BINDING MEMBERS FOR HUMAN CYTOMEGALOVIRUS

Field of the Invention

This invention relates to binding members, especially antibody molecules, which neutralise the biological effects of human cytomegalovirus (hCMV). The binding members are useful for the treatment and prophylaxis of hCMV infection.

Background

Human cytomegalovirus (hCMV) is a widely distributed pathogen that usually establishes asymptomatic, life-long persistence in 40-80% of the human population depending on geographical and socioeconomic origin. However, in immunocompromised patients, such as transplant recipients and HIV infected individuals, and also in newborns, hCMV infection is a major cause of morbidity and mortality and puts a significant economic burden on health care systems.

hCMV is the most significant infection impacting on the outcome of solid organ transplantation (SOT) and hematopoietic stem cell transplantation (SCT) (Razonable & Paya, 2003). After transplantation, an active hCMV infection occurs in approximately 60-70% of hCMV-seropositive patients or seronegative patients who receive organ transplants from a seropositive donor (Razonable & Paya, *ibid*). If no preventative measures are taken, the risk of developing hCMV disease is 20-30%. Considering the fact that approximately 26,000 allogenic SCTs were performed worldwide in 2008 (www.cibmtr.org), the success of this therapy and the reduction of post-transplant morbidity and mortality have considerable financial implications. hCMV related complications can result in additional costs of EUR 25,000 to 50,000 per patient.

In addition, HCMV infection in transplant patients is associated with transplant related atherosclerosis and accelerated graft loss (Streblow *et al.*, 2007).

As mentioned before, hCMV is relevant as a perinatal pathogen. Each year, approximately 1% of susceptible women seroconvert during pregnancy. Approximately 40% of these transmit hCMV to their children resulting in 40,000 infected newborns annually in the USA (Kenneson & Cannon, 2007). 10-20% of the infected children have acute symptoms at birth. Of these, up to 20% die and the remainder typically have moderate-to-severe complications, including CNS related conditions, like blindness, deafness and mental retardation. Apart from the devastating consequences for affected patients and their families, the healthcare costs for those patients are significant, in particular, if the perinatal infection results in severe and permanent disabilities.

To date, five antiviral agents are approved for use in hCMV infection: Ganciclovir/Valganciclovir, Cidofovir, Foscarnet and Fomivirsen. All compounds suffer from dose dependent side effects and the development of resistant virus strains (Schreiber *et al.*, 2009). None of the drugs are licensed for use in children or in pregnant women. In addition, intravenous immunoglobulin preparations (IVIG) e.g. CytoGam® (CSL Behring) and Cytotect® (Biotest) are used for prophylaxis and treatment of patients at risk of hCMV infection. However, uncertainty about benefits of this treatment in the transplant situation is evident (Sokos *et al.*, 2002; Raanani *et al.*, 2009). The adoptive transfer of hCMV-specific cytotoxic T-cells has been used with success in hematopoetic SCT patients (Moss & Rickinson, 2005), but this treatment is extremely expensive and will be limited to a few transplant

centres having the necessary expertise. Moreover, this type of treatment is restricted to transplant recipients who are seropositive for hCMV. In contrast, IVIG has been reported to be effective in the treatment and prevention of congenital CMV infection (Nigro *et al.*, 2005). However, IVIG for hCMV treatment is isolated and purified from hCMV seropositive donors, resulting in variable titers and therefore batch-to-batch variation for hCMV specific antibodies in these preparations. In addition, human blood-derived drug products always bear the risk for transmission of human pathogens. As a consequence, a recombinant antibody product, allowing the efficient neutralisation of hCMV for the prophylaxis and treatment of diseases caused by hCMV infection is desired.

Targets for antibody therapy of hCMV infections are proteins expressed in the surface of the hCMV virion. The composition of the hCMV virion envelope is very complex and whilst many structural proteins that comprise the envelope have been identified, it is still not fully defined. During the development of antibodies for the therapy of hCMV, antigenic determinants have been identified in surface glycoprotein complexes gp58/l16 (gB or gC-1), gp 47-52 (gC-II; gM and gN) (Shimamura *et al.*, 2006) and gp 86 (gH or gC-III) (Urban *et al.*, 1996). The majority of neutralising antibodies identified to date bind to gB protein, which has been shown to contain the majority of neutralising epitopes (Britt *et al.*, 1990). The gB complex is synthesised as a 130kD precursor, which is cleaved into two covalently linked molecules, named gp58 and gpl16. The N-terminal fragment (gpl16) contains one linear, neutralising epitope, called antigenic domain-2 (AD-2) of 20 amino acids (amino acids 67-86), which does not require complement for antibody- mediated biological activity (Meyer *et al.*, 1990). The gp58 moiety of gB carries the neutralising domain AD-1, which comprises 74

amino acids (amino acids 557-630) and most likely represents a conformational epitope (Ohlin *et al.*, 1993; Wagner *et al.*, 1992).

The advent of monoclonal antibodies initially gave rise to the identification of a variety of neutralising mouse monoclonal antibodies against hCMV. However, mouse monoclonal antibodies are unsuitable for use in human therapy since these proteins are recognised by the human immune system as being foreign, and are consequently eliminated after a very short period of time, resulting in low or no clinical efficacy. Chimeric antibodies have been developed against hCMV proteins and EP664834B (Harris *et al*) describes a chimeric antibody targeted to the 86kD glycoprotein of hCMV termed gH; however such antibodies have not been successful in clinical settings.

Technologies using heteromyelomas for the generation of hybridomas have been used to generate a variety of human monoclonal antibodies recognising various hCMV glycoproteins, which are found both in the viral envelope. US5,043,281 (Masuho *et al*) describes a neutralising human monoclonal antibody that recognises a CMV antigen protein having a molecular weight of between 130,000 and 55,000. US5,750,106 (Ostberg) describes a human monoclonal antibody to CMV termed SDZ MSL 109, which recognises the gH glycoprotein, as well as a hybridoma cell line for the production of this antibody. One of the virus-neutralising human monoclonal antibodies, SDZ MSL-109 has been evaluated in Phase I/II clinical trials for hCMV induced retinitis in immunocompromised patients, but due to lack of efficacy the clinical trials were not continued (Borucki *et al.*, 2004; Hamilton *et al.*, 1997; Boeckh *et al.*, 2001). One plausible explanation for the

failure of these trials is the antigenic variability of hCMV. hCMV is unique among the human herpes viruses in that it is antigenic variable and most human monoclonal antibodies, reacting with the envelope antigens, show strain-specific neutralisation capacity. This is especially true for the gH-specific human monoclonal antibodies, like SDZ MSL-109. This obstacle can only be overcome by the use of monoclonal antibodies directed against epitopes on hCMV that are conserved between different isolates.

In the past, progress in the isolation of hCMV neutralising monoclonal antibodies was slow, due to the fact that high-throughput screens of antibodies for neutralising capacity were not available. In addition, the method of Epstein Barr virus (EBV) immortalisation has been used frequently to generate immortalised B cells producing an antibody of interest, for a number of years. This technique has been successful for the generation of antibody-secreting cells from different sources of human B cells such as the peripheral blood of healthy subjects using antigen-specific selection (Casali *et al.*, 1986), lymph nodes, spleen or peripheral blood from patients (Yamaguchi *et al.*, 1987; Posner *et al.*, 1991; Raff *et al.*, 1988; Steenbakkers *et al* 1993 and 1994). This technique was used for the immortalisation of peripheral blood mononuclear cells isolated from CMV-seropositive blood donors and the subsequent isolation of three antibodies: ITC52, ITC63b and ITC88 (WO 93/021952 A1). ITC52 and ITC63b are reactive with the conformational AD-1 epitope of CMV consisting of amino acid sequence 557-630 of CMV gp58 and ITC88 is reactive against AD-2 comprising the amino acid sequence 67-86 (AD-2) of CMV gp116 (WO 93/021952 A1).

Improvements on the method of EBV transformation have been published by Lanzavecchia (WO 04/076677 A2) and Funaro *et al* (WO 07/068758 A1) and these methods have been used for the generation of antibodies to hCMV. WO 08/084410 A2 (Lanzavecchia & Macagno) describes antibodies produced from EBV cell lines 1F11, 2F4, 5A2 and 9A11 that neutralise hCMV infection of endothelial cells, epithelial cells, retinal cells and dendritic cells and are directed towards a conformational epitope formed by gpUL130 and gpUL131A. However, the antibodies from these EBV lines do not have any detectable hCMV neutralising capacity, if fibroblasts are used as target cells for infection. WO 08/084410 A2 also mentions EBV lines 10C6, 5F1, 6B4 and 7H3, producing antibodies that neutralise hCMV infection of fibroblasts and endothelial cells at half-maximal inhibitory concentrations (IC_{50}) ranging between 0.3 and 2.0µg/ml. The antibodies produced from these EBV lines are described to bind to a functional epitope of gB. However, although antibody heavy and light chain sequences have been deducted from some of the above mentioned EBV cell lines, this data has not been confirmed for recombinantly expressed and purified antibodies encoded by the published sequences. A more recent patent application from Lanzavecchia & Macagno (WO 10/007463 A1) describes the antibody 6G4, which binds to an epitope determined by a combination of the UL128, UL130 and UL131A proteins and which neutralises hCMV infection of endothelial, retinal and dendritic cells. Furthermore, WO 10/007533 A1 (Lanzavecchia & Macagno) describes hCMV neutralising antibodies that bind to an epitope in the hCMV UL128 protein, an epitope formed by gH, gL, UL128 and UL130 proteins, an epitope formed by UL128, UL130 and UL131A proteins or an epitope formed by UL130 and UL131A proteins.

