HUMAN CYTOMEGALOVIRUS NEUTRALISING ANTIBODIES AND USE THEREOF

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention relates to antibodies having specificity for human cytomegalovirus, suitably monoclonal antibodies having that specificity and immortalised B cells that produce such monoclonal antibodies. The invention also relates to the epitopes that the antibodies bind to as well as the use of the antibodies and the epitopes in screening methods as well as the diagnosis, prophylaxis and therapy of disease.

BACKGROUND ART

Human cytomegalovirus (hCMV) is a widely distributed pathogen that may cause severe pathology in immunosuppressed adults and upon infection of the fetus and has been implicated in chronic diseases such as atherosclerosis. hCMV infects multiple cell types including fibroblasts, endothelial, epithelial and hematopoietic cells [1]. *In vitro* propagated attenuated strains of hCMV, which are being developed as candidate vaccines, have lost the tropism for endothelial cells, while retaining the capacity to infect fibroblasts [2]. Recently two viral glycoprotein complexes have been shown to control the cellular tropism of hCMV. A complex of gH, gL and gO is required for infection of fibroblasts, while a complex of gH, gL and proteins encoded by the UL131-UL128 genes are responsible for infection of endothelial cells, epithelial cells and dendritic cells [2-8].

Hyperimmune globulins are already commercialised for the prophylaxis of hCMV disease associated with transplantation and recent evidence indicates that they have therapeutic effect in pregnant women [9]. This therapeutic approach is limited by the low amount of neutralising antibody that can be transferred and for this reason the availability of human antibodies (such as human monoclonal antibodies) with high neutralising capacity would be highly desirable. However the target of hCMV neutralising antibodies remains to be established. Previous work identified gB and gH as potential targets. However, a humanised antibody to gH (MSL 109) did not show any significant effect in a clinical trial [10, 11]. All neutralising antibodies described so far had low neutralising capacity as they neutralise hCMV infection only at high concentrations. For instance, the antibody MSL-109 only displayed a 50% neutralising activity at a concentration of 10 μg/ml, a fact that may explain the lack of an *in vivo* effect [11]. The neutralising potency of anti-hCMV antibodies is typically measured using fibroblasts as target cells. However, hCMV is known to cause pathology by infecting other cell types such as endothelial, epithelial cells and leukocytes. There do not appear to be any monoclonal antibodies available that would be capable of neutralising with high potency infection of non-fibroblast target cells. The recently described neutralising antibodies to UL128 and UL130 also showed very low potency in neutralising infection of endothelial cells [7].

There is therefore a need for the production of neutralising antibodies against hCMV as well as the elucidation of the target to which such antibodies bind.

DISCLOSURE OF THE INVENTION

The invention is based on the production of antibodies and antibody fragments that neutralise hCMV infection and which have a particularly high potency in neutralising hCMV infection. Such antibodies are desirable, as only low concentrations are required in order to neutralise a given amount of virus. This facilitates higher levels of protection whilst administering lower amounts of antibody. Human monoclonal antibodies and the immortalised B cell clones that secrete such antibodies are also included within the scope of the invention.

The inventors have discovered that antibodies directed to a combination of UL130 and UL131A are particularly effective in neutralising hCMV. The combination may be a complex of UL130 and UL131A forming an epitope recognised by the antibody or an antibody may be directed to one of UL130 and UL131A, the presence of the other protein being necessary for specificity.

The invention also relates to the characterisation of the epitope to which the antibodies bind and the use of that epitope in raising an immune response.

The invention also relates to various methods and uses involving the antibodies of the invention and the epitopes to which they bind.

Antibodies of the invention

The invention provides monoclonal or recombinant antibodies having particularly high potency in neutralising hCMV. The invention also provides fragments of these recombinant or monoclonal antibodies, particularly fragments that retain the antigen-binding activity of the antibodies, for example which retain at least one complementarity determining region (CDR) specific for hCMV proteins UL130 and UL131A.

In this specification, by "high potency in neutralising hCMV" is meant that an antibody molecule of the invention neutralises hCMV in a standard assay at a concentration much lower than antibodies known in the art, for example compared to MSL-109.

Preferably, the antibody molecule of the present invention can neutralise at a concentration of 0.16μg/ml or lower (*i.e.* 0.15, 0.125, 0.1, 0.075, 0.05, 0.025, 0.02, 0.016, 0.015, 0.0125, 0.01, 0.0075, 0.005, 0.004 or lower), preferably 0.016μg/ml or lower (an antibody concentration of 10^{-8} or lower, preferably 10^{-9} M or lower, preferably 10^{-10} M or lower, *i.e.* 10^{-11}M, 10^{-12}M, 10^{-13}M or lower). This means that only very low concentrations of antibody are required for 50% neutralisation of a clinical isolate of hCMV *in vitro* compared to the concentration of MSL-109 required for neutralisation of the same titre of hCMV. Preferably, the concentration of antibody of the invention required for 50% neutralisation of infection of endothelial cells, epithelial cells and dendritic cells by a clinical isolate of hCMV is 50 times or more (*i.e.* 75, 100, 150, 200 or more) lower than that required by MSL-109. Potency can be measured using a standard neutralisation assay as described in the art.

The antibodies of the invention are able to neutralise hCMV. Preferably, an antibody according to the invention prevents infection of fibroblasts or endothelial cells. More preferably, an antibody according to the invention prevents infection of endothelial cells. Preferably, an antibody according to the invention prevents infection of both fibroblasts and endothelial cells. The antibodies of the invention preferably also prevent infection of dendritic cells and epithelial cells such as retinal cells.

These antibodies can be used as prophylactic or therapeutic agents upon appropriate formulation, or as a diagnostic tool.

A "neutralising antibody" is one that can neutralise the ability of that pathogen to initiate and/or perpetuate an infection in a host. The invention provides a neutralising monoclonal human antibody, wherein the antibody recognises an antigen from human cytomegalovirus (hCMV).

Preferably, an antibody according to the invention has specificity for a combination of UL130 and UL131A.

Preferably an antibody according to the invention is a monoclonal antibody referred to herein as 1F11 or 2F4. These antibodies were initially isolated from a hCMV infected donor, and are produced by the immortalised B cell clones referred to as 1F11 or 2F4. These antibodies have been shown to neutralise hCMV infection of endothelial cells, epithelial cells, retinal cells and dendritic cells. In addition, the antibodies 5A2 and 9A11, isolated from a different hCMV infected donor, show the same specificity for a combination of UL130 and UL131A and the ability to neutralise hCMV infection of endothelial, epithelial, retinal and dendritic cells. These antibodies are produced by the immortalised B cell clones referred to as 5A2 and 9A11, respectively.

1F11 consists of a heavy chain having the amino acid sequence recited in SEQ ID NO: 7 and a light chain having the amino acid sequence recited in SEQ ID NO: 8. 2F4 consists of a heavy chain having the amino acid sequence recited in SEQ ID NO: 17 and a light chain having the amino acid sequence recited in SEQ ID NO: 18. The CDRs of the antibody heavy chains are referred to as CDRH1, CDRH2 and CDRH3, respectively. Similarly, the CDRs of the antibody light chains are referred to as CDRL1, CDRL2 and CDRL3, respectively. The position of the CDR amino acids are defined according to the IMGT numbering system [12, 13, 14] as: CDR1 – IMGT positions 27 to 38, CDR2 – IMGT positions 56 to 65 and CDR3 – IMGT positions 105 to 117.

