequence having at least 95% identity thereto.

11. The antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof comprises a light chain comprising three CDR sequences selected from the group consisting of SEQ ID NOs: 36-38, or a sequence having at least 95% identity thereto.

12. The antibody or fragment thereof according to claim 1, wherein the antibody comprises a heavy chain sequence at least 95% identical to SEQ ID NO: 30.

13. The antibody or fragment thereof according to claim 1, wherein the antibody comprises a light chain sequence at least 95% identical to SEQ ID NO: 35.

14. The antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof binds to an antigen with an affinity constant (K_D) of less than 1 x 10^{-8} M.

15. The antibody or fragment thereof according to claim 1, wherein the antibody or fragment thereof binds to an antigen with an affinity constant (K_D) of less than 1 x 10^{-9} M.

16. A pharmaceutical composition comprising the antibody or fragment thereof according to any one of claims 1-15 and a pharmaceutically acceptable carrier effective to reduce EBV infected tumor cells in a subject.

17. A method of passive immunization comprising administration to a subject an effective amount of the antibody or fragment thereof according to any one of claims 1-15.

18. A method of treatment of EBV virus infection comprising administration to a subject in need thereof an amount of antibody or fragment thereof according to any one of claims 1-15, effective to reduce or prevent the disease.

19. The method of claim 18, wherein the antibody is administered intravenously (IV), subcutaneously (SC), intramuscularly (IM), transdermally, or orally.

20. The method of claim 18, wherein the antibody is administered in an amount in the range of 1 to 100 milligrams per kilogram of the subject's body weight.

21. A method of generating a recombinant antibody or fragment thereof that binds to a HLA/peptide complex, the method comprising the steps of:

(a) generating a complex of HLA associated with a peptide;

(b) immunizing an animal with the complex of step (a);

(c) isolating B cells specific to the complex; and

(d) immortalizing the B-cells from (c).