WO 08/071806 A1 (Funaro *et al*) describes the antibody 26A1, which binds to and neutralises hCMV but does not show significant binding to either antigens gB or gH when tested by ELISA. A half-maximal inhibitory concentration (IC_{50}) of the antibody 26A1 is reported to be in the range of 1µg/ml for both primary fibroblasts and endothelial cells, and therefore in a range that has been reached by antibodies described in the prior art. A further patent application by Funaro and colleagues, describes the antibody 1F7, which recognises gH (WO 09/003975 A1). Similar to antibody 26A1, as described in WO 08/071806 A1, the half-maximal inhibitory concentration (IC_{50}) of the antibody 1F7 is reported to be in the range of 1µg/ml for both primary fibroblasts and endothelial cells, and therefore in a range that has been reached by antibodies described in the prior art. Yet another patent application by Funaro and colleagues (WO 09/024445 A1) describes the antibodies 8C10, 37B7, 8A11 and 10B7, which either recognise the AD-2 domain of gB (clones 8C10, 8A11, 10B7), or a protein unrelated to gB or gH (clone 37B7). As in the patent applications of Funaro and colleagues (WO 08/071806 A1 and WO 09/003975 A1), the antibodies described in WO 09/024445 A1 also exhibit a half-maximal inhibitory concentration (IC_{50}) in the range of 1µg/ml for both primary fibroblasts and endothelial cells (10B7, 8A11, 37B7) or higher at about 10µg/ml (8C10), and therefore in the range of previously published hCMV neutralising antibodies.

Additional recent patent applications, describe hCMV neutralising antibodies with similar features. For instance, WO 09/114560 A2 (Olsen) describes antibody clones 2F10, 2M16, 2N9, 3C21, 3G7, 4P12, 5P9, 9C16, which all bind to the AD-2 epitope of gB and display half-maximal inhibitory concentration (IC_{50}) of hCMV infection of fibroblasts in the

range of 1µg/ml. US20090004198 (Nakajima *et al*) discloses a high affine antibody for the gB AD-1 domain, with apparent pM binding affinity, and 80% hCMV neutralising activity on fibroblasts, if used at concentrations of 1µg/ml and higher (10µg/ml and 100µg/ml). Two recent applications WO 10/114105 A1 and WO 10/114106 A1, both from Evec Inc., describe antibodies that bind to AD-2 and a discontinuous epitope in AD-1, respectively.

As disclosed in the present invention, we have developed hCMV neutralising human antibodies, which bind with high affinity to the gB protein of hCMV. In addition, these antibodies display similar high potency (IC_{50}s below 0.5µg/ml) in hCMV neutralisation using a broad range of hCMV susceptible cell types (fibroblasts, endothelial, epithelial and dendritic cells) and high potency not only on laboratory strain AD-169, but also on all clinical isolates tested so far. In addition, the antibodies disclosed herein recognise and define a novel neutralising epitope of the gB protein that has not been described previously. Owing to their high affinity and potency, and their novel epitope binding characteristics determined by functional studies as described herein, the binding members of the invention are particularly suitable for use in the therapeutic treatment, prophylaxis and/or diagnosis of hCMV infections in human patients. The binding members are useful for treating various disorders associated with CMV infection, as described in detail elsewhere herein.

Summary of Invention

Several, highly potent, gB specific and hCMV neutralising human antibodies are described herein, which also recognise a completely new hCMV neutralising epitope and therefore act by a different therapeutic principle in comparison to all other

known antibodies specific for hCMV. Their discovery and functional characterization are disclosed further below. As described in more detail in the Examples, 50 fully human, hCMV neutralising antibodies have been identified. They have been isolated from EBV transformed human peripheral blood derived B cells derived from hCMV-infected donors. CDR sequences of the antibodies 1 to 50 are as detailed in Tables 19 and 20. V_H domain and V_L domain combinations for a panel of antibodies 1 to 46 are as detailed in Table 7. All sequences referred to in Tables 7 and 19 are also shown in the appended sequence listing that forms part of the present disclosure.

As described in more detail below, binding members according to the invention have been shown to neutralise hCMV infection of target cells at therapeutically relevant concentrations, i.e. with IC_{50} below 1µg/ml. The most active binding members neutralise hCMV even at IC_{50}s below 0.5µg/ml. The neutralising capacity has also been observed with different clinical isolates (e.g. Towne and Altu; Table 16), representing different gB genotypes. Binding members of the invention may neutralise one or more activities of hCMV. For example, the inhibited biological activity may be prevention of infection of fibroblasts, endothelial cells, epithelial cells, retinal cells and/or dendritic cells. The prevention of infection of fibroblasts has been demonstrated in an *in vitro* neutralisation assay utilising a recombinant strain of hCMV, AD169 that expresses the reporter gene luciferase. Upon infection with this genetically modified hCMV strain, target cells become positive for luciferase enzyme expression, which can be detected by appropriate substrate-conversion in a standard luminometer. The binding members described in this invention were shown to neutralise the infection of hCMV strain AD169 when first incubated with the recombinant,

luciferase positive virus strain and then seeded onto monolayers of primary human foreskin fibroblasts (HFFs). Following further incubation, luminescence was detected using a luminometer and the relative light units (RLU) detected. The percentage neutralisation was then calculated, wherein the neutralising titre is indicated as the concentration of binding member (µg/ml) that gives a 50% or 100% reduction of hCMV infection of target cells. Binding members may give a 50% reduction of hCMV infection at concentrations of 0.1 to 5.0µg/ml, preferably 0.1 to 2.0µg/ml, more preferably from 0.3 to 1.3µg/ml or more preferably from 0.1 to 0.6µg/ml. The binding members have been shown to result in a 50% reduction of hCMV infection at therapeutically relevant concentrations of 0.1, 0.5, 1.0, 1.1, 1.2, 1.3, 1.5 or 2.0µg/ml.

Other methods that may be used for determining the neutralisation of infectivity of hCMV include ELISA, FACS, Western blotting, immunoprecipitation, and visual inspection based on plaque forming and counting.

The binding members described herein were shown to neutralise human hCMV not only in fibroblasts, but with similar efficacy also in endothelial, epithelial and dendritic cells (as shown in an assay using primary foreskin fibroblasts, Example 4 and in an assay using human umbilical vein endothelial cells (HUVEC), human ARPE-19 retinal pigment epithelial cells and primary dendritic cells, as shown in Example 7).

The invention discloses high affinity binding members for hCMV and specific for the hCMV gB protein. Binding members of the invention may bind hCMV gB protein with a K_D of not more than 50nM, e.g. not more than 25nM, 15nM, 10nM, 5nM, 3nM, 1.5nM, 1nM, 0.5nM, 0.1nM, 75pM or 57pM. Preferably the

binding member has a K_D of 1nM or less, preferably less than 0.5nM, preferably less than 0.1nM and more preferably less than 75pM. The K_D may be determined by surface plasmon resonance, e.g. Biacore®. Biacore® measurements of affinity are described herein in Example 5.

As described elsewhere herein, surface plasmon resonance involves passing an analyte in fluid phase over a ligand attached to a solid support, and determining association rates (k_a) and dissociation rates (k_d) between analyte and ligand. Surface plasmon resonance may for example be performed whereby a binding member is passed in fluid phase over gB protein attached to a support. Surface plasmon resonance data may be fitted to a monovalent analyte data model. The affinity may be expressed as the dissociation constant, K_D, which is calculated from the ratio of the dissociation and the association rate constants k_d/k_a as determined by surface plasmon resonance using a monovalent analyte data model.

The binding members described herein are shown to bind to a specific region of hCMV gB protein. Known epitopes of hCMV gB protein lie within the antigenic domain 1 (AD-1; between amino acids 552-635) and/or antigenic domain 2 (AD-2; between amino acids 67-86) of gB strain AD169, SEQ ID No: 239. In the present invention we describe binding members that bind to two new antigenic domains of hCMV gB protein, antigenic domain 4 (AD-4; a discontinuous region between amino acids 121-132 and 344-438 of gB strain AD169; SEQ ID No: 239) and antigenic domain 5 (AD-5; between amino acids 133 to 343 of gB strain AD169; SEQ ID No: 239). In initial experiments, the ability of six of the binding members described herein, monoclonal, recombinant antibodies Ab-04, Ab-11, Ab-14, Ab-

19, Ab-28 and Ab-42, to bind to specific regions of hCMV gB protein was investigated. In particular, the epitope binding specificity of these six binding members was first investigated in a Biacore® competition assay with a selection of anti-hCMV antibodies known in the art, which bind either to the AD-1 or to the AD-2 epitope of gB protein. As the binding members of the present invention specifically bound to gB protein with high affinity, but could not compete for gB binding with AD-1 and AD-2 specific antibodies, it was clear that the binding members of the present invention recognise a novel neutralising epitope of the gB protein. Further support for this finding was obtained by expressing a truncated version of gB protein comprising amino acid residues 100 to 447 (gB strain AD169; SEQ ID No: 239), which upon expression in COS cells was recognised by binding members of the invention.