5A2 consists of a heavy chain having the amino acid sequence recited in SEQ ID NO: 39 and a light chain having the amino acid sequence recited in SEQ ID NO: 40.

The amino acid sequences of the CDRs of these antibodies are shown in Table 1.

Table 1

	1F11	2F4	5A2
CDRH1	GFTFSSYA (SEQ ID NO:1)	GFSFNTYG (SEQ ID NO:11)	GGTFSSYV (SEQ ID NO:33)
CDRH2	ISFDGDNK (SEQ ID NO:2)	IWDDGSKM (SEQ ID NO:12)	IIPIFNTA (SEQ ID NO:34)
CDRH3	AREELVGLMPPYYNYGLDV (SEQ ID NO:3)	ARDEGAIMLHAMTDYGLDV (SEQ ID NO:13)	ARDFLSGPMEMPGGYYGL DV (SEQ ID NO:35)
CDRL1	SSNIGNNF (SEQ ID NO:4)	NLGDEF (SEQ ID NO:14)	QSVLYSSNNKNY (SEQ ID NO:36)
CDRL2	DND (SEQ ID NO:5)	QDS (SEQ ID NO:15)	WAS (SEQ ID NO:37)
CDRL3	ETWDGSLNPAVV (SEQ ID NO:6)	QAWDSSTAHYV (SEQ ID NO:16)	QQYYSTPIT (SEQ ID NO:38)

The invention also includes an antibody comprising a heavy chain comprising one or more (*i.e.* one, two or all three) heavy chain CDRs from 1F11 or 2F4 (SEQ ID NOs: 1-3 or 11-13). Also included is an antibody comprising a heavy chain comprising one or more (*i.e.* one, two or all three) heavy chain CDRs from 5A2 (SEQ ID NOs:33-35).

Preferably an antibody according to the invention comprises a heavy chain comprising (i) SEQ ID NO: 1 for CDRH1, SEQ ID NO: 2 for CDRH2 and SEQ ID NO: 3 for CDRH3, or (ii) SEQ ID NO: 11 for CDRH1, SEQ ID NO: 12 for CDRH2 and SEQ ID NO: 13 for CDRH3. A further preferred antibody according to the invention comprises a heavy chain comprising SEQ ID NO: 33 for CDRH1, SEQ ID NO: 34 for CDRH2 and SEQ ID NO: 35 for CDRH3.

The invention also includes an antibody comprising a light chain comprising one or more (*i.e.* one, two or all three) light chain CDRs from 1F11 or 2F4 (SEQ ID NOs: 4-6 or 14-16). Also included is an antibody comprising a light chain comprising one or more (*i.e.* one, two or all three) light chain CDRs from 5A2 (SEQ ID NOs:36-38).

Preferably an antibody according to the invention comprises a light chain comprising (i) SEQ ID NO: 4 for CDRL1, SEQ ID NO: 5 for CDRL2 and SEQ ID NO: 6 for CDRL3, or (ii) SEQ ID NO: 14 for CDRL1, SEQ ID NO: 15 for CDRL2 and SEQ ID NO: 16 for CDRL3. A further preferred antibody according to the invention comprises a light chain comprising SEQ ID NO: 36 for CDRL1, SEQ ID NO: 37 for CDRL2 and SEQ ID NO: 38 for CDRL3.

Preferably an antibody according to the invention comprises a heavy chain having the sequence recited in SEQ ID NO: 7, 17 or 39. Preferably an antibody according to the invention comprises a light chain having the sequence recited in SEQ ID NO: 8, 18 or 40.

Hybrid antibody molecules may also exist that comprise one or more CDRs from 1F11 and one or more CDRs from 2F4. Preferably, such hybrid antibodies comprise three CDRs from 1F11 and three CDRs from 2F4. Thus, preferred hybrid antibodies comprise i) the three light chain CDRs from 1F11 and the three heavy chain CDRs from 2F4, or ii) the three heavy chain CDRs from 1F11 and the three light chain CDRs from 2F4. In an alternative, such hybrids may contain one or more CDRs from 5A2.

The invention also includes nucleic acid sequences encoding part or all of the light and heavy chains and CDRs of the present invention. Preferred nucleic acid sequences according to the invention include SEQ ID NO: 9 (encoding the 1F11 heavy chain variable region), SEQ ID NO: 10 (encoding the 1F11 light chain variable region), SEQ ID NO: 19 (encoding the 2F4 heavy chain variable region), and SEQ ID NO: 20 (encoding the 2F4 light chain variable region). Preferred nucleic acid sequences encoding the various CDRs include SEQ ID NO: 21 (encoding 1F11 CDRH1), SEQ ID NO: 22 (encoding 1F11 CDRH2), SEQ ID NO:23 (encoding 1F11 CDRH3), SEQ ID NO:24 (encoding 1F11 CDRL1), SEQ ID NO:25 (encoding 1F11 CDRL2), SEQ ID NO:26 (encoding 1F11 CDRL3), SEQ ID NO:27 (encoding 2F4 CDRH1), SEQ ID NO:28 (encoding 2F4 CDRH2), SEQ ID NO:29 (encoding 2F4 CDRH3), SEQ ID NO:30 (encoding 2F4 CDRL1), SEQ ID NO:31 (encoding 2F4 CDRL2) and SEQ ID NO:32 (encoding 2F4 CDRL3). Further preferred nucleic acid sequences according to the invention include SEQ ID NO: 41 (encoding the 5A2 heavy chain variable region), SEQ ID NO: 42 (encoding the 5A2 light chain variable region), SEQ ID NO: 43 (encoding 5A2 CDRH1), SEQ ID NO: 44 (encoding 5A2 CDRH2), SEQ ID NO:45 (encoding 5A2 CDRH3), SEQ ID NO:46 (encoding 5A2 CDRL1), SEQ ID NO:47 (encoding 5A2 CDRL2), SEQ ID NO:48 (encoding 5A2 CDRL3). Due to the redundancy of the genetic code, variants of these sequences will exist that encode the same amino acid sequences.

Variant antibodies are also included within the scope of the invention. Thus, variants of the sequences recited in the application are also included within the scope of the invention. Such variants may arise due to the degeneracy of the genetic code, as mentioned above. Alternatively, natural variants may be produced due to errors in transcription or translation. A variant of 2F4 is also disclosed herein. This variant comprises an additional two serine residues at the C-terminal end of the 2F4 heavy chain amino acid sequence (SEQ ID NO: 17). Thus, this variant of 2F4 consists of a heavy chain having the amino acid sequence recited in SEQ ID NO:49 and a light chain having the amino acid sequence recited in SEQ ID NO: 18. The nucleic acid sequence encoding the variant heavy chain is recited in SEQ ID NO:50. Thus, antibodies comprising the 2F4 variant heavy chain (SEQ ID NO: 49) are included within the scope of the invention.

Further variants of the antibody sequences having improved affinity may be obtained using methods known in the art and are included within the scope of the invention. For example, amino acid substitutions may be used to obtain antibodies with further improved affinity. Alternatively, codon optimisation of the nucleotide sequence may be used to improve the efficiency of translation in expression systems for the production of the antibody.