22. The method of claim 21, wherein the isolation in step (c) is by immuno-magnetic selection.

23. The method of claim 21, wherein the peptide is derived from Epstein-Barr virus Latent Membrane Protein 2 (LMP2).

24. The method of claim 21, wherein the peptide has the sequence CLGGLLTMV (SEQ ID NO: 1).

25. The method of claim 21, wherein the the HLA is HLA-A0201.

26. An isolated nucleic acid encoding the antibody or fragment thereof according to any one of claims 1-15.

27. An expression vector comprising the nucleic acid of claim 26.

28. A host cell comprising the expression vector of claim 27.

29. The host cell of claim 28, wherein the host cell is a bacterial cell.

30. The host cell of claim 28, wherein the host cell is a eukaryotic cell.

31. The host cell of claim 28, wherein the host cell is a mammalian cell.

Methodology Schematic

Figure 1

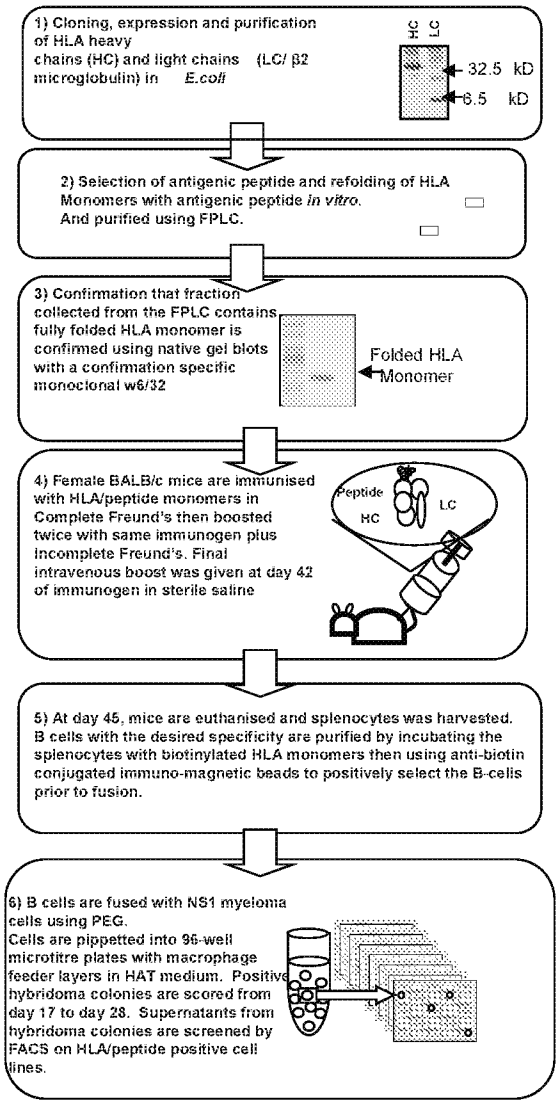


Figure 2

Generation and purification of HLA-A201/LMP2 monomers

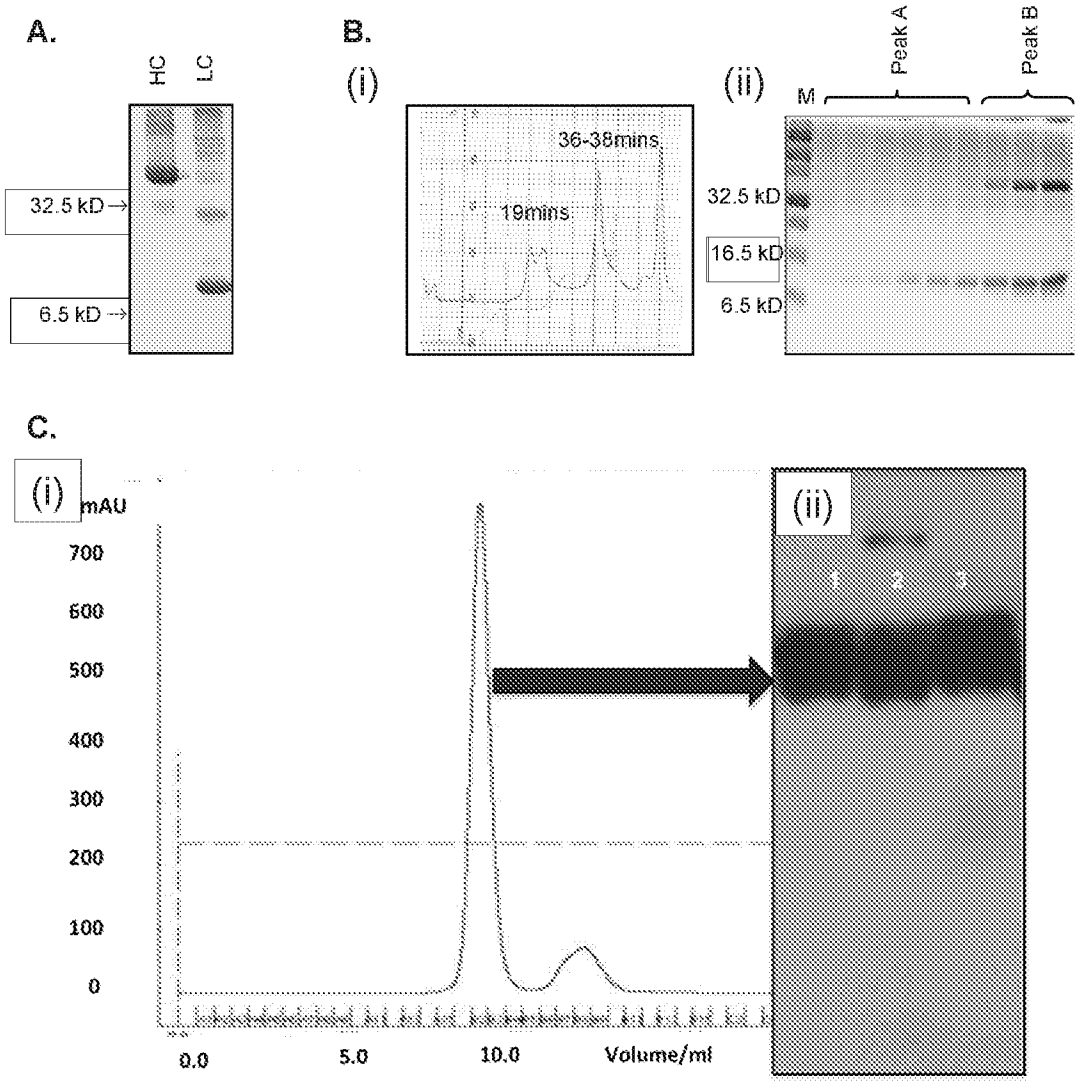
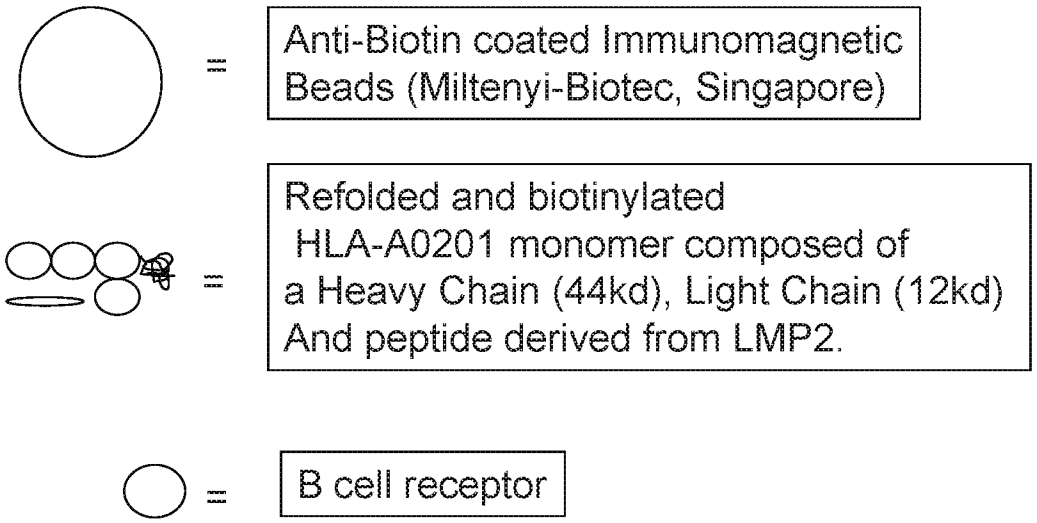