Following the generation of a molecular model of HCMV gB (strain AD169; SEQ ID No: 239), surface exposed protein domains were identified and a discontinuous amino acid sequence between amino acid residues 121-132 and 344-438 was predicted to be a likely epitope, to which binding members of the invention could bind. When this predicted epitope was expressed as amino acids 116 to 132 and 344 to 440 (gB strain AD169; SEQ ID No: 239), which were coupled by a synthetic amino acid linker, it was found that this recombinant protein was specifically recognised by binding members Ab-11, Ab-14 or Ab-28 of the invention. This new epitope has been termed AD-4. Therefore the binding members of the invention do not bind to the region AD-1 of hCMV gB protein. Also, the binding members of the invention do not bind to the region AD-2 of hCMV gB protein. In contrast, binding members Ab-01 to Ab-46 of the invention bind to a new conformational epitope termed

AD-4 (also termed Domain II (Dom II)), between amino acid residues 100 to 447,and preferably between amino acid residues 121 to 438. More preferably, binding members of the invention bind to discontinuous amino acid stretches 116-132 and 344-440 of gB strain AD169 (SEQ ID No: 239) and most preferably stretches 121-132 and 344-438 of gB strain AD169 (SEQ ID No: 239). In this regard it has to be understood in accordance with the invention that the discontinuous epitope generated by amino acids stretches 121-132 and 344-438 of gB strain AD169 (SEQ ID No: 239) constitutes the same epitope as the discontinuous epitope generated by amino acids stretches 116-132 and 344-440 of gB strain AD169 (SEQ ID No: 239).

Since Antibodies Ab-01 to Ab-46 all have structurally related CDRs (in particular HCDR3 of identical length and related sequence), and are derived from a single donor, these antibody molecules are most likely somatic mutants of an original gB-reactive clones, and are therefore expected to bind the same or very similar overlapping epitope on the hCMV gB protein. Accordingly, the epitope characterisation results obtained with recombinant antibodies Ab-11, Ab-14 or Ab-28 are also expected to be representative for all of the antibodies Ab-01 to Ab-46 disclosed herein. The present invention therefore relates to a binding member, preferably an antibody, that binds to a conformational epitope of the gB protein recognised by antibodies Ab-11, Ab-14 or Ab-28 and also to a binding member that competes with any of antibodies Ab-11, Ab-14 or Ab-28 for binding to a conformational epitope of the gB protein recognised by these antibodies.

Therefore in a first embodiment, a binding member of the invention may bind hCMV gB protein at a region comprising amino acids 116 to 132 or amino acids 121 to 132 as predicted

from the structural model (Example 9). A binding member of the invention may also bind hCMV gB protein at a region comprising amino acids 344 to 440 or amino acids 344 to 438 as predicted from the structural model (Example 9). Optionally a binding member may bind flanking residues or structurally neighbouring residues in the hCMV gB amino acid sequence, in addition to binding amino acids 116 to 132 and/or amino acids 344 to 440. By convention, residue numbering corresponds to hCMV gB strain AD169 (SEQ ID No: 239).

In further experiments, the ability of four of the binding members described herein, monoclonal, recombinant antibodies Ab-47, Ab-48, Ab-49, Ab-50, to bind to specific regions of hCMV gB protein was also investigated. In particular, the epitope binding specificity of these four binding members was first investigated in an ELISA competition assay (Example 8.3) and then using a capture ELISA (Example 10.2). It was clear that these four binding members recognise a further novel neutralising epitope of the gB protein.

Following the generation of a molecular model of HCMV gB (strain AD169; SEQ ID No: 239), surface exposed protein domains were identified and an amino acid sequence between amino acid residues 133 and 343 was predicted to be a likely epitope, to which binding members of the invention could bind. This predicted epitope was subdivided and expressed as two subdomains: Subdomain 1 (amino acids 133-144 and 251-343) and Subdomain 2 (amino acids 140 to 255) (gB strain AD169; SEQ ID No: 239). When tested in a capture ELISA, Subdomain 1 was recognised by binding members Ab-47, Ab-49 or Ab-50 of the invention. The new epitope region of amino acids 134 to 344 (gB strain AD169; SEQ ID No: 239) has been termed AD-5.

Therefore binding members Ab-47, Ab-48, Ab-49 or Ab-50 of the invention do not bind to the region AD-1 of hCMV gB protein. Also, the binding members of the invention do not bind to the region AD-2 of hCMV gB protein. In contrast, binding members Ab-47 to Ab-50 of the invention bind to a new conformational epitope termed AD-5 (also termed Domain I (Dom I)), between amino acid residues 133 to 343 of gB strain AD169 (SEQ ID No: 239).

The present invention therefore relates to a binding member, preferably an antibody, that binds to an epitope of the gB protein recognised by antibodies Ab-47, Ab-48, Ab-49 or Ab-50 and also to a binding member that competes with any of these four antibodies for binding to a conformational epitope of the gB protein recognised by these antibodies.

Therefore in a second embodiment, a binding member of the invention may bind hCMV gB protein at a region comprising amino acids 133 to 343, as predicted from the structural model (Example 9). Optionally a binding member may bind flanking residues or structurally neighbouring residues in the hCMV gB amino acid sequence, in addition to binding amino acids 133 to 343. By convention, residue numbering corresponds to hCMV gB strain AD169 (SEQ ID No: 239).

A binding member of the invention may comprise an antibody molecule, e.g. an antibody molecule with fully human amino acid sequence. The binding member normally comprises an antibody V_H and/or V_L domain. V_H and V_L domains of binding members are also disclosed as part of the invention. Each of the V_H and V_L domains comprises complementarity determining regions, (CDRs), and framework regions, (FRs). An antibody V_H domain comprises three HCDR regions, designated HCDR1, HCDR2,

and HCDR3. An antibody V_L domain comprises three LCDR regions, designated LCDR1, LCDR2, and LCDR3. A V_H or V_L domain framework comprises four framework regions, FWR1, FWR2, FWR3 and FWR4, interspersed with CDRs in the following structure: FWR1 - CDR1 - FWR2 - CDR2 - FWR3 - CDR3 - FWR4.

Examples of antibody V_H and V_L domains and CDRs according to the present invention are as listed in the appended sequence listing that forms part of the present disclosure. Further CDRs are disclosed below and in Table 19. All V_H and V_L sequences, CDR sequences, sets of CDRs and sets of HCDRs and sets of LCDRs disclosed herein represent aspects and embodiments of the invention. As described herein, a 'set of CDRs' comprises CDR1, CDR2 and CDR3. Thus, a set of heavy chain CDRs refers to HCDR1, HCDR2 and HCDR3, and a set of light chain CDRs refers to LCDR1, LCDR2 and LCDR3. Unless otherwise stated, a 'set of CDRs' includes HCDRs and LCDRs.

Typically, binding members of the invention are monoclonal antibodies.

A binding member of the invention may comprise an antigen-binding site within a non-antibody molecule, normally provided by one or more CDRs e.g. a set of CDRs in a non-antibody protein scaffold, as discussed further below.

Binding members Ab-01 to Ab-46, according to the invention, were initially isolated from an hCMV infected donor and were isolated from EBV immortalised B cell lines, referred to as SM1, SM3, SM4, SM5, SM6, SM7, SM9, SM10 or SM11. From these nine cell lines 37 different V_H and 62 different V_L coding sequences of human antibodies could be identified (Table 6). The combination of all identified V_H and V_L coding sequences

from each cell line as IgH and IgL chains can theoretically generate 295 different antibodies. Of these, 46 different recombinant antibodies have been identified, which were hCMV neutralising in a first-line biological screening assay using luciferase-expressing, hCMV laboratory-strain AD-169 and primary human foreskin fibroblasts. Six of these recombinant antibodies were found to neutralise hCMV with high potency (IC_{50}s below 1µg/ml) and to bind gB protein with high affinity of K_D of 15nM or less (Tables 13 & 15 below).

The structures and locations of binding member variable domains may be determined by reference to Kabat *et al.*, (1991) and updates thereof. Described herein is a panel of binding members each comprising the set of CDRs as specified in Tables 19 and 20, in which HCDR1 has Kabat residues 31-35; HCDR2 has Kabat residues 50-65; HCDR3 has Kabat residues 95-102. LCDR1 has Kabat residues 24-34; LCDR2 has Kabat residues 50-56 and LCDR3 has Kabat residues 89-97. The Kabat numbering was determined using the website: http://www.bioinf.org.uk/abs/abnum/

A binding member of a first embodiment of the invention may comprise one or more CDRs as described herein, e.g. a CDR3, and optionally also a CDR1 and CDR2 to form a set of CDRs. The CDR or set of CDRs may be a CDR or set of CDRs of any of antibodies Ab-01 to Ab-46, or may be a variant thereof as described herein.

A binding member may comprise a set of H and/or L CDRs of any of antibodies Ab-01 to Ab-46 with one or more amino acid mutations within the disclosed set of H and/or L CDRs. Amino acid mutations are substitutions, deletions or insertions of one amino acid. Based on the examples provided and the

disclosed sequences, there may be, e.g., up to 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations e.g. substitutions, within the set of H and/or L CDRs. Furthermore, there may be up to 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations, in HCDR3 and/or there may be up to 6, 5, 4, 3, 2 or 1 mutations, in HCDR2 and/or there may be up to 3, 2 or 1 mutations in HCDR1 and/or there maybe up to 6, 5, 4, 3, 2 or 1 mutations in LCDR3 and/or there maybe 1 mutation in LCDR2 and/or LCRD1. The mutation may be a substitution or the H and/or L CDRs may optionally contain an insertion or deletion of one amino acid as compared with the disclosed set of H and/or L CDRs.