Preferably, such variant antibody sequences will share 70% or more (*i.e.* 80, 85, 90, 95, 97, 98, 99% or more) sequence identity with the sequences recited in the application. Preferably such sequence identity is calculated with regard to the full length of the reference sequence (*i.e.* the sequence recited in the application). Preferably, percentage identity, as referred to herein, is as determined using BLAST version 2.1.3 using the default parameters specified by the NCBI (the National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/) [Blosum 62 matrix; gap open penalty=11 and gap extension penalty=1].

Further included within the scope of the invention are vectors, for example expression vectors, comprising a nucleic acid sequence according to the invention. Cells transformed with such vectors are also included within the scope of the invention.

The invention also relates to monoclonal antibodies that bind to an epitope capable of binding the monoclonal antibody 1F11 or 2F4. The invention also relates to monoclonal antibodies that bind to an epitope capable of binding the monoclonal antibody 5A2.

Monoclonal and recombinant antibodies are particularly useful in identification and purification of the individual polypeptides or other antigens against which they are directed. The antibodies of the invention have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme. The antibodies may also be used for the molecular identification and characterisation (epitope mapping) of antigens.

Antibodies of the invention can be coupled to a drug for delivery to a treatment site or coupled to a detectable label to facilitate imaging of a site comprising cells of interest, such as cells infected with hCMV. Methods for coupling antibodies to drugs and detectable labels are well known in the art, as are methods for imaging using detectable labels. Labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Detection of the formation of an antibody-antigen complex between an antibody of the invention and an epitope of interest (an hCMV epitope) can be facilitated by attaching a detectable substance to the antibody. Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material is luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of

suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H. Such labeled reagents may be used in a variety of well-known assays, such as radioimmunoassays, enzyme immunoassays, *e.g.*, ELISA, fluorescent immunoassays, and the like. See for example, references 15-18.

An antibody according to the invention may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive metal ion or radioisotope. Examples of radioisotopes include, but are not limited to, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, Bi-213, Pd-109, Tc-99, In-111, and the like. Such antibody conjugates can be used for modifying a given biological response; the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon *et al.* (1985) "Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy," in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld *et al.* (Alan R. Liss, Inc.), pp. 243-256; ed. Hellstrom *et al.* (1987) "Antibodies for Drug Delivery," in *Controlled Drug Delivery*, ed. Robinson *et al.* (2d ed; Marcel Dekker, Inc.), pp. 623-653; Thorpe (1985) "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological and Clinical Applications*, ed. Pinchera *et al.* pp. 475-506 (Editrice Kurtis, Milano, Italy, 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy," in *Monoclonal Antibodies for Cancer Detection and Therapy*, ed. Baldwin *et al.* (Academic Press, New York, 1985), pp. 303-316; and Thorpe *et al.* (1982) *Immunol. Rev.* 62:119-158.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in reference 19. In addition, linkers may be used between the labels and the antibodies of the invention [20]. Antibodies or, antigen-binding fragments thereof may be directly labeled with radioactive iodine, indium, yttrium, or other radioactive particle known in the art [21]. Treatment may consist of a combination of treatment with conjugated and nonconjugated antibodies administered simultaneously or subsequently [22, 23].

Antibodies of the invention may be attached to a solid support.

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers, and methods to attach them to peptides, are shown in references 24-27. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O--CH_2--CH_2)_n O--R$ where R can be hydrogen, or a protective group such as an alkyl or

alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1,000 and 40,000, more preferably between 2,000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Water-soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), and the like. POG is preferred. One reason is because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is shown in reference 28, and a discussion of POG/IL-2 conjugates is found in reference 24.

Another drug delivery system for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in references 29, 30 and 31. Other drug delivery systems are known in the art and are described in, for example, references 32 and 33.

Antibodies of the invention are preferably provided in purified form. Typically, the antibody will be present in a composition that is substantially free of other polypeptides *e.g.* where less than 90% (by weight), usually less than 60% and more usually less than 50% of the composition is made up of other polypeptides.

Antibodies of the invention may be immunogenic in non-human (or heterologous) hosts *e.g.* in mice. In particular, the antibodies may have an idiotope that is immunogenic in non-human hosts, but not in a human host. Antibodies of the invention for human use include those that cannot be obtained from hosts such as mice, goats, rabbits, rats, non-primate mammals, *etc.* and cannot be obtained by humanisation or from xeno-mice.

Antibodies of the invention can be of any isotype (*e.g.* IgA, IgG, IgM *i.e.* an α, γ or μ heavy chain), but will generally be IgG. Within the IgG isotype, antibodies may be IgG1, IgG2, IgG3 or IgG4 subclass. Antibodies of the invention may have a κ or a λ light chain.

Production of antibodies

Monoclonal antibodies according to the invention can be made by one of the methods known in the art. The general methodology for making monoclonal antibodies using hybridoma technology is well

known [34, 35]. Preferably, the alternative EBV immortalisation method described in reference 36 is used.

Using the method described in reference 36, B-cells producing the antibody of the invention can be transformed with EBV in the presence of a polyclonal B cell activator. Transformation with EBV is a standard technique and can easily be adapted to include polyclonal B cell activators.

Additional stimulants of cellular growth and differentiation may be added during the transformation step to further enhance the efficiency. These stimulants may be cytokines such as IL-2 and IL-15. In a particularly preferred aspect, IL-2 is added during the immortalisation step to further improve the efficiency of immortalisation, but its use is not essential.

The immortalised B cells produced using these methods can then be cultured using methods known in the art and antibodies isolated therefrom.

Monoclonal antibodies may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography. Techniques for purification of monoclonal antibodies, including techniques for producing pharmaceutical-grade antibodies, are well known in the art.

Fragments of the monoclonal antibodies of the invention can be obtained from the monoclonal antibodies by methods that include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, fragments of the monoclonal antibodies can be obtained by cloning and expression of part of sequences of the heavy or light chains. Antibody "fragments" include Fab, Fab', $F(ab')_2$ and Fv fragments. The invention also encompasses single-chain Fv fragments (scFv) derived from the heavy and light chains of a monoclonal antibody of the invention *e.g.* the invention includes a scFv comprising the CDRs from an antibody of the invention. Also included are heavy or light chain monomers and dimers as well as single chain antibodies, *e.g.* single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibodies or fragments of the antibodies of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecules of the present invention or fragments thereof. Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab and $F(ab')_2$ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, *e.g.* mammalian, host cell expression systems may be used for

production of larger antibody molecules, including complete antibody molecules. Suitable mammalian host cells include CHO, HEK293T, PER.C6, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell comprising a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

Alternatively, antibodies according to the invention may be produced by i) expressing a nucleic acid sequence according to the invention in a cell, and ii) isolating the expressed antibody product. Additionally, the method may include iii) purifying the antibody.

Screening and isolation of B cells

Transformed B cells are screened for those producing antibodies of the desired antigen specificity, and individual B cell clones can then be produced from the positive cells.

The screening step may be carried out by ELISA, by staining of tissues or cells (including transfected cells), a neutralisation assay or one of a number of other methods known in the art for identifying desired antigen specificity. The assay may select on the basis of simple antigen recognition, or may select on the additional basis of a desired function *e.g.* to select neutralising antibodies rather than just antigen-binding antibodies, to select antibodies that can change characteristics of targeted cells, such as their signalling cascades, their shape, their growth rate, their capability of influencing other cells, their response to the influence by other cells or by other reagents or by a change in conditions, their differentiation status, *etc.*

The cloning step for separating individual clones from the mixture of positive cells may be carried out using limiting dilution, micromanipulation, single cell deposition by cell sorting or another method known in the art. Preferably the cloning is carried out using limiting dilution.