Figure 3

Principle of pre-selection of antigen specific B cells prior to hybridoma generation



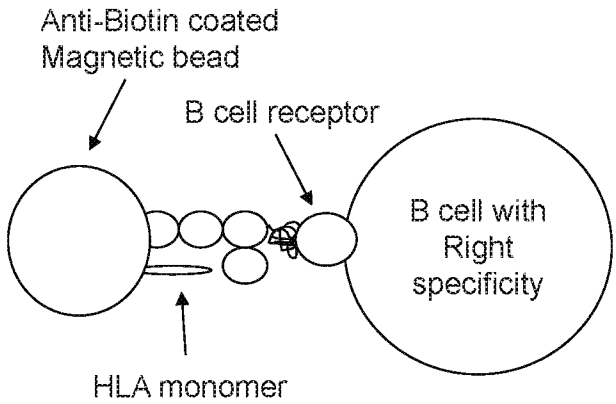


Figure 4

Screening B cell Hybridomas for TCR-like HLA-A0201/LMP2 specificity

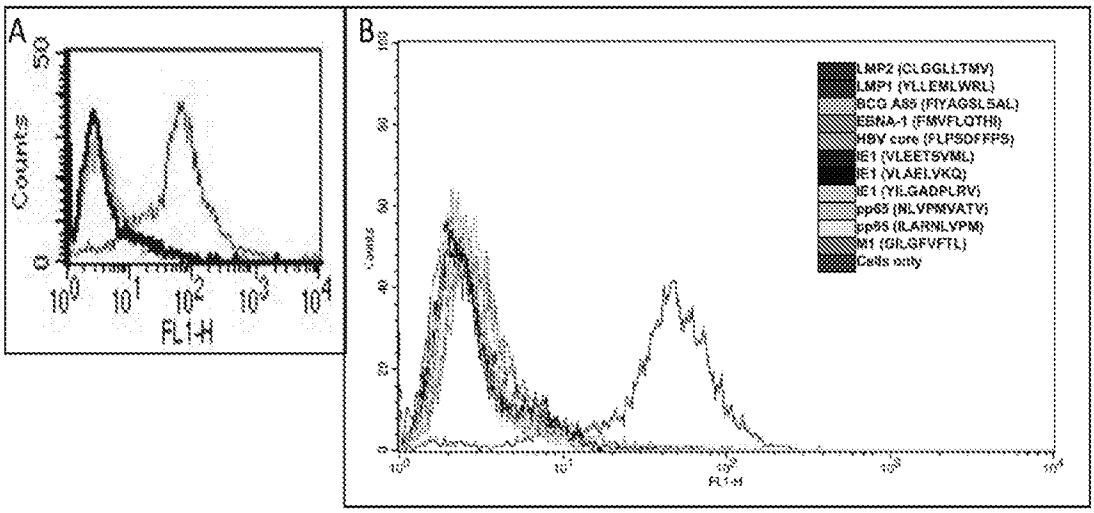


Figure 5

Pre-selection of B cells for required specificity significantly enhances the percentage of A0201/LMP2 specific hybridomas versus unselected splenocytes