Substitutions, insertions or deletions may be made at any point in the CDRs. For example, in HCDR1 substitutions may be of any of Kabat residues 31-35, e.g. any of Kabat residues 31, 32, 34 and/or 35, in HCDR2 substitutions may be of any of Kabat residues 50-65, e.g. any of Kabat residues 50, 53, 54, 58, 60,and/or 64 and in HCDR3 substitutions may be of any of Kabat residues 99-102, e.g. any of Kabat residues 99-100C, 100E, 100F and/or 100K-102. For example, in LCDR1 a substitution may be of any of Kabat residues 24-34 e.g. Kabat residues 26 or 27, in LCDR2 substitutions may be of any of Kabat residues 50-56, e.g. Kabat residue 56 and in LCDR3 substitutions may be of any of Kabat residues 89 to 97, e.g. any of Kabat residues 89 and there may be an insertion at position 95B. Details of specific amino acid mutations compared with the sequence of antibody Ab-28 can be found in Tables 20a and 20b for the HCDRs and LCDRs respectively, e.g. amino acid substitutions or insertions.

For example, the present invention provides an isolated binding member for hCMV, comprising a set of CDRs: HCDR1,

HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the set of CDRs has 22 or fewer amino acid alterations from a set of CDRs in which:
HCDR1 has amino acid sequence SEQ ID No: 3;
HCDR2 has amino acid sequence SEQ ID No: 4;
HCDR3 has amino acid sequence SEQ ID No: 5;
LCDR1 has amino acid sequence SEQ ID No: 93;
LCDR2 has amino acid sequence SEQ ID No: 94; and
LCDR3 has amino acid sequence SEQ ID No: 95

For example a binding member or V_H domain according to the invention may comprise the HCDR1 of antibody Ab-28 with one or more of the following mutations:
Kabat residue Asp 31 replaced by Gly;
Kabat residue His 32 replaced by Phe or Tyr;
Kabat residue Met 34 replaced by Ile or Leu; and
Kabat residue Val 35 replaced by Asn.

A binding member or V_H domain according to the invention may comprise the HCDR2 of antibody Ab-28 with one or more of the following mutations:
Kabat residue Trp 50 replaced by Ser or Cys;
Kabat residue Gln 53 replaced by Asn or His;
Kabat residue Ser 54 replaced by Thr;
Kabat residue Gly 58 replaced by Lys, Asn or His;
Kabat residue Gly 60 replaced by Ala; and
Kabat residue Gln 64 replaced by Arg.

A binding member or V_H domain according to the invention may comprise the HCDR3 of antibody Ab-28 with one or more of the following mutations:
Kabat residue Thr 99 replaced by Ala;
Kabat residue Val 100 replaced by Met;

Kabat residue Ser 100A replaced by Thr;
Kabat residue Asn 100B replaced by Thr;
Kabat residue Ser 100C replaced by Phe;
Kabat residue Leu 100E replaced by Met or Ala;
Kabat residue Ser 100F replaced by Gly;
Kabat residue His 100K replaced by Tyr;
Kabat residue Asn 100L replaced by Ser or Asp;
Kabat residue Arg 100M replaced by Val or Ile;
Kabat residue Leu 100N replaced by Met;
Kabat residue Asp 101 replaced by Gly; and
Kabat residue Ala 102 replaced by Val or Ile.

A binding member or V_L domain according to the invention may comprise the LCDR1 of antibody Ab-28 in which Kabat residue Ser 26 is replaced by Asn or Kabat residue Ser 27 is replaced by Arg.

A binding member or V_L domain according to the invention may comprise the LCDR2 of antibody Ab-28 in which Kabat residue Ser 56 is replaced by Pro.

A binding member or V_L domain according to the invention may comprise the LCDR3 of antibody Ab-28 with one or more of the following mutations:
Kabat residue Gly 89 replaced by Ala;
Kabat residue Pro 91 replaced by Trp;
Kabat residue Arg 93 replaced by Ser;
Kabat residue Ser 94 replaced by Asp;
Kabat residue Ser 95a replaced by Gly or Ala;
Ala inserted at Kabat residue 95b;
Kabat residue Val 96 replaced by Tyr; and
Kabat residue Ile 97 replaced by Val.

Thus a binding member of the invention may comprise a LCDR3 wherein Kabat residue 95b is Ala or wherein Kabat residue 95b is absent.

The invention provides binding members comprising an HCDR1, HCDR2 and/or HCDR3 of any of antibodies Ab-01 to Ab-46, and/or an LCDR1, LCDR2 and/or LCDR3 of any of antibodies 1 to 46 e.g. a set of CDRs of any of antibodies Ab-01 to Ab-46 shown in Table 19 or 20.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 8; HCDR2 is SEQ ID No: 9; HCDR3 is SEQ ID No: 10; LCDR1 is SEQ ID No: 98; LCDR2 is SEQ ID No: 99; and LCDR3 is SEQ ID No: 100, representing the CDRs of antibody Ab-02.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 13; HCDR2 is SEQ ID No: 14; HCDR3 is SEQ ID No: 15; LCDR1 is SEQ ID No: 103; LCDR2 is SEQ ID No: 104; and LCDR3 is SEQ ID No: 105, representing the CDRs of antibody Ab-04.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 18; HCDR2 is SEQ ID No: 19; HCDR3 is SEQ ID No: 20; LCDR1 is SEQ ID No: 108; LCDR2 is SEQ ID No: 109; and LCDR3 is SEQ ID No: 110, representing the CDRs of antibody Ab-11.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3,

wherein: HCDR1 is SEQ ID No: 23; HCDR2 is SEQ ID No: 24; HCDR3 is SEQ ID No: 25; LCDR1 is SEQ ID No: 113; LCDR2 is SEQ ID No: 114; and LCDR3 is SEQ ID No: 115, representing the CDRs of antibody Ab-14.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 3; HCDR2 is SEQ ID No: 4; HCDR3 is SEQ ID No: 5; LCDR1 is SEQ ID No: 93; LCDR2 is SEQ ID No: 94; and LCDR3 is SEQ ID No: 95, representing the CDRs of antibody Ab-28.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 28; HCDR2 is SEQ ID No: 29; HCDR3 is SEQ ID No: 30; LCDR1 is SEQ ID No: 108; LCDR2 is SEQ ID No: 109; and LCDR3 is SEQ ID No: 110, representing the CDRs of antibody Ab-42.

The binding member may comprise a set of V_H CDRs of one of these antibodies. Optionally it may also comprise a set of V_L CDRs of one of these antibodies, and the V_L CDRs may be from the same or a different antibody as the V_H CDRs.

A V_H domain comprising a set of HCDRs of any of antibodies Ab-01 to Ab-46, and/or a V_L domain comprising a set of LCDRs of any of antibodies Ab-01 to Ab-46 are also provided by the invention.

Typically, a V_H domain is paired with a V_L domain to provide an antibody antigen-binding site, although as discussed further below a V_H or V_L domain alone may be used to bind antigen. The V_H domain of antibody Ab-28 may be paired with

the V_L domain of antibody Ab-28, so that an antibody antigen-binding site is formed comprising both the antibody Ab-28 V_H and V_L domains. Analogous embodiments are provided for the other V_H and V_L domains disclosed herein. In other embodiments, the antibody Ab-28 V_H is paired with a V_L domain other than the antibody V_L. Light-chain promiscuity is well established in the art (Kang *et al.*, 1991). Again, analogous embodiments are provided by the invention for the other V_H and V_L domains disclosed herein.

Thus, an IgH chain containing the V_H of any of antibodies 1 to 46 may be paired with the IgL chain containing V_L of any of antibodies Ab-01 to Ab-46 to generate a gB specific binding member.

A binding member may comprise an antibody molecule having one or more CDRs, e.g. a set of CDRs, within an antibody framework. The framework regions may be of human germline gene segment sequences. Human germline gene segment sequences are known to those skilled in the art and can be accessed for example from the VBase compilation or the IMGT online database (http://imgt.cines.fr).

A binding member of the invention may be an isolated human antibody molecule having a V_H domain comprising a set of HCDRs in a human germline framework, e.g. IGHV1-2. Thus, the VH domain framework regions FWR1, FWR2 and/or FWR3 may comprise framework regions of human germline gene segment IGHV1-2. FWR4 may comprise a framework region of human germline J segments selected from, for example, SEQ ID Nos: 188 to 191. The amino acid sequence of V_H FWR1 may be SEQ ID No: 181. The amino acid sequence of V_H FWR2 may be SEQ ID No: 182. The amino acid sequence of V_H FWR3 may be SEQ ID No: 183 or 184.

An antibody molecule or a V_H domain of the invention may comprise the following set of heavy chain framework regions: FWR1 SEQ ID No: 181; FWR2 SEQ ID No: 182; FWR3 SEQ ID No: 183 or 184; or may comprise the said set of heavy chain framework regions with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid alterations, such as a substitution, an insertion or a deletion.

Furthermore, an antibody of the invention may include a V_H domain that is encoded by a nucleic acid sequence that is at least 80% homologous to the IGHV1-2 germline gene sequence, Preferably the nucleic acid sequence is at least 90%, 95%, 96%, 97% homologous to the IGHV1-2 germline gene sequence, and more preferably, at least 98%, 99% homologous to the IGHV1-2 germline gene sequence. The V_H domain of an antibody of the invention may be at least 80% homologous to the amino acid sequence of the V_H domain encoded by the IGHV1-2 germline gene sequence. Preferably the amino acid sequence of the V_H domain is at least 90%, 95%, 96%, 97% homologous to the amino acid sequence encoded by the IGHV1-2 germline gene sequence, and more preferably, at least 98%, 99% homologous to the amino acid sequence encoded by IGHV1-2 germline gene sequence.