The immortalised B cell clones of the invention can be used in various ways *e.g.* as a source of monoclonal antibodies, as a source of nucleic acid (DNA or mRNA) encoding a monoclonal antibody of interest, for research, *etc.*

The invention provides a composition comprising immortalised B memory lymphocytes, wherein the lymphocytes produce antibodies with high neutralising potency specific for hCMV, and wherein the antibodies are produced at ≥5pg per cell per day. The invention also provides a composition comprising clones of an immortalised B memory lymphocyte, wherein the clones produce a monoclonal antibody with a high affinity specific for hCMV, and wherein the antibody is produced at $\geq 10^N$ ng per clone per day. Preferably said clones produce a monoclonal antibody with a high potency in neutralizing hCMV infection.

Preferred immortalised B cell clones according to the invention are 1F11 and 2F4. Further preferred clones are 5A2 and 9A11.

Epitopes

As mentioned above, the antibodies of the invention can be used to map the epitopes to which they bind. The Applicants have discovered that the antibodies 1F11, 2F4, 5A2 and 9A11 that neutralise hCMV infection of endothelial cells, epithelial cells, retinal cells and dendritic cells are directed towards an epitope found on a combination of UL130 and UL131A. Although the Applicant does not wish to be bound by this theory, it is believed that the antibodies 1F11 and 2F4 bind to a conformational epitope formed by these two proteins. It is also believed that 5A2 and 9A11 also bind to such a conformational epitope formed by UL130 and UL131A.

Due to the fact that 1F11, 2F4, 5A2, and 9A11 do not neutralise infection of fibroblasts, it is postulated that these antibodies recognise a different epitope to the human monoclonal antibodies 10C6, 5F1, 6B4 and 7H3. Furthermore, it is believed that the monoclonal antibodies 10C6, 5F1, 7H3 and 6B4 bind to a functional epitope of gB.

The epitopes recognised by these antibodies may have a number of uses. The epitopes and mimotopes in purified or synthetic form can be used to raise immune responses (*i.e.* as a vaccine, or for the production of antibodies for other uses) or for screening patient serum for antibodies that immunoreact with the epitopes or mimotopes. Preferably, such an epitope or mimotope, or antigen comprising such an epitope or mimotope is used as a vaccine for raising an immune response. The antibodies of the invention can also be used in a method to monitor the quality of vaccines in particular to check that the antigen in a vaccine contains the correct immunogenic epitope in the correct conformation.

The epitopes may also be useful in screening for ligands that bind to said epitopes. Such ligands preferably block the epitopes and so prevent infection. Such ligands are encompassed within the scope of the invention.

Recombinant expression

The immortalised memory B lymphocytes of the invention may also be used as a source of nucleic acid for the cloning of antibody genes for subsequent recombinant expression. Expression from recombinant sources is more common for pharmaceutical purposes than expression from B cells or hybridomas *e.g.* for reasons of stability, reproducibility, culture ease, *etc.*

Thus the invention provides a method for preparing a recombinant cell, comprising the steps of: (i) obtaining one or more nucleic acids (*e.g.* heavy and/or light chain genes) from the B cell clone that encodes the antibody of interest; and (ii) inserting the nucleic acid into an expression host in order to permit expression of the antibody of interest in that host.

Similarly, the invention provides a method for preparing a recombinant cell, comprising the steps of: (i) sequencing nucleic acid(s) from the B cell clone that encodes the antibody of interest; and (ii) using the sequence information from step (i) to prepare nucleic acid(s) for inserting into an expression host in order to permit expression of the antibody of interest in that host.

The invention also provides a method of preparing a recombinant cell, comprising the step of transforming a host cell with one or more nucleic acids that encode a monoclonal antibody of interest, wherein the nucleic acids are nucleic acids that were derived from an immortalised B cell clone of the invention. Thus the procedures for first preparing the nucleic acid(s) and then using it to transform a host cell can be performed at different times by different people in different places (*e.g.* in different countries).

These recombinant cells of the invention can then be used for expression and culture purposes. They are particularly useful for expression of antibodies for large-scale pharmaceutical production. They can also be used as the active ingredient of a pharmaceutical composition. Any suitable culture techniques can be used, including but not limited to static culture, roller bottle culture, ascites fluid, hollow-fiber type bioreactor cartridge, modular minifermenter, stirred tank, microcarrier culture, ceramic core perfusion, *etc.*

Methods for obtaining and sequencing immunoglobulin genes from B cells are well known in the art *e.g.* see reference 37).

The expression host is preferably a eukaryotic cell, including yeast and animal cells, particularly mammalian cells (*e.g.* CHO cells, human cells such as PER.C6 [Crucell; reference 38] or HKB-11 [Bayer; references 39 & 40] cells, myeloma cells [41 & 42], *etc.*), as well as plant cells. Preferred expression hosts can glycosylate the antibody of the invention, particularly with carbohydrate structures that are not themselves immunogenic in humans. Expression hosts that can grow in serum-free media are preferred. Expression hosts that can grow in culture without the presence of animal-derived products are preferred.

The expression host may be cultured to give a cell line.

The invention provides a method for preparing one or more nucleic acid molecules (*e.g.* heavy and light chain genes) that encodes an antibody of interest, comprising the steps of: (i) preparing an immortalised B cell clone according to the invention; (ii) obtaining from the B cell clone nucleic acid that encodes the antibody of interest. The invention also provides a method for obtaining a nucleic acid sequence that encodes an antibody of interest, comprising the steps of: (i) preparing an immortalised B cell clone according to the invention; (ii) sequencing nucleic acid from the B cell clone that encodes the antibody of interest.

The invention also provides a method of preparing nucleic acid molecule(s) that encodes an antibody of interest, comprising the step of obtaining the nucleic acid from a B cell clone that was obtained from a transformed B cell of the invention. Thus the procedures for first obtaining the B cell clone and then preparing nucleic acid(s) from it can be performed at very different times by different people in different places (*e.g.* in different countries).

The invention provides a method for preparing an antibody (*e.g.* for pharmaceutical use), comprising the steps of: (i) obtaining and/or sequencing one or more nucleic acids (*e.g.* heavy and light chain genes) from the selected B cell clone expressing the antibody of interest; (ii) inserting the nucleic acid(s) into or using the nucleic acid(s) to prepare an expression host that can express the antibody of interest; (iii) culturing or sub-culturing the expression host under conditions where the antibody of interest is expressed; and, optionally, (iv) purifying the antibody of the interest.

The invention also provides a method of preparing an antibody comprising the steps of: culturing or sub-culturing an expression host cell population under conditions where the antibody of interest is expressed and, optionally, purifying the antibody of the interest, wherein said expression host cell population has been prepared by (i) providing nucleic acid(s) encoding a selected B cell the antibody of interest that is produced by a population of B memory lymphocytes prepared as described above, (ii) inserting the nucleic acid(s) into an expression host that can express the antibody of interest, and (iii) culturing or sub-culturing expression hosts comprising said inserted nucleic acids to produce said expression host cell population. Thus the procedures for first preparing the recombinant expression host and then culturing it to express antibody can be performed at very different times by different people in different places (*e.g.* in different countries).