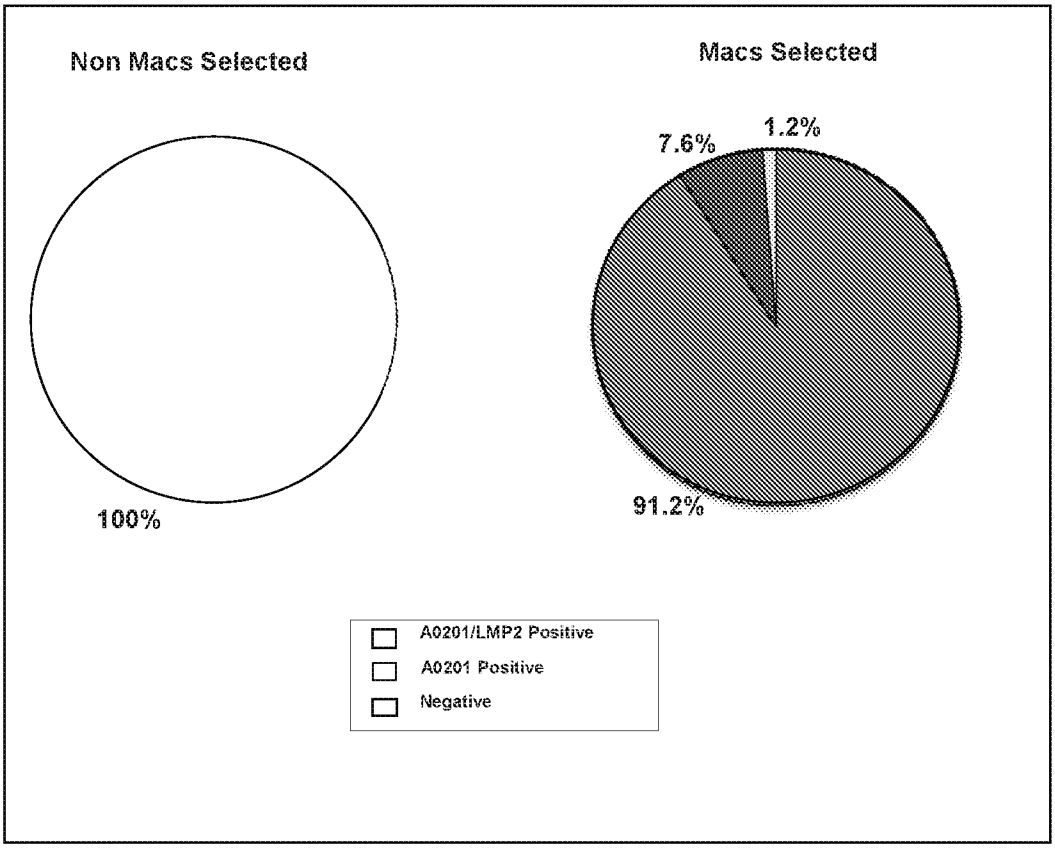


Figure 6

Immunoglobulin Isotype Test for anti-HLA-A2/LMP2 specific monoclonal

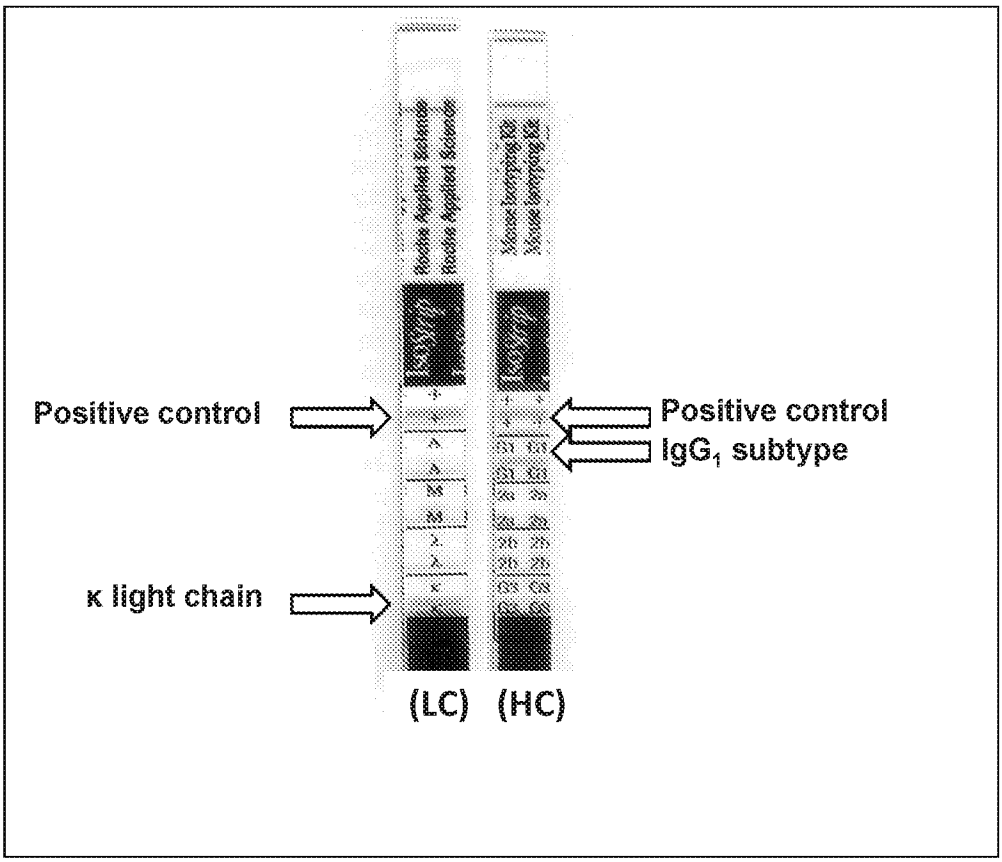


Figure 7

Affinity curve for TCR-like HLA-A0201/LMP2 antibody

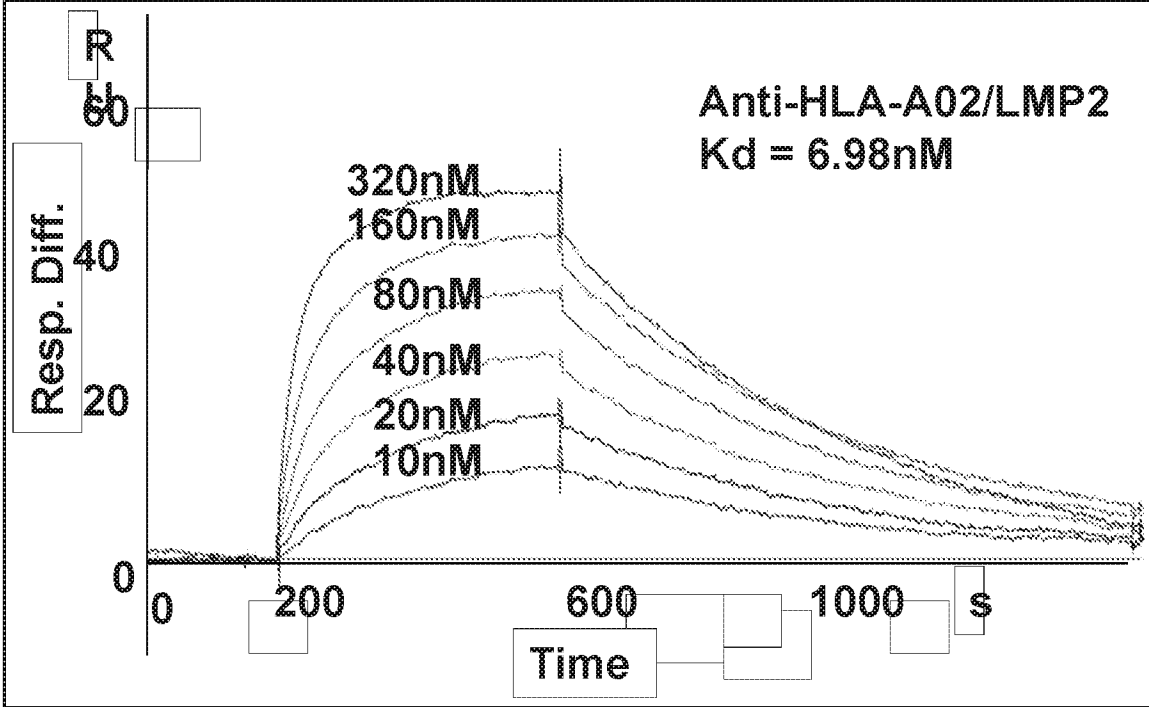
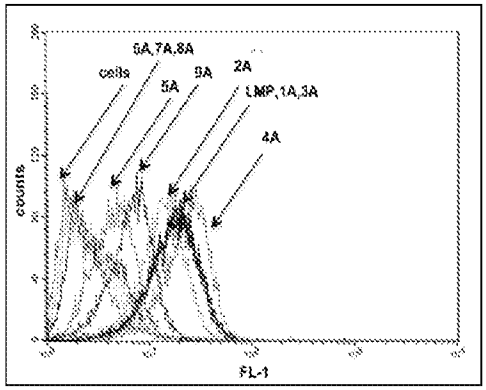


Figure 8