Normally the binding member also has a V_L domain comprising a set of LCDRs, e.g. in a human germline framework, e.g. IGLV1-51. Thus, the V_L domain framework regions may comprise framework regions FWR1, FWR2 and/or FWR3 of human germline gene segment IGLV1-51. FWR4 may comprise a framework region of human germline J segment IGLJ2 (SEQ ID No: 193). The amino acid sequence of V_L FWR1 may be SEQ ID No: 185. The amino acid

sequence of V_L FWR2 may be SEQ ID No: 186. The amino acid sequence of V_L FWR3 may be SEQ ID No: 187.

An antibody molecule or a V_L domain of the invention may comprise the following set of light chain framework regions: FWR1 SEQ ID No: 185; FWR2 SEQ ID No: 186; FWR3 SEQ ID No: 187; or may comprise the said set of light chain framework regions with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 amino acid alterations, such as a substitution, an insertion or a deletion.

Furthermore, an antibody of the invention may include a V_L domain that is encoded by a nucleic acid sequence that is at least 80% homologous to the IGLV1-51 germline gene sequence. Preferably the nucleic acid sequence is at least 90%, 95%, 96%, 97% homologous to the IGLV1-51 germline gene sequence, and more preferably, at least 98%, 99% homologous to the IGLV1-51 germline gene sequence. The V_L domain of an antibody of the invention may be at least 80% homologous to the amino acid sequence of the V_L domain encoded by the IGLV1-51 germline gene sequence. Preferably the amino acid sequence of the V_L domain is at least 90%, 95%, 96%, 97% homologous to the amino acid sequence encoded by the IGLV1-51 germline gene sequence, and more preferably, at least 98%, 99% homologous to the amino acid sequence encoded by IGLV1-51 germline gene sequence.

For example, an antibody molecule of the invention may comprise a set of heavy and light chain framework regions, wherein heavy chain FWR1 is SEQ ID No: 181; heavy chain FWR2 is SEQ ID No: 182; heavy chain FWR3 is SEQ ID No: 183; light chain FWR1 is SEQ ID No: 185; light chain FWR2 is SEQ ID No: 186; light chain FWR3 is SEQ ID No: 187; or may comprise the

said set of heavy and light chain framework regions with 10 or fewer, e.g. five or fewer, amino acid alterations, e.g. substitutions.

Binding members Ab-47 to Ab-50, according to the invention, were initially isolated from three hCMV infected donors and were isolated from EBV immortalised B cell lines, referred to as SM10, SM12, 2C2 or 1G2. From these four cell lines, four different V_H and five different V_L coding sequences of human antibodies could be identified (Table 12). The combination of all identified V_H and V_L coding sequences from each cell line as IgH and IgL chains can theoretically generate 20 different antibodies. Of these, four different recombinant antibodies have been identified, which were hCMV neutralising in a first-line biological screening assay using luciferase-expressing, hCMV laboratory-strain AD-169 and primary human foreskin fibroblasts. All of these recombinant antibodies were found to neutralise hCMV with high potency (IC_{50}s below 0.6µg/ml; Table 14 below).

The structures and locations of binding member variable domains may be determined by reference to Kabat *et al.*, (1991) and updates thereof. Described herein are binding members Ab-46, Ab-47, Ab-48 and Ab-50 each comprising the set of CDRs as specified in Table 19, in which the CDRs were identified by the Kabat numbering system (Kabat & Wu, 1991) and determined using the website: http://www.bioinf.org.uk/abs/abnum/

A binding member of a second embodiment of the invention may comprise one or more CDRs as described herein, e.g. a CDR3, and optionally also a CDR1 and CDR2 to form a set of CDRs. The CDR or set of CDRs may be a CDR or set of CDRs of any of

antibodies Ab-47 to Ab-50, or may be a variant thereof as described herein.

A binding member may comprise a set of H and/or L CDRs of any of antibodies Ab-47 to Ab-50 with one or more amino acid mutations within the disclosed set of H and/or L CDRs. Amino acid mutations are substitutions, deletions or insertions of one amino acid. Based on the examples provided and the disclosed sequences, there may be, e.g., up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations within the set of H and/or L CDRs. The mutation may be a substitution or the H and/or L CDRs may optionally contain an insertion or deletion of one amino acid as compared with the disclosed set of H and/or L CDRs. Substitutions, insertions or deletions may be made at any point in the CDRs.

The invention provides binding members comprising an HCDR1, HCDR2 and/or HCDR3 of any of antibodies Ab-47 to Ab-50, and/or an LCDR1, LCDR2 and/or LCDR3 of any of antibodies Ab-47 to Ab-50, e.g. a set of CDRs of any of antibodies Ab-47 to Ab-50 shown in Table 19.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 243; HCDR2 is SEQ ID No: 244; HCDR3 is SEQ ID No: 245; LCDR1 is SEQ ID No: 263; LCDR2 is SEQ ID No: 264; and LCDR3 is SEQ ID No: 265, representing the CDRs of antibody Ab-47.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 248; HCDR2 is SEQ ID No: 249; HCDR3 is SEQ ID No: 250; LCDR1 is SEQ ID No: 268; LCDR2 is

SEQ ID No: 269; and LCDR3 is SEQ ID No: 270, representing the CDRs of antibody Ab-48.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 253; HCDR2 is SEQ ID No: 254; HCDR3 is SEQ ID No: 255; LCDR1 is SEQ ID No: 273; LCDR2 is SEQ ID No: 274; and LCDR3 is SEQ ID No: 275, representing the CDRs of antibody Ab-49.

For example, a binding member of the invention may comprise a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein: HCDR1 is SEQ ID No: 258; HCDR2 is SEQ ID No: 259; HCDR3 is SEQ ID No: 260; LCDR1 is SEQ ID No: 278; LCDR2 is SEQ ID No: 279; and LCDR3 is SEQ ID No: 280, representing the CDRs of antibody Ab-50.

A binding member of the invention may be an isolated human antibody molecule having a V_H domain comprising a set of HCDRs in a human germline framework, e.g. IGHV4-39 or IGHV4-59. Thus, the VH domain framework regions FWR1, FWR2 and/or FWR3 may comprise framework regions of human germline gene segment IGHV4-39 or IGHV4-59. FWR4 may comprise a framework region of human germline J segments selected from any of the six heavy chain J segments (see Ravetch *et al.*, 1981).

The amino acid sequence of Ab-47 or Ab-50 V_H domain may comprise the following set of heavy chain framework regions of IGHV4-39: FWR1 SEQ ID No: 281, FWR2 SEQ ID No: 282; FWR3 SEQ ID No: 283; or may comprise the said set of heavy chain framework regions with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 amino acid alterations, such as a substitution, an insertion or a deletion.

The amino acid sequence of Ab-48 or Ab-48 V_H domain may comprise the following set of heavy chain framework regions of IGHV4-59: FWR1 SEQ ID No: 284, FWR2 SEQ ID No: 285; FWR3 SEQ ID No: 286; or may comprise the said set of heavy chain framework regions with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 amino acid alterations, such as a substitution, an insertion or a deletion.

Furthermore, an antibody of the invention may include a V_H domain that is encoded by a nucleic acid sequence that is at least 75% homologous to the IGHV4-39 or IGHV4-59 germline gene sequence, Preferably the nucleic acid sequence is at least 80%, 85%, 90%, 95%, 96%, 97% homologous to the IGHV4-39 or IGHV4-59 germline gene sequence, and more preferably, at least 98%, 99% homologous to the IGHV4-39 or IGHV4-59 germline gene sequence. The V_H domain of an antibody of the invention may be at least 75% homologous to the amino acid sequence of the V_H domain encoded by the IGHV4-39 or IGHV4-59 germline gene sequence. Preferably the amino acid sequence of the V_H domain is at least 80%, 85%, 90%, 95%, 96%, 97% homologous to the amino acid sequence encoded by the IGHV4-39 or IGHV4-59 germline gene sequence, and more preferably, at least 98%, 99% homologous to the amino acid sequence encoded by IGHV1-2 germline gene sequence.

A binding member of the invention may also comprise a V_L domain comprising a set of kappa light chain CDRs in a human germline framework, e.g. IGKV2D-28 or IGKV1D-33. Thus, the VL domain framework regions FWR1, FWR2 and/or FWR3 may comprise framework regions of human germline gene segment IGKV2D-28 or IGKV1D-33. FWR4 may comprise a framework region of human

germline J segments selected from any of the five kappa J segments (see Hieter *et al.*, 1982).

The amino acid sequence of Ab-47 or Ab-48 V_L domain may comprise the following set of kappa light chain framework regions of IGKV2D-28: FWR1 SEQ ID No: 287, FWR2 SEQ ID No: 288; FWR3 SEQ ID No: 289; or may comprise the said set of light chain framework regions with 1 or 2 amino acid alterations, such as a substitution, an insertion or a deletion.

The amino acid sequence of Ab-49 V_L domain may comprise the following set of light chain framework regions of IGKV1D-33: FWR1 SEQ ID No: 290, FWR2 SEQ ID No: 291; FWR3 SEQ ID No: 292; or may comprise the said set of light chain framework regions with 1, 2, 3, 4, 5, 6, 7, or 8 amino acid alterations, such as a substitution, an insertion or a deletion.