Pharmaceutical compositions

The use of antibodies as the active ingredient of pharmaceuticals is now widespread, including the products Herceptin™ (trastuzumab), Rituxan™, Campath™, Remicade™, ReoPro™, Mylotarg™, Zevalin™, Omalizumab, Synagis™ (Palivizumab), Zenapax™ (daclizumab), *etc.*

The invention thus provides a pharmaceutical composition containing the antibodies of the invention and/or nucleic acid encoding such antibodies and/or immortalised B cells that express such antibodies and/or the epitopes recognised by the antibodies of the invention. A pharmaceutical composition may also contain a pharmaceutically acceptable carrier to allow administration. The carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

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, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may 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.

Preferred forms for administration include forms suitable for parenteral administration, *e.g.* by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. It is preferred that the compositions are adapted for administration to human subjects.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intraperitoneal, intrathecal, intraventricular, transdermal, transcutaneous, topical, subcutaneous, intranasal, enteral, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose

schedule or a multiple dose schedule. Known antibody-based pharmaceuticals provide guidance relating to frequency of administration *e.g.* whether a pharmaceutical should be delivered daily, weekly, monthly, *etc.* Frequency and dosage may also depend on the severity of symptoms.

Compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition, like Synagis™ and Herceptin™, for reconstitution with sterile water containing a preservative). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. For example, a lyophilised antibody can be provided in kit form with sterile water or a sterile buffer.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Gennaro (2000) *Remington: The Science and Practice of Pharmacy,* 20th edition, ISBN: 0683306472.

Pharmaceutical compositions of the invention generally have a pH between 5.5 and 8.5, preferably between 6 and 8, and more preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen free. The composition may be isotonic with respect to humans. Pharmaceutical compositions of the invention are preferably supplied in hermetically-sealed containers.

Pharmaceutical compositions will include an effective amount of one or more antibodies of the invention and/or one or more immortalised B cells of the invention and/or a polypeptide comprising an epitope that binds an antibody of the invention *i.e.* 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 the therapeutics or combination of therapeutics selected for administration. The effective amount for a

given situation is determined by routine experimentation and is within the judgment of a clinician. For purposes of the present invention, an effective dose will generally be from about 0.01mg/kg to about 50mg/kg, or about 0.05 mg/kg to about 10 mg/kg of the compositions of the present invention in the individual to which it is administered. Known antibody-based pharmaceuticals provide guidance in this respect *e.g.* Herceptin™ is administered by intravenous infusion of a 21 mg/ml solution, with an initial loading dose of 4mg/kg body weight and a weekly maintenance dose of 2mg/kg body weight; Rituxan™ is administered weekly at 375mg/m^2; *etc.*

Compositions can include more than one (*e.g.* 2, 3, 4, 5, *etc.*) antibody of the invention, particularly where such antibodies bind to different antigens (or to different epitopes in the same antigen) to provide an additive or synergistic therapeutic effect. For example, one antibody may bind to the UL130-UL131A combination (or complex) while another may bind to gH. In a further example, one antibody may bind to the UL130-UL131A combination (or complex) while another may bind to gB. Thus, one antibody may be targeted to the mechanism that mediates infection of fibroblasts, while the other antibody may be targeted to the mechanism that mediates infection of endothelial cells. For optimal clinical effect it may well be advantageous to address both mechanisms of hCMV infection and maintenance.

Antibodies of the invention may be administered (either combined or separately) with other therapeutics *e.g.* with chemotherapeutic compounds, with radiotherapy, *etc.* Preferred therapeutic compounds include anti-viral compounds such as ganciclovir, foscarnet and cidofovir. Such combination therapy provides an additive or synergistic improvement in therapeutic efficacy relative to the individual therapeutic agents when administered alone. The term "synergy" is used to describe a combined effect of two or more active agents that is greater than the sum of the individual effects of each respective active agent. Thus, where the combined effect of two or more agents results in "synergistic inhibition" of an activity or process, it is intended that the inhibition of the activity or process is greater than the sum of the inhibitory effects of each respective active agent. The term "synergistic therapeutic effect" refers to a therapeutic effect observed with a combination of two or more therapies wherein the therapeutic effect (as measured by any of a number of parameters) is greater than the sum of the individual therapeutic effects observed with the respective individual therapies.

Antibodies may be administered to those patients who have previously shown no response to treatment for hCMV infection, *i.e.* have been shown to be refractive to anti-hCMV treatment. Such treatment may include previous treatment with an anti-viral agent. This may be due to, for example, infection with an anti-viral resistant strain of hCMV.

In compositions of the invention that include antibodies of the invention, the antibodies preferably make up at least 50% by weight (*e.g.* 60%, 70%, 80%, 90%, 95%, 97%, 98%, 99% or more) of the total protein in the composition. The antibodies are thus in purified form.

The invention provides a method of preparing a pharmaceutical, comprising the steps of: (i) preparing an antibody of the invention; and (ii) admixing the purified antibody with one or more pharmaceutically-acceptable carriers.

The invention also provides a method of preparing a pharmaceutical, comprising the step of admixing an antibody with one or more pharmaceutically-acceptable carriers, wherein the antibody is a monoclonal antibody that was obtained from a transformed B cell of the invention. Thus the procedures for first obtaining the monoclonal antibody and then preparing the pharmaceutical can be performed at very different times by different people in different places (*e.g.* in different countries).

As an alternative to delivering antibodies or B cells for therapeutic purposes, it is possible to deliver nucleic acid (typically DNA) to a subject that encodes the monoclonal antibody (or active fragment thereof) of interest, such that the nucleic acid can be expressed in the subject *in situ* to provide a desired therapeutic effect. Suitable gene therapy and nucleic acid delivery vectors are known in the art.

Compositions of the invention may be immunogenic compositions, and are more preferably vaccine compositions comprising an antigen comprising an epitope found on a combination of hCMV proteins UL130 and UL131A. Alternative compositions may comprise (i) an antigen comprising an epitope found on a combination of hCMV proteins UL130 and UL131A, and (ii) an antigen comprising an epitope found on hCMV gB. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

Compositions may include an antimicrobial, particularly if packaged in a multiple dose format.

Compositions may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

Compositions may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2mg/ml NaCl is typical.

Compositions may comprise a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at around 15-30mg/ml (*e.g.* 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

The compositions of the invention may also comprise one or more immunoregulatory agents. Preferably, one or more of the immunoregulatory agents include(s) an adjuvant.

The compositions of the invention will preferably elicit both a cell mediated immune response as well as a humoral immune response in order to effectively address a hCMV infection. This immune response will preferably induce long lasting (*e.g.* neutralising) antibodies and a cell mediated immunity that can quickly respond upon exposure to hCMV.

Medical treatments and uses

The antibodies of the invention or fragments thereof may be used for the treatment of hCMV infection, for the prevention of hCMV infection or for the diagnosis of hCMV infection.

Methods of diagnosis may include contacting an antibody or an antibody fragment with a sample. Such samples may be tissue samples taken from, for example, salivary glands, lung, liver, pancrease, kidney, ear, eye, placenta, alimentary tract, heart, ovaries, pituitary, adrenals, thyroid, brain or skin. The methods of diagnosis may also include the detection of an antigen/antibody complex.