Fine mapping of antigen-antibody interaction using alanine walking

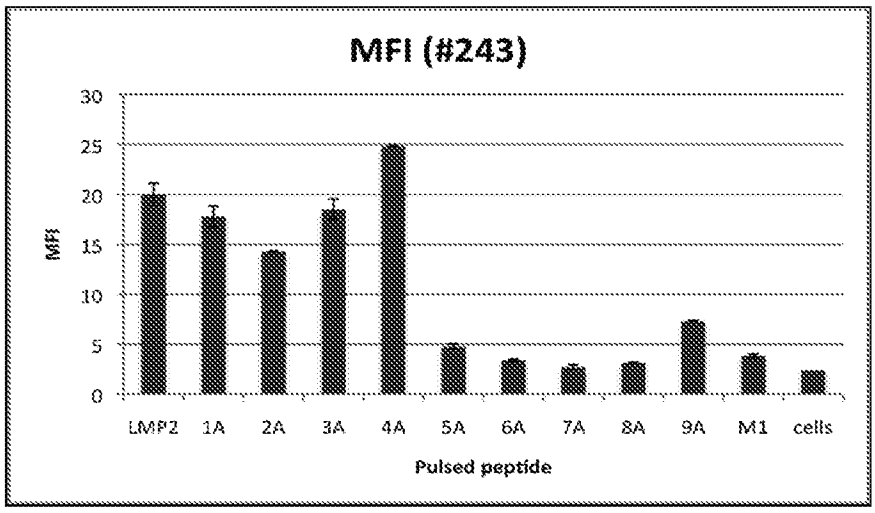
A.



B.

	P1	P2	P3	P4	P5	P6	P7	P8	P9
LMP2	C	L	S	G	L	L	T	M	V
1A	A	-	-	-	-	-	-	-	-
2A	-	A	-	-	-	-	-	-	-
3A	-	-	A	-	-	-	-	-	-
4A	-	-	-	A	-	-	-	-	-
5A	-	-	-	-	A	-	-	-	-
6A	-	-	-	-	-	A	-	-	-
7A	-	-	-	-	-	-	A	-	-
8A	-	-	-	-	-	-	-	A	-
9A	-	-	-	-	-	-	-	-	A
M1	G	I	L	G	F	V	F	T	L

C.