A binding member of the invention may also comprise a V_L domain comprising a set of lambda light chain CDRs in a human germline framework, e.g. IGLV1-47. Thus, the VL domain framework regions FWR1, FWR2 and/or FWR3 may comprise framework regions of human germline gene segment IGLV1-47. FWR4 may comprise a framework region of human germline J segments selected from any of the four lambda J segments (see Udey & Blomberg 1987; Vasicek & Leder, 1990).

The amino acid sequence of Ab-50 V_L domain may comprise the following set of lambda light chain framework regions of IGLV1-47: FWR1 SEQ ID No: 293, FWR2 SEQ ID No: 294; FWR3 SEQ ID No: 295; or may comprise the said set of light chain

framework regions with 1 or 2 amino acid alterations, such as a substitution, an insertion or a deletion.

Furthermore, an antibody of the invention may include a V_L domain that is encoded by a nucleic acid sequence that is at least 90% homologous to the IGKV2D-28, IGKV1D-33 or IGLV1-47 germline gene sequence. Preferably the nucleic acid sequence is at least 95%, 96%, 97% homologous to the IGKV2D-28, IGKV1D-33 or IGLV1-47 germline gene sequence, and more preferably, at least 98%, 99% homologous to the IGKV2D-28, IGKV1D-33 or IGLV1-47 germline gene sequence. The V_L domain of an antibody of the invention may be at least 90% homologous to the amino acid sequence of the V_L domain encoded by the IGKV2D-28, IGKV1D-33 or IGLV1-47 germline gene sequence. Preferably the amino acid sequence of the V_L domain is at least 95%, 96%, 97% homologous to the amino acid sequence encoded by the IGKV2D-28, IGKV1D-33 or IGLV1-47 germline gene sequence, and more preferably, at least 98%, 99% homologous to the amino acid sequence encoded by IGKV2D-28, IGKV1D-33 or IGLV1-47 germline gene sequence.

A binding member of the invention may be one which competes for binding to hCMV with any binding member that (i) binds hCMV and (ii) comprises a binding member, V_H and/or V_L domain, CDR e.g. HCDR3, and/or set of CDRs disclosed herein.

Competition between binding members may be assayed *in vitro*, for example using binding assays, like ELISA, surface plasmon resonance, and/or by tagging a specific reporter molecule to one binding member which can be detected in the presence of one or more other untagged binding members, to enable identification of binding members which bind the same epitope or an overlapping epitope. Such methods are readily known to

one of ordinary skill in the art, and are described in more detail herein (see the Examples). Thus, a further aspect of the present invention provides a binding member comprising an antibody antigen-binding site that competes with an antibody molecule, for example an antibody molecule comprising a V_H and/or V_L domain, CDR e.g. HCDR3 or set of CDRs of any of antibodies Ab-01 to Ab-50, for binding to hCMV.

In further aspects, the invention provides an isolated nucleic acid which comprises a sequence encoding a binding member, comprising a V_H domain and/or V_L domain according to the present invention, and methods of preparing a binding member, comprising a V_H domain and/or a V_L domain of the invention, encoded by said nucleic acid under conditions to bring about production of said binding member, comprising V_H domain and/or V_L domain, and recovering it.

Another aspect of the present invention provides isolated nucleic acids encoding any of the V_H CDR or V_L CDR sequences disclosed herein.

A further aspect provides a host cell containing or transfected with nucleic acid of the invention.

Further aspects of the present invention describe compositions containing binding members of the invention, and their use in methods of binding, inhibiting and/or neutralising hCMV infection, including methods of treatment of the human or animal body by therapy.

Binding members according to the invention may be used in a method of treatment or diagnosis, such as a method of treatment (which may include prophylactic treatment) of a

disease or disorder in the human or animal body (e.g. in a human patient), which comprises administering to said human or animal body an effective amount of a binding member of the invention or a combination of several binding members of the invention. Conditions treatable in accordance with the present invention include any in which hCMV plays a role, as discussed in detail elsewhere herein.

These and other aspects of the invention are described in further detail below.

Terminology

It is convenient to point out here that 'and/or' where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example 'A and/or B' is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

hCMV

The full-length amino acid sequence of human cytomegalovirus (hCMV) has GenBank Acc. No. X17403 (Human cytomegalovirus strain AD169 complete genome) and comprises a 229354 base pair sequence (Chee *et al.*, 1990; Bankier *et al.*, 1991).

gB

gB complex is a surface glycoprotein complex of the virion envelope of CMV. There are a number of different strains of gB protein:

gB strain AD169 - SwissProt Acc. No. P06473 (SEQ ID No: 239)
gB strain Towne - SwissProt Acc. No. P13201 (SEQ ID No: 240)
Known neutralising domains of gB include antigenic domain-1 (AD-1; amino acids 552-635 of SEQ ID No: 239 [AD169]) and

antigenic domain-2 (AD-2; amino acids 67-86 of SEQ ID No: 239 [AD169]). A further antigenic domain, AD-3 also exists (amino acids 783-906 of SEQ ID No: 239 [AD169]). This domain is located intravirally and is not the target of neutralising antibodies.

Binding member

This describes one member of a pair of molecules that bind one another. The members of a binding pair may be naturally derived or wholly or partially synthetically produced. One member of a binding pair may be a polypeptide, nucleic acid, carbohydrate, lipid, small molecular weight compound, an oligonucleotide, an oligopeptide, RNA interference (RNAi; see Milhavet *et al.*, 2003), antisense (see Opalinska & Gewirtz, 2003), a recombinant protein, an antibody, or fragments thereof or conjugates or fusion proteins thereof.

Antisense or RNAi inhibitors for use in the present invention may comprise nucleic acid molecules capable of modulating gene expression, for example capable of down regulating expression of a sequence encoding a hCMV gB protein. Such nucleic acid molecules may include, but are not limited to antisense molecules, short interfering nucleic acid (siNA), double-stranded RNA (dsRNA), micro RNA, short hairpin RNA (shRNA), nucleic acid sensor molecules, allozymes, enzymatic nucleic acid molecules and triplex oligonucleotides and any other nucleic acid molecule which can be used in mediating RNA interference 'RNAi' or gene silencing in a sequence-specific manner.

One member of the pair of molecules may have an area on its surface, or a cavity, which binds to and is therefore complementary to a particular spatial and polar organization

of the other member of the pair of molecules. Examples of types of binding pairs are antigen-antibody, receptor-ligand and enzyme-substrate.

A binding member normally comprises a molecule having a binding site. For example, a binding member may be an antibody molecule or a non-antibody protein that comprises a binding site. A binding site may be provided by means of arrangement of CDRs on antibody framework regions and/or on non-antibody protein scaffolds, such as fibronectin or cytochrome B etc. (Haan & Maggos 2004; Koide *et al.*, 1998; Nygren *et al.*, 1997), or by randomising or mutating amino acid residues of a loop within a protein scaffold to confer binding specificity for a desired target. Scaffolds for engineering novel binding sites in proteins have been reviewed in detail by Nygren *et al.*, *ibid.* Protein scaffolds for antibody mimics are disclosed in WO 00/034784 A1 (Lipovsek), in which proteins (antibody mimics) are described that include a fibronectin type III domain having at least one randomised loop. A suitable scaffold into which to graft one or more CDRs, e.g. a set of HCDRs, may be provided by any domain member of the immunoglobulin gene superfamily. The scaffold may be a human or non-human protein. An advantage of a non-antibody protein scaffold is that it may provide an antigen-binding site in a scaffold molecule that is smaller and/or easier to manufacture than at least some antibody molecules. Small size of a binding member may confer useful physiological properties, such as an ability to enter cells, penetrate deep into tissues or reach targets within other structures, or to bind within protein cavities of the target antigen. Use of antigen binding sites in non-antibody protein scaffolds is reviewed in Wess, 2004. Typical are proteins having a stable backbone and one or more variable loops, in

which the amino acid sequence(s) of the loop or loops is/are specifically or randomly mutated to create an antigen-binding site that binds the target. Such proteins include the IgG-binding domains of protein A from *S. aureus*, transferrin, tetranectin, fibronectin, lipocalins as well as gamma-crystalline and other Affilin™ scaffolds (Scil Proteins).

Examples of other approaches include synthetic 'Microbodies' based on cyclotides - small proteins having intra-molecular disulphide bonds, Microproteins (Versabodies™, Amunix) and ankyrin repeat proteins (DARPins, Molecular Partners).

In addition to antibody sequences and/or an antigen-binding site, a binding member according to the present invention may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Binding members of the invention may carry a detectable label, or may be conjugated to a toxin or a targeting moiety or enzyme (e.g. via a peptidyl bond or linker). For example, a binding member may comprise a catalytic site (e.g. in an enzyme domain) as well as an antigen binding site, wherein the antigen binding site binds to the antigen and thus targets the catalytic site to the antigen. The catalytic site may inhibit biological function of the antigen, e.g. by cleavage.

Although, as noted, CDRs can be carried by non-antibody scaffolds, the structure for carrying a CDR or a set of CDRs of the invention will generally be an antibody heavy or light chain sequence or substantial portion thereof in which the CDR or set of CDRs is located at a location corresponding to the CDR or set of CDRs of naturally occurring V_H and V_L

antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of immunoglobulin variable domains may be determined by reference to Kabat & Wu, (1991) and updates thereof. A number of academic and commercial on-line resources are available to query this database. For example, see Martin, 1996 and the associated on-line resource, currently at the web address of http://www.bioinf.org.uk/abs/simkab.html.