The invention therefore provides (i) an antibody according to the invention, (ii) an immortalised B cell clone according to the invention, (iii) an epitope capable of binding one of 1F11 or 2F4, or (iv) an epitope capable of binding one of 5A2 or 9A11, for use in therapy.

Also provided is a method of treating a patient comprising administering to that patient (i) an antibody according to the invention, (ii) an epitope capable of binding one of 1F11 or 2F4, or (iii) an epitope capable of binding one of 5A2 or 9A11.

The invention also provides the use of (i) an antibody according to the invention, (ii) an immortalised B cell clone according to the invention, (iii) an epitope capable of binding one of 1F11 or 2F4, (iv) an antibody that binds to an epitope capable of binding one of 1F11 or 2F4, (v) an epitope capable of binding one of 5A2 or 9A11, or (vi) an antibody that binds to an epitope capable of binding one of 5A2 or 9A11, in the manufacture of a medicament for the prevention or treatment of hCMV infection.

The invention provides a composition of the invention for use as a medicament. It also provides the use of an antibody of the invention and/or a protein comprising an epitope to which such an antibody binds in the manufacture of a medicament for treatment of a patient and/or diagnosis in a patient. It also provides a method for treating a subject and/or of performing diagnosis on a subject, comprising the step of administering to them a composition of the invention. The subject is preferably a human. One way of checking efficacy of therapeutic treatment involves monitoring disease symptoms after administration of the composition of the invention. Treatment can be a single dose schedule or a multiple dose schedule. The invention is useful for CMV infection.

Preferably, an antibody, immortalised B cell clone, epitope or composition according to the invention is administered to groups of subjects particularly at risk of or susceptible to hCMV infection. Such subjects groups include immunocompromised subjects, such as those suffering from HIV or undergoing immunosuppressive therapy, such as transplant patients.

Antibodies of the invention can be used in passive immunisation.

Antibodies and fragments thereof as described in the present invention may also be used in a kit for the diagnosis of hCMV infection.

Epitopes capable of binding the monoclonal antibody 1F11 or 2F4 described in the present invention may be used in a kit for monitoring the efficacy of vaccination procedures by detecting the presence of protective anti-hCMV antibodies.

Epitopes capable of binding the monoclonal antibody 5A2 or 9A11 described in the present invention may be used in a kit for monitoring the efficacy of vaccination procedures by detecting the presence of protective anti-hCMV antibodies.

Antibodies and fragments thereof as described in the present invention may also be used in a kit for monitoring vaccine manufacture with the desired immunogenicity.

The invention also provides a method of preparing a pharmaceutical, comprising the step of admixing a monoclonal antibody with one or more pharmaceutically-acceptable carriers, wherein the monoclonal antibody is a monoclonal antibody that was obtained from an expression host of the invention. Thus the procedures for first obtaining the monoclonal antibody (*e.g.* expressing it and/or purifying it) and then admixing it with the pharmaceutical carrier(s) can be performed at very different times by different people in different places (*e.g.* in different countries).

Starting with a transformed B cell of the invention, various steps of culturing, sub-culturing, cloning, sub-cloning, sequencing, nucleic acid preparation *etc.* can be performed in order to perpetuate the antibody expressed by the transformed B cell, with optional optimisation at each step. In a preferred embodiment, the above methods further comprise techniques of optimisation (*e.g.* affinity maturation or optimisation) applied to the nucleic acids encoding the antibody. The invention encompasses all cells, nucleic acids, vectors, sequences, antibodies *etc.* used and prepared during such steps.

In all these methods, the nucleic acid used in the expression host may be manipulated between steps (ii) and (iii) to insert, delete or amend certain nucleic acid sequences. Changes from such manipulation include, but are not limited to, changes to introduce restriction sites, to amend codon usage, to add or optimise transcription and/or translation regulatory sequences, *etc.* It is also possible to change the nucleic acid to alter the encoded amino acids. For example, it may be useful to introduce one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) amino acid substitutions, one or more

(*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) amino acid deletions and/or one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) amino acid insertions into the antibody's amino acid sequence. Such point mutations can modify effector functions, antigen-binding affinity, post-translational modifications, immunogenicity, *etc.*, can introduce amino acids for the attachment of covalent groups (*e.g.* labels) or can introduce tags (*e.g.* for purification purposes). Mutations can be introduced in specific sites or can be introduced at random, followed by selection (*e.g.* molecular evolution).

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "about" in relation to a numerical value x means, for example, $x \pm 10\%$.

The term "disease" as used herein is intended to be generally synonymous, and is used interchangeably with, the terms "disorder" and "condition" (as in medical condition), in that all reflect an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

As used herein, reference to "treatment" of a patient is intended to include prevention and prophylaxis. The term "patient" means all mammals including humans. Examples of patients include humans, cows, dogs, cats, horses, goats, sheep, pigs, and rabbits. Preferably, the patient is a human.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows a SDS-PAGE which demonstrates that human monoclonal antibodies (1) 1F11 and (2) 2F4 precipitate complexes of hCMV proteins, whereas irrelevant IgG does not.

Figure 2 shows a FACS analysis which demonstrates that human monoclonal antibodies (A) 1F11 and 2F4 and (B) 5A2 and 9A11 recognise a conformational epitope made by UL130 and UL131A gene products.

Figure 3 shows the nucleotide and amino acid sequences of the heavy and light chains of 1F11 and 2F4. The CDR sequences are in bold.

Figure 4 shows a FACS analysis which demonstrates that the human monoclonal antibodies 7H3, 10C6, 5F1 and 6B4 recognise an epitope on hCMV protein gB.

Figure 5 shows the nucleotide and amino acid sequences of the heavy and light chains of 5A2. The CDR sequences are in bold.

MODES FOR CARRYING OUT THE INVENTION

Example 1: Cloning of B cells and screening for hCMV neutralising activity

Two donors with high hCMV neutralising antibody titres in the serum were identified. Memory B cells were isolated and immortalised using EBV and CpG as described in reference 36. Briefly, memory B cells were isolated by negative selection using CD22 beads, followed by removal of IgM^+, IgD^+ IgA^+ B cells using specific antibodies and cell sorting. The sorted cells (IgG^+) were immortalised with EBV in the presence of CpG 2006 and irradiated allogeneic mononuclear cells. Replicate cultures each containing 50 memory B cells were set up in twenty 96 well U bottom plates. After two weeks the culture supernatants were collected and tested for their capacity to neutralise hCMV infection of either fibroblasts or epithelial cells in separate assays. B cell clones were isolated from positive polyclonal cultures as described in reference 36. IgG concentrations in the supernatant of selected clones were determined using an IgG-specific ELISA.

For the viral neutralisation assay a titrated amount of a clinical hCMV isolate was mixed with an equal volume of culture supernatant or with dilutions of human sera containing neutralising antibodies. After 1 hour incubation at room temperature the mixture was added to confluent monolayers of either endothelial cells (*e.g.* HUVEC cells) or fibroblasts in 96 well flat bottom plates and incubated at 37°C for two days. The supernatant was discarded, the cells were fixed with cold methanol and stained with a mixture of mouse monoclonal antibodies to hCMV early antigens, followed by a fluorescein-labelled goat anti mouse Ig. The plates were analyzed using a fluorescence microscope. In the absence of neutralising antibodies the infected cells were ~1000/field, while in the presence of saturating concentrations of neutralising antibodies the infection was completely inhibited. The neutralising titer is indicated as the concentration of antibody (μg/ml) that gives a 50% reduction of hCMV infection.