Data are given as mean ± SD of MFI in 3 independent experiments
* *p* values <0.05 were considered statistically significant

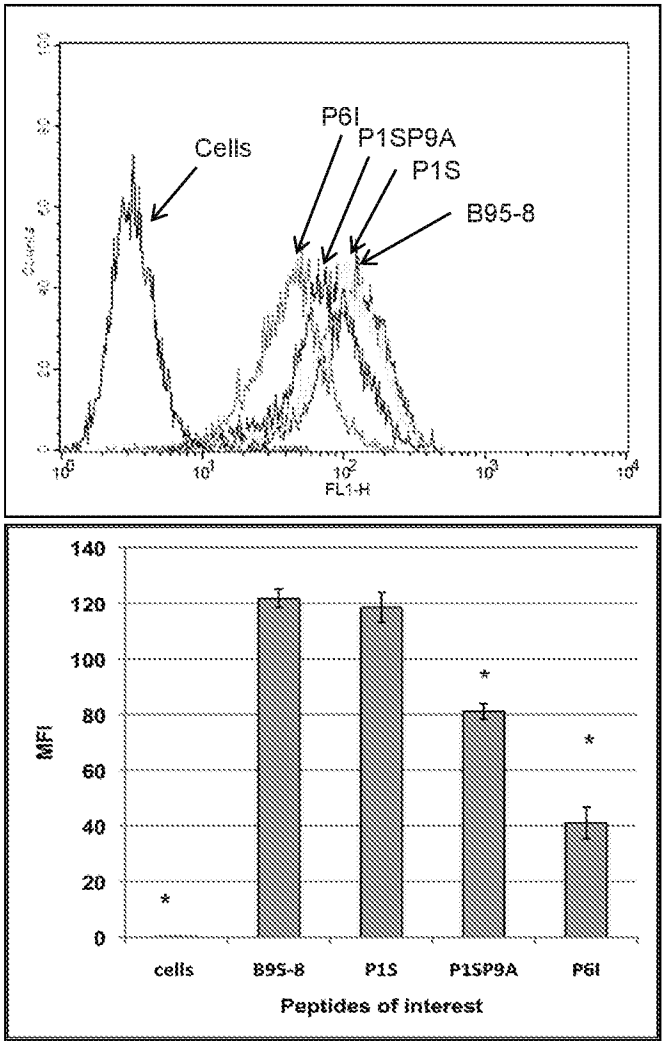
Figure 9

Variants of HLA-0201 restricted LMP2 peptide (CLGGLLTMV) of interest

Position/Peptide name	P1	P2	P3	P4	P5	P6	P7	P8	P9
B95-8	C	L	G	G	L	L	T	M	V
P1S	S	-	-	-	-	-	-	-	-
P6I	-	-	-	-	-	I	-	-	-
P1SP9A	S	-	-	-	-	-	-	-	A

Figure 10

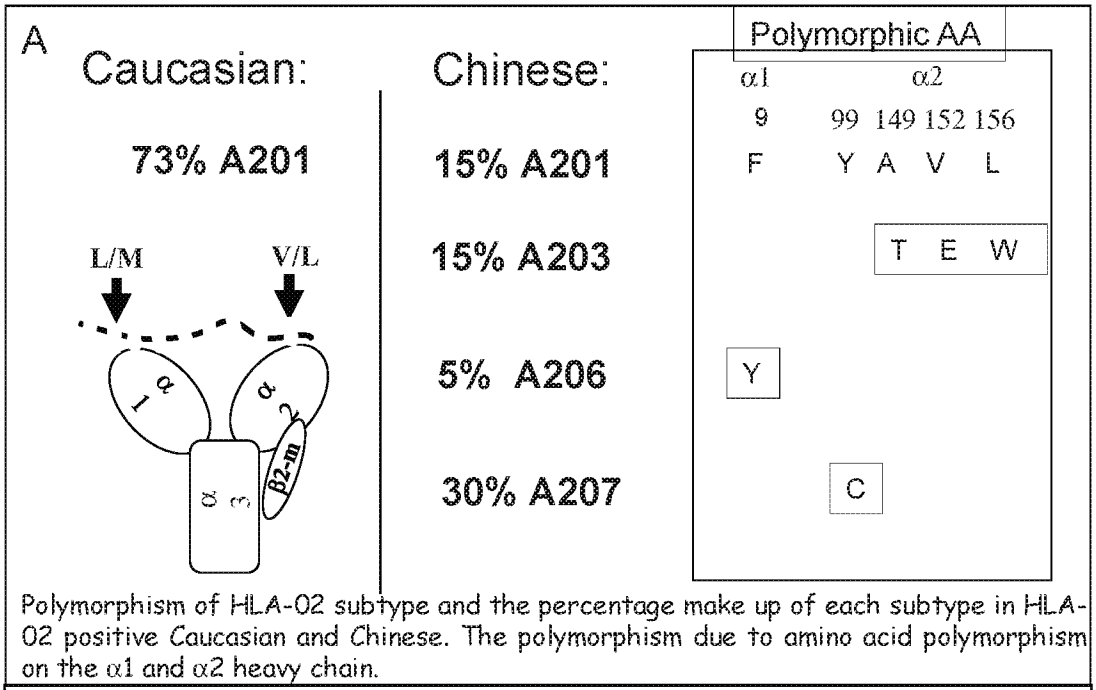
Surface binding of TCR-like monoclonal to T2 cells pulsed with peptides with various clinically observed peptide variant to CLGGLLTMV