By CDR region or CDR, it is intended to indicate the hypervariable regions of the heavy and light chains of the immunoglobulin as defined by Kabat *et al., ibid.* An antibody typically contains 3 heavy chain CDRs, termed HCDR1, HCDR2, and HCDR3, and 3 light chain CDRs, termed LCDR1, LCDR2 and LCDR3. The term CDR or CDRs is used here in order to indicate one of these regions or several, or even the whole, of these regions which contain the majority of the amino acid residues responsible for the binding by affinity of the antibody for the antigen or the epitope which it recognises.

Among the six CDR sequences, the third CDR of the heavy chain (HCDR3) has a greatest size variability i.e. greater diversity, essentially due to the mechanism known in the art as V(D)J rearrangement of the V, D and J gene segments of the germline immunoglobulin heavy chain gene locus. The HCDR3 may be as short as two amino acids or as long as 26 amino acids, or may have any length in between these two extremes. CDR length may also vary according to the length that can be accommodated by the particular underlying framework. Functionally, HCDR3 can play an important role in the determination of the specificity of the antibody (Segal *et al.*, 1974; Amit *et al.*, 1986; Chothia *et al.*, 1987, 1989;

Caton *et al.*, 1990; Sharon 1990a, Sharon 1990b, Kabat *et al.*, 1991).

In binding members Ab-01 to Ab-46 of the present invention, as indicated in Tables 20a and b, HCDR1 may be 5 amino acids long, consisting of Kabat residues 31-35. HCDR2 may be 17 amino acids long, consisting of Kabat residues 50-65. HCDR3 may be 22 amino acids long, consisting of Kabat residues 95-102. LCDR1 may be 13 amino acids long, consisting of Kabat residues 24-34. LCDR2 may be 7 amino acids long, consisting of Kabat residues 50-56. LCDR3 may be 10 amino acids long, consisting of Kabat residues 89-97.

In binding members Ab-47 to Ab-50 of the present invention, HCDR1 may be 7 or 5 amino acids long, consisting of Kabat residues 31-37 or 31-35, respectively. HCDR2 may be 16 amino acids long and HCDR3 may be 10, 15, 17 or 22 amino acids long. LCDR1 may be 11 amino acids long, consisting of Kabat residues 24-34; or 13 amino acids long, consisting of Kabat residues 23-35; or 16 amino acids long, consisting of Kabat residues 24-39. LCDR2 may be 7 amino acids long and LCDR3 may be 9 amino acids long.

Antibody Molecule

This describes an immunoglobulin whether naturally, or partly, or wholly synthetically produced. The term also covers any polypeptide or protein comprising an antibody antigen-binding site. It must be understood here that the invention does not relate to the antibodies in natural form, that is to say they are not in their natural environment but that they have been able to be isolated or obtained by purification from natural sources, or else obtained by genetic recombination, or by chemical synthesis, and that

they can then contain unnatural amino acids. Antibody fragments that comprise an antibody antigen-binding site include, but are not limited to, molecules such as Fab, Fab', $F(ab')_2$, Fab' -SH, scFv, Fv, dAb and Fd. Various other antibody molecules including one or more antibody antigen-binding sites have been engineered, including for example Fab2, Fab3, diabodies, triabodies, tetrabodies and minibodies and also bispecific and trispecific antibodies. Antibody molecules and methods for their construction and use are described in Hollinger & Hudson (2005).

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules that bind the target antigen. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the CDRs, of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See for instance, EP0184187A (Kudo *et al*) or EP0239400A (Winter). A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term 'antibody molecule' should be construed as covering any binding member or substance having an antibody antigen-binding site with the required specificity and/or binding to antigen. Thus, this term covers bispecifc or trispecific antibodies as well as antibody fragments and derivatives, including any polypeptide comprising an antibody antigen-binding site, whether natural or wholly or partially synthetic. Chimeric molecules comprising an antibody antigen-binding site, or equivalent, fused to another polypeptide

(e.g. derived from another species or belonging to another antibody class or subclass) are therefore included. Cloning and expression of chimeric antibodies are described for example in EP0120694A (Boss *et al*) and EP0125023A (Cabilly *et al*).

Further techniques available in the art of antibody engineering have made it possible to isolate human and humanised antibodies. For example, human hybridomas can be made as described by Kontermann & Dubel (2001). Phage display, another established technique for generating binding members has been described in detail in many publications, such as Kontermann & Dubel, *ibid* and WO 92/01047 A1 (McCafferty *et al*).

Transgenic mice in which the mouse antibody genes are inactivated and functionally replaced with human antibody genes while leaving intact other components of the mouse immune system, can be used for isolating human antibodies (Mendez *et al*., 1997). Alternatively, the method described by Grawunder & Melchers (WO 03/068819 A1) can be used to generate genetically modified vertebrate precursor lymphocytes for the production of heterologous antibodies or binding proteins. Humanised antibodies can be produced using techniques known in the art such as those disclosed in for example WO 91/09967 A1 (Adair *et al*). Further, WO 04/006955 A1 (Foote) describes methods for humanising antibodies, based on selecting variable region framework sequences from human antibody genes by comparing canonical CDR structure types for CDR sequences of the variable region of a non-human antibody to canonical CDR structure types for corresponding CDRs from a library of human antibody sequences, e.g. germline antibody gene segments. Human antibody variable regions having similar

canonical CDR structure types to the non-human CDRs form a subset of member human antibody sequences from which to select human framework sequences. The subset members may be further ranked by amino acid similarity between the human and the non-human CDR sequences. In the method of WO 04/006955 A1 *ibid*, top ranking human sequences are selected to provide the framework sequences for constructing a chimeric antibody that functionally replaces human CDR sequences with the non-human CDR counterparts using the selected subset member human frameworks, thereby providing a humanized antibody of high affinity and low immunogenicity without need for comparing framework sequences between the non-human and human antibodies. Chimeric antibodies made according to the method are also disclosed.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of V_L, V_H, C_L and C_H1 domains; (ii) the Fd fragment consisting of the V_H and C_H1 domains; (iii) the Fv fragment consisting of the V_L and V_H domains of a single antibody; (iv) the dAb fragment (Ward *et al.*, 1989; McCafferty *et al.*, 1990; Holt *et al.*, 2003), which consists of a V_H or a V_L domain; (v) isolated CDR regions; (vi) $F(ab')_2$ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a V_H domain and a V_L domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, 1998; Huston *et al* 1988); (viii) bispecific single chain Fv dimers (WO 93/011161 A1 (Whitlow *et al*)) and (ix) 'diabodies', multivalent or multispecific fragments constructed by gene fusion (Holliger *et al.*, 1993 & WO 94/13804 A1). Fv, scFv or diabody molecules may be stabilized by the incorporation of disulphide bridges

linking the V_H and V_L domains (Reiter *et al.*, 1996). Minibodies comprising an scFv joined to a C_H3 domain may also be made (Hu *et al;* 1996). Other examples of binding fragments are Fab', which differs from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain C_H1 domain, including one or more cysteines from the antibody hinge region, and Fab' -SH, which is a Fab' fragment in which the cysteine residue(s) of the constant domains bear a free thiol group. Antibody molecules have also been described that containing just two CDRs linked by a framework region (Qui *et al.*, 2007). CDR3 from the V_H or V_L domain was linked to the CDR1 or CDR2 loop of the other domain with linkage through the C terminus of the selected CDR1 or CDR2 to the N terminus of the CDR3, via a framework region.

A domain antibody (dAb) is a small monomeric antigen-binding fragment of an antibody, namely the variable region of an antibody heavy or light chain (Holt *et al.*, 2003). V_H dAbs occur naturally in camelids (e.g. camel, llama) and may be produced by immunising a camelid with a target antigen, isolating antigen-specific B cells and directly cloning dAb genes from individual B cells; however dAbs can also be produced in cell culture. A binding member of the present invention may be a dAb comprising a V_H or V_L domain substantially as set out herein, or a V_H or V_L domain comprising a set of CDRs substantially as set out herein.

Antibody fragments of the invention can be obtained starting from any of antibodies Ab-01 to Ab-50, by methods such as digestion by enzymes e.g. pepsin or papain and/or by cleavage of the disulfide bridges by chemical reduction. In another manner, the antibody fragments comprised in the present invention can be obtained by techniques of genetic

recombination well known to the person skilled in the art or else by peptide synthesis or by nucleic acid synthesis and expression.

Functional antibody fragments according to the present invention include any functional fragment whose half-life is increased by a chemical modification, especially by PEGylation, or by incorporation in a liposome for example.

Bispecific or bifunctional antibodies form a second generation of monoclonal antibodies in which two different variable regions are combined in the same molecule (Holliger & Bohlen, 1999). Therefore, a bispecific antibody may have two different binding specificities encoded by the variable regions and therefore bind to two different epitopes on single or multiple target antigens. Their use has been demonstrated both in the diagnostic field and in the therapy field from their capacity to recruit new effector functions or to target several molecules on the surface of tumour cells. For example, antibodies can be armed with additional cytotoxic mechanisms such as radioisotopes, bacterial toxins, inflammatory cytokines, chemotherapuetics or prodrugs. Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger & Winter, 1993). Examples of bispecific antibodies include those of the BiTE® technology (Micromet, Inc.) in which the binding domains of two antibodies with different specificity can be used and directly linked via short flexible peptides. This combines two antibodies on a short single polypeptide chain. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific antibodies can be constructed as entire IgG, as quadroma (dual-specific antigen binding fragment (Fab) plus Fcγ), as bispecific $F(ab')_2$, as Fab'PEG, as heterodimeric Fab, as diabodies or as bispecific or heterodimeric scFv (reviewed in Kufer *et al.*, 2004). Further, two bispecific antibodies can be linked using routine methods known in the art to form tetravalent antibodies. Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E.coli*.