Table 2 shows that three different types of antibodies have been identified. Those that can neutralise infection of fibroblasts, those that can neutralise infection of endothelial cells and those that can neutralise infection of both. This agrees with previous data that different proteins are responsible for tropism towards a particular cell type [7]. In addition to neutralisation of endothelial cells, 1F11 and 2F4 were observed to neutralise infection of epithelial cells such as retinal cells, and dendritic cells (data not shown).

Table 2

		50% neutralisation (µg/ml)	
Clone	**Specificity**	**Fibroblasts**	**Endothelial cells**
1F11	**UL130/UL131A**	*	**0.001**
2F4	**UL130/UL131A**	*	**0.003**
5A2	**UL130/UL131A**	*	**0.002**
9A11	**UL130/UL131A**	*	**0.001**
7H3	**gB**	**2**	*
10C6	**gB**	**0.3**	**0.3**
5F1	**gB**	**0.3**	**0.3**
6B4	**gB**	**0.5**	**2**
Cytotec^		**5000**	**50**
Donor's Serum		**33**	**1**

*no neutralisation at the highest concentration tested (*i.e.* >2 µg/ml).

^ Cytotect (Biotest) is a pool of hCMV hyperimmune IgG.

Some antibodies neutralized infection of both fibroblasts and endothelial cells at IgG concentrations ranging from 0.3 to 0.5 µg/ml. Other antibodies (1F11, 5A2, 9A11 and 2F4) failed to neutralize hCMV infection of fibroblasts, but neutralized the infection of endothelial cells and did so at extremely low concentrations ranging from 0.001 to 0.004 µg/ml (more than 1000 fold more potent than previously known antibodies).

Note that since the initial characterization, it has been determined that 5F1 binds to an epitope of gB rather than gH. This is consistent with the results which demonstrate that blocking gB allows neutralization of infection of fibroblasts as observed for 7H3, 10C6 and 6B4.

Example 2: Identification of the target antigens recognised by the monoclonal antibodies

Human MRC-9 fibroblasts were infected with a clinical hCMV isolate. After 3 days the cells were metabolically labeled with ^{35}S Methionine and Cysteine. After preclearance of the lysate human monoclonal antibodies 1F11 and 2F4 were added and immunocomplexes were precipitated by the addition of Protein A beads and resolved on SDS-PAGE (Figure 1). A human monoclonal IgG antibody with irrelevant specificity was used as negative control. The results show that human monoclonal antibodies 1F11 and 2F4 precipitate complexes of CMV proteins.

To map the specificity of the human monoclonal antibodies expression vectors encoding hemagglutinin (HA)-tagged UL128Δ1-27, UL130Δ1-25 and UL131AΔ1-18 hCMV proteins lacking signal peptides were constructed. HEK293T cells were transfected with these vectors alone or in combination. After 36h, cells were fixed, permeabilized and stained with an anti-HA antibody (to control for efficiency of transfection) and with monoclonal antibodies followed by a goat anti-human IgG. A HuMab IgG with irrelevant specificity was used as negative control. Figure 2A shows that the human monoclonal antibodies 1F11 and 2F4 recognise a conformational epitope made by UL130 and UL131A gene products. Figure 2B shows that the human monoclonal antibodies 5A2 and 9A11 recognise a conformational epitope made by the UL130 and UL131A gene products.

Conclusions

The above results define two human monoclonal antibodies that are capable of neutralizing with high potency and selectivity the hCMV infection of human endothelial cells. To identify the epitope recognized, the antibodies were tested for their capacity to immunoprecipitate proteins from hCMV infected cells (Figure 1). Human Mabs 1F11 and 2F4 precipitated several proteins with apparent molecular weights of ~15, 33-35 and ~100 KDa. These patterns are compatible with the precipitation of a complex containing gH, gL and UL128, UL130 and possibly UL131A.

To better define the target of these antibodies we characterized their capacity to stain HEK293T cells transfected with vectors encoding HA-tagged UL128, UL130 and UL131A. As shown in Figure 2A, 1F11 and 2F4 stained only cells coexpressing UL130 and UL131A, suggesting that they recognize a conformational epitope determined by the two gene products. This conclusion is supported by the fact that these antibodies do not react in a western blot with lysates of infected or transfected cells run under reducing, denaturing, conditions (data not shown).

Similar results were observed for 5A2 and 9A11. Figure 2b shows that these antibodies stained only cells coexpressing UL130 and UL131A, suggesting that they recognize a conformational epitope determined by the two gene products.

Example 3:Further identification of the target antigens recognised by the monoclonal antibodies

To map specificities of human monoclonal antibodies neutralizing infection of fibroblasts an expression vector encoding full length gB was constructed. HEK293T cells were transfected with this vector. After 36h, cells were fixed, permeabilized and stained with human monoclonal antibodies (HuMab) followed by goat anti-human IgG. Figure 4 shows that monoclonal antibodies 7H3, 10C6, 5F1, and 6B4 (but not an IgG antibody of an irrelevant specificity) specifically stained cells transfected with gB, indicating that they recognize an epitope of gB. Of note, the monoclonal antibodies 10C6, 5F1 and 6B4 neutralise infection of fibroblasts and endothelial cells, whereas the monoclonal antibody 7H3 neutralises the infection of fibroblasts (but not of endothelial cells). This

notion suggests that the monoclonal antibodies 10C6, 5F1, and 6B4 bind to a functional epitope of gB that is distinct from the epitope bound by the monoclonal antibody 7H3.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. A neutralising antibody having high potency in neutralising human cytomegalovirus.

2. An antibody according to claim 1, wherein the antibody has an affinity of 10^{-8}M or less.

3. An antibody according to claim 1, wherein the concentration of antibody required for 50% neutralisation of a clinical isolate of hCMV is 0.1μg/ml or less.

4. An antibody of claim 1, which has an affinity for hCMV at least 50 times greater than that of MSL109.

5. An antibody according to any one of claims 1-4, wherein said antibody is specific for a combination of the hCMV proteins UL130/UL131A.

6. An antibody according to claim 1 comprising one or more of SEQ ID NOs: 1-6, 11-16 or 33-38.

7. An antibody according to claim 1 comprising a heavy chain comprising one or more of SEQ ID NOs: 1-3, 11-13 or 33-35.

8. An antibody according to claim 1 comprising a heavy chain comprising SEQ ID NO: 1 for CDRH1, SEQ ID NO: 2 for CDRH2 and SEQ ID NO: 3 for CDRH3.

9. An antibody according to claim 1 comprising a heavy chain comprising SEQ ID NO: 11 for CDRH1, SEQ ID NO: 12 for CDRH2 and SEQ ID NO: 13 for CDRH3.

10. An antibody according to claim 1 comprising a heavy chain comprising SEQ ID NO: 33 for CDRH1, SEQ ID NO: 34 for CDRH2 and SEQ ID NO: 35 for CDRH3.

11. An antibody according to claim 1 comprising a light chain comprising one or more of SEQ ID NOs: 4-6, 14-16 or 36-38.

12. An antibody according to claim 1 comprising a light chain comprising SEQ ID NO: 4 for CDRL1, SEQ ID NO: 5 for CDRL2 and SEQ ID NO: 6 for CDRL3.