Data are given as mean ± SD of MFI in 3 independent experiments
* *p* values <0.05 were considered statistically significant

Figure 11

Anti-HLA-A02/LMP2 recognises LMP2 epitope presented on HLA-0201, HLA-0206 and HLA-0207



Polymorphism of HLA-02 subtype and the percentage make up of each subtype in HLA-02 positive Caucasian and Chinese. The polymorphism due to amino acid polymorphism on the α1 and α2 heavy chain.

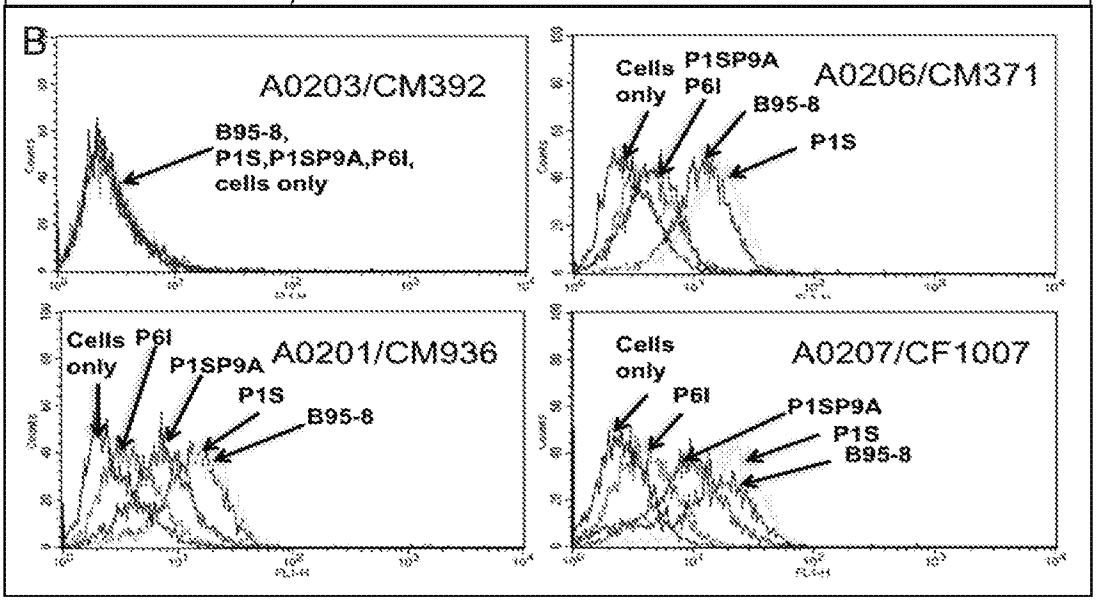


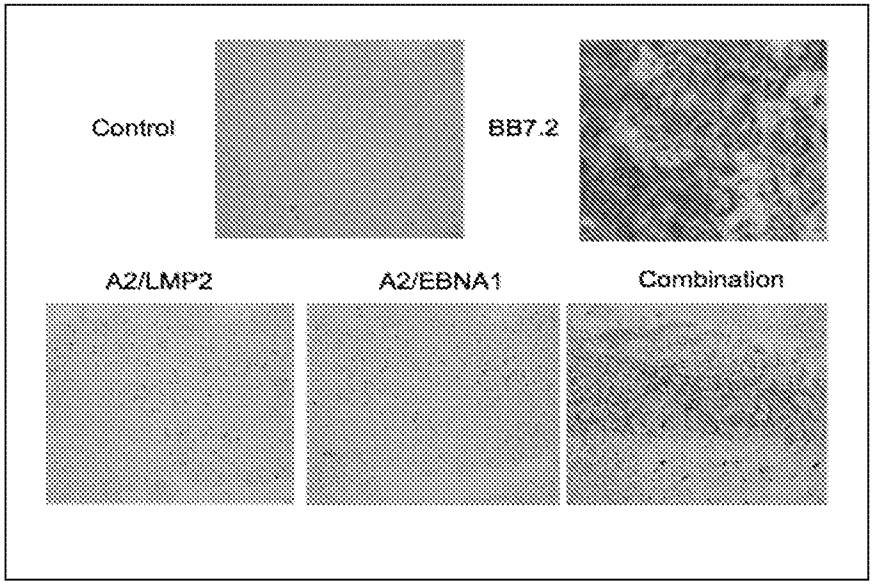
FIGURE 12

Amino acid sequences monoclonal antibodies derived from murine hybridoma single cell clones with specificity for HLA-A0201/LMP2 (CLGGLLTMV)