Recent work on multi-specific antibodies has led to the development of mixtures antibodies wherein three to five recombinant human monoclonal antibodies are produced by a single, clonal cell. The component antibodies share the same immunoglobulin light chain variable region to ensure that all binding sites associated with the antibody species in the mixture are functional (Oligoclonics™; Merus Biopharmaceuticals BV; WO 04/106375 A1). The component antibodies may comprise different formats such as whole IgG or Fab fragments or mixtures of both full-length immunoglobulin and fragments of antibodies. The component antibodies are selected for superior biological activities such as increased potency in neutralisation of virus, improved neutralisation and removal of cytokines and chemokines, enhanced tumour cell killing and prevention of escape and improved breath of viral protection.

Various methods are available in the art for obtaining antibodies against hCMV. The antibodies may be monoclonal antibodies, especially of human origin, which can be obtained according to the standard methods well known to the person

skilled in the art. In general, for the preparation of monoclonal antibodies or their functional fragments, especially of murine origin, it is possible to refer to techniques which are described in particular in the manual 'Antibodies' (Harlow & Lane, 1988) or to the technique of preparation from hybridomas described by Kohler and Milstein, 1975.

Monoclonal antibodies can be obtained, for example, from the B cells of an animal or human immunized against hCMV, or one of its fragments, for example gB, containing the epitope recognised by said monoclonal antibodies. Suitable fragments and peptides or polypeptides comprising them are described herein, and may be used to immunise animals to generate antibodies against hCMV. hCMV or one of its fragments can be produced according to the usual working methods, by genetic recombination starting with a nucleic acid sequence contained in the cDNA sequence coding for hCMV or fragment thereof and/or by peptide synthesis starting from a sequence of amino acids comprised in the peptide sequence of the hCMV and/or fragment thereof.

The monoclonal antibodies can, for example, be purified on an affinity column on which hCMV protein or one of its component proteins containing the epitope recognised by said monoclonal antibodies has previously been immobilized. More particularly, the monoclonal antibodies can be purified by chromatography on protein A and/or G, followed or not followed by ion-exchange chromatography aimed at eliminating the residual protein contaminants as well as the DNA and the LPS, in itself, followed or not followed by exclusion chromatography on Sepharose gel in order to eliminate the potential aggregates due to the presence of dimers or of

other multimers. Any of these techniques can be used simultaneously or successively.

Antigen-binding site

This describes the part of a molecule that binds to and is complementary to all or part of the target antigen. In an antibody molecule it is referred to as the antibody antigen-binding site, and comprises the part of the antibody that binds to and is complementary to all or part of the target antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antibody antigen-binding site may be provided by one or more antibody variable domains. An antibody antigen-binding site may comprise an antibody light chain variable region (V_L) and an antibody heavy chain variable region (V_H).

An antigen binding site may be engineered in a region of an antibody molecule separate from the natural location of the CDRs, e.g. in a framework region of a V_H or V_L domain, or in an antibody constant domain e.g. but not limited to C_H1 and/or C_H3. An antigen binding site engineered in a structural region may be additional to, or instead of, an antigen binding site formed by sets of CDRs of a V_H and V_L domain. Where multiple antigen binding sites are present in an antibody molecule, they may bind the same antigenic domain on hCMV, for example, thereby increasing valency of the binding member and thereby increasing its avidity. Alternatively, multiple antigen binding sites may bind different antigens on hCMV and/or one or more other antigens, and this may be used to add effector functions, prolong half-life or improve *in vivo* delivery of the antibody molecule.

Isolated

This refers to the state in which binding members of the invention, or nucleic acid encoding such binding members, will generally be in accordance with the present invention. Thus, binding members, V_H and/or V_L domains, and encoding nucleic acid molecules and vectors according to the present invention may be provided isolated and/or purified, e.g. from their natural environment, in substantially pure or homogeneous form, or, in the case of nucleic acid, free or substantially free of nucleic acid or genes of origin other than the sequence encoding a polypeptide with the required function. Isolated members and isolated nucleic acid will be free or substantially free of material with which they are naturally associated, such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (e.g. cell culture) when such preparation is by recombinant DNA technology practised *in vitro* or *in vivo*. Members and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the members will normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Binding members may be glycosylated, either naturally or by systems of heterologous eukaryotic cells (e.g. CHO or NS0 cells), or they may be non-glycosylated, if, for example, they are produced by expression in a prokaryotic cell.

Heterogeneous preparations comprising anti-hCMV antibody molecules also form part of the invention. For example, such preparations may be mixtures of antibodies with full-length heavy chains and heavy chains lacking the C-terminal lysine, with various degrees of glycosylation and/or with derivatized

amino acids, such as cyclisation of an N-terminal glutamic acid to form a pyroglutamic acid residue.

As used herein, the phrase 'substantially as set out' refers to the characteristic(s) of the relevant CDRs of the VH or VL domain of binding members described herein will be either identical or highly similar to the specified regions of which the sequence is set out herein. As described herein, the phrase 'highly similar' with respect to specified region(s) of one or more variable domains, it is contemplated that from 1 to about 5, e.g. from 1 to 4, including 1 to 3, or 1, 2, 3 or 4, amino acid substitutions may be made in the CDR and/or VH or VL domain.

Brief Description of the Figures

Figure 1. This figure shows a schematic overview of the gB protein (strain AD169; SEQ ID No: 239), indicating the location of known antigenic domains AD-1, AD-2 and AD-3 (Ohlin *et al.*, 1993; Wagner *et al.*, 1992). There is a cleavage site at amino acid 460 and a disulphide bond linking the two moieties together, as indicated by the brackets. Signal: signal sequence (amino acids 1-22), TM: transmembrane domain (amino acids 751-771).

Figure 2. This figure shows gB-specific memory B cell enrichment by preparative fluorescent activated cell sorting (FACS). In order to isolate gB-specific, IgG-positive memory B cells, anti-CD20 MACS-enriched (MACS=magnetic activated cell sorting) B cells were stained with the following antibodies: a. Anti-human CD19 (B cell marker); b. Anti-human CD27 (memory B cell marker); c. Anti-human IgG. Additionally, B cells were incubated with a recombinant glycoprotein B labelled with a fluorescent dye. CD19+/CD27+/IgG+/gB+

reactive B cells were sorted onto irradiated feeder cells and antibody-producing cell lines were established as described in Example 1.

Figure 3. Domain architecture of HCMV gB. The regions representing individual domains are displayed in different shades and the numbers of the starting residues are given. Brackets indicate disulfide bonds. Signal: signal sequence, TM: transmembrane domain.

Figure 4. Competition ELISA between anti-hCMV antibodies and Ab-50. Figure 4a shows Ab-50 in competition with antibodies Ab-47, Ab-48, Ab-49 and C23 (gB specific control antibody). Competition for binding to gB protein is observed for Ab-50 vs Ab-47 and Ab-49, but not for Ab-48 (or C23 control antibody). Figure 4b shows Ab-50 in competition with antibody ITC52, ITC88, 89-104 and 89-109. No competition for binding is observed between Ab-50 and any of these tested antibodies. The dashed line across the top part of graphs 4a and 4b indicates Ab-50 at a concentration of 0.5ng/well.

Figure 5. Antibody titers in human sera against gB and gB fragments. Eighty randomly selected sera from HCMV seropositive individuals were analysed in an ELISA for reactivity against recombinant gB and the antigenic domains 1 (AD-1), 2 (AD-2), 4 (AD-4/Dom II) and 5 (AD-5/Dom I). The horizontal line represents the cut off for the individual antigens.

Figure 6. This figure shows the correlation between antibody titer against the different antigenic regions (as measured by ELISA) and 50% neutralisation titer. r: Spearman rank correlation coefficient.

Figure 7. Specificity and neutralisation capacity of affinity purified anti-AD-4 polyclonal antibodies.
a) ELISA plates were coated with gB, AD-1, AD-2 and AD-4 respectively, and tested with various antibodies. E3: affinity purified IgG fraction; Pre: serum pool before affinity purification; Post: serum pool after affinity purification; C23: human AD-2-specific monoclonal antibody; 89-104: human anti-AD-1 specific monoclonal antibody; anti-GST: murine monoclonal antibody specific for GST.
b) Neutralising assay using the serum pool, the human monoclonal antibody C23 and the affinity purified AD-4 specific IgG fraction (E3).

Figure 8. This figure shows the amino acid sequence of AD-4 and mutant proteins. The amino acid sequence of AD-4 as used for the mammalian cell expression is shown in the top lane. Residues that were exchanged to alanine are indicated in lanes A-Q. Dashes indicate identity with the wild type sequence.

Figure 9. Recognition of AD-4 and AD-4 mutant proteins by human monoclonal antibodies (Ab-28, Ab-11, Ab-14) and affinity purified IgG from hCMV seropositive donors.
ELISA plates were coated with the indicated AD-4 fusion proteins and used to analyse binding of human monoclonal antibodies (Ab-28, Ab-11, Ab-14; Figure 9a) or the affinity purified IgG fraction (E3; Figure 9b). An anti-GST antibody was used to control for coating efficiency of