13. An antibody according to claim 1 comprising a light chain comprising SEQ ID NO: 14 for CDRL1, SEQ ID NO: 15 for CDRL2 and SEQ ID NO: 16 for CDRL3.

14. An antibody according to claim 1 comprising a light chain comprising SEQ ID NO: 36 for CDRL1, SEQ ID NO: 37 for CDRL2 and SEQ ID NO: 38 for CDRL3.

15. An antibody according to claim 8, wherein said heavy chain has the sequence recited in SEQ ID NO: 7.

16. An antibody according to claim 9, wherein said heavy chain has the sequence recited in SEQ ID NO: 17.

17. An antibody according to claim 10, wherein said heavy chain has the sequence recited in SEQ ID NO: 39.

18. An antibody according to claim 12, wherein said light chain has the sequence recited in SEQ ID NO: 8.

19. An antibody according to claim 13, wherein said light chain has the sequence recited in SEQ ID NO: 18.

20. An antibody according to claim 14, wherein said light chain has the sequence recited in SEQ ID NO: 40.

21. An antibody according to any preceding claim wherein the antibody is a human monoclonal antibody.

22. An antibody according to claim 15 or claim 18, wherein said antibody is monoclonal antibody 1F11.

23. An antibody according to claim 16 or claim 19, wherein said antibody is monoclonal antibody 2F4.

24. An antibody according to claim 17 or claim 20, wherein said antibody is monoclonal antibody 5A2.

25. An antibody that binds to an epitope capable of binding to an antibody according to claim 7 or claim 11.

26. A fragment of an antibody according to any previous claim.

27. A fragment according to claim 26 which is a Fab, Fab', $F(ab')_2$ or Fv fragment.

28. A nucleic acid molecule encoding an antibody or fragment according to any previous claim.

29. A nucleic acid molecule according to claim 28, wherein said nucleic acid molecule comprises any one of SEQ ID NOs: 9, 10, 19, 20, 21-32, or 41-48.

30. A vector comprising a nucleic acid molecule according to claim 28 or claim 29.

31. A cell comprising a vector according to claim 30.

32. An immortalised B cell clone expressing an antibody according to any one of claims 1-25.

33. An epitope which binds to an antibody according to any one of claims 1-25.

34. An immunogenic polypeptide comprising an epitope according to claim 33.

35. A method for producing antibodies according to any one of claims 1-25 comprising (i) culturing an immortalised B cell clone according to claim 10 and (ii) isolating antibodies.

36. A pharmaceutical composition comprising an antibody or fragment according to any one of claims 1-27, a nucleic acid according to claim 28 or claim 29, an immortalised B cell clone according to claim 32 or an immunogenic polypeptide according to claim 34.

37. A pharmaceutical composition according to claim 36 comprising a first antibody or fragment specific for a first epitope and second antibody or fragment specific for a second epitope.

38. A pharmaceutical composition according to claim 36 or claim 37, further comprising a pharmaceutically acceptable diluent or carrier.

39. An antibody or fragment according to any one of claims 1-27, a nucleic acid according to claim 28 or claim 29, an immortalised B cell clone according to claim 32 or an immunogenic polypeptide according to claim 34 for use in therapy or diagnosis.

40. Use of an antibody or fragment according to any one of claims 1-27, a nucleic acid according to claim 28 or claim 29, an immortalised B cell clone according to claim 32 or an immunogenic polypeptide according to claim 34 (i) in the manufacture of a medicament for the treatment of hCMV infection, or (ii) in a vaccine.

41. Use of a first antibody or fragment according to any one of claims 1-27 specific for a first epitope and a second antibody or fragment according to any one of claims 1-27 specific for a second epitope in the manufacture of a medicament for the treatment of hCMV infection.

42. Use according to claim 40 or claim 41, wherein the patient is refractive to conventional treatment against hCMV.

43. Use according to claim 40 or claim 41, wherein the patient is pre-administered, post-administered or simultaneously administered with an anti-viral agent.

44. Use according to claim 43, wherein said anti-viral agent is selected from ganciclovir, foscarnet or cidofovir.

45. Use according to claim 40 or claim 41, wherein said patient is immunocompromised.

46. Use according to claim 45, wherein said patient has HIV or is a transplant patient.

47. A composition of claim 37 or the use of claim 41, wherein said first antibody or fragment is specific for the UL130/UL131A complex.

48. The composition or use of claim 47, wherein said second antibody or fragment is specific for gH.

49. A method for treating a subject, comprising the step of administering a pharmaceutical composition according to any one of claims 36-38 or 47-48.

50. A kit for the diagnosis of hCMV infection comprising antibodies or fragments according to any one of claims 1-27 or the nucleic acid of claim 28 or claim 29.

51. A method for preparing a recombinant cell, comprising the steps of: (i) sequencing nucleic acid from a B cell clone as defined in claim 32; (ii) using the sequence information from step (i) to prepare nucleic acid for inserting into an expression host in order to permit expression of the antibody of interest in that host.

52. The method of claim 51, wherein the nucleic acid is manipulated between steps (i) and (ii) to introduce restriction sites, to change codon useage, and/or to optimise transcription and/or translation regulatory sequences.

53. The method of claim 51, wherein said expression host is a eukaryotic cell.

54. The method of claim 53, wherein said eukaryotic cell is a yeast cell, an animal cell or a plant cell.

55. The method of claim 54, wherein the animal cell is a mammalian cell.

56. The method of claim 55, wherein the animal cell is a CHO or human cell.

57. The method of claim 56, wherein the human cell is a PER.C6 cell, a HEK293T cell or a HKB-11 cell.

58. A method for producing antibodies according to any one of claims 1-25 comprising culturing or sub-culturing an expression host obtainable by the method of claim 51 under conditions where the antibody of interest is expressed; and, optionally, (iv) purifying the antibody of the interest.

59. A method of screening for polypeptides that can induce an immune response against hCMV, comprising screening polypeptide libraries using an antibody or fragment according to any one of claims 1-27.

60. Use of an antibody or fragment according to any one of claims 1-27 for monitoring the quality of anti-hCMV vaccines by checking that the antigen said vaccine contains the correct epitope in the correct conformation.

61. The use according to any one of claims 40-46 wherein the antibody prevents infection of endothelial cells, epithelial cells such as retinal cells and myeloid cells such as dendritic cells.

62. An antibody according to claim 1, wherein the concentration of antibody required for 50% neutralisation of infection of endothelial cells, epithelial cells and dendritic cells by a clinical isolate of hCMV is 0.1 μg/ml or less.

63. The antibody according to claim 62, wherein the concentration of antibody is 0.01μg/ml or less.

64. The antibody according to claim 62, wherein the epithelial cells are retinal cells.

65. The antibody according to claim 62, wherein the antibody is specific for a combination of the hCMV proteins UL130/UL131A.

66. An epitope which specifically binds to an antibody according to any one of claims 1-25 for use (i) in therapy, (ii) in the manufacture of a medicament for treating hCMV infection or (iii) as a vaccine.

67. An epitope which specifically binds to an antibody according to any one of claims 1-25 for screening for ligands able to neutralise hCMV infection.

68. An antibody according to claim 1, wherein the concentration of antibody required for 50% neutralisation of infection of endothelial cells, epithelial cells and dendritic cells by a clinical isolate of hCMV is 50 times or more lower than that required by MSL-109.