HLA-A0201/LMP2 hybridoma #243	
Heavy chain	CAGGTGCAGCTGAAGCAGTCAGGACCTGGCCTAGTGCAGCCCTCACAGAGC CTGTCCATCACCTGCACAGTCTCT**GGTTTCTCATTAACTAACTATGGT**GTACA CTGGGTTCGCCAGTCTCCAGGAAAGGGTCTGGAGTGGCTGGGAGTG**ATATG** **GAGTGGTGGAAGCACA**GACTATAATGCAGCTTTCATATCCAGATTGAGCATC AGCAAGGACAATTCCAAGAGCCAAGTTTTCTTTAAAATGAACAGTCTGCAAGC TAATGACACAGCCATATATTACTGT**GCCAGAAATTGGGTCCCTTACTACTTTG** **ACTACTGG**GGCCAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACGACACC CCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATG GTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGA CCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCT GCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACC TGGCCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACC AAGGTGGACAAGAAAATTGTGCCCAGGGATTGT QVQLKQSGPGLVQPSQSLSITCTVS**GFSLTNYG**VHWVRQSPGKGLEWLGV**IWS** **GGST**DYNAAFISRLSISKDNSKSQVFFKMNSLQANDTAIYYC**ARNWVPYYFDYW** GQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVP RDC
Light chain	GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACATCAGTTGGAGACAG GGTCAGCGTCACCTGCAGGGCCAGT**CAGAATGTGTTTACTAAT**GTAGCCTG GTATCAACAGAAACCAGGGCAAGCTCCTAAAGCACTGATTTAC**TCGACATCC** TACCGGTACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGGACAG ATTTCACTCTCACCATCAGCAATGTGCAGTCTGAAGACTTGGCAGAGTATTTC TGT**CAGCAATATATCAGCTATCCTCTCACGTTC**GGTGCTGGGACCAAGCTGG AGCTGAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAG TGAGCAGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCT ACCCCAAAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAA TGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGC ATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCT ATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTC AACAGGAATGAGTGT DIVMTQSQKFMSTSVGDRVSVTCRAS**QNVFTN**VAWYQQKPGQAPKALIY**STS**Y RYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFC**QQYISYPLTF**GAGTKLELKR ADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC

Figure 13

Immunological staining of nasopharyngeal carcinoma (NPC) biopsy with TCR-like monoclonal antibodies



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2015/050181

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/08 (2006.01) C07K 16/28 (2006.01) A61K 39/395 (2006.01) A61K 39/42 (2006.01) A61P 31/22 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

EPODOC, WPI-AP, MEDLINE, CAPLUS, BIOSIS, EMBASE GENOMEQUEST: Keywords: Macary P, Hanson B, Antibody immunoglobulin, Epstein Barr, EBV, HLA, MHC, TCR Mimic, LMP2, Complex, Fusion, Conjugat, hybridoma, SEQ ID NOs: 1, 30-33, 35-38 and like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 August 2015	10 August 2015
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	PAMPA RAY AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 2967

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/SG2015/050181**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIM, A.C.N. et al. 'Defining the Expression Hierarchy of Latent T-Cell Epitopes in Epstein-Barr Virus Infection with TCR-like Antibodies', Scientific Reports, 2013, Nov 18, 3:3232 doi: 10.1038/srep03232 Abstract; page 1, last paragraph; page 2, left hand column; page 3, Figure 1; page 5, left hand column	1-31
X Y	WO 2011/062560 A1 (NATIONAL UNIVERSITY OF SINGAPORE et al.) 26 May 2011 Abstract, paragraphs 63, 197-206, Examples 1-4 as above and paragraphs 130, 134, 137, 142-145, 234, 137, 145, 151, 152, 168	21, 22, 25 1-7, 14-20, 23, 24, 26-31
X	WO 2005/116072 A2 (WEIDANZ, J.A. and WITTMAN, V.) 08 December 2005 Abstract, paragraphs 102, 143, 144	21, 22, 25
Y	WO 2011/039508 A2 (UCL BUSINESS PLC) 07 April 2011 Abstract, pages 20, lines 9-13, pages 21, lines 17-19	1-7, 14-20, 23, 24, 26-31
Y	WO 2004/041849 A1 (THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH) 21 May 2004 SEQ ID 59, table 5, page 50, claim 16	1-7, 14-20, 23, 24, 26-31
A	WO 2012/123755 A1 (THE UNIVERSITY OF BIRMINGHAM) 20 September 2012 The Whole Document in particular Table 3, Page 29, lines 14-20, Page 30, lines 32-36, Claim 12	1-31

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/SG2015/050181**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/SG2015/050181**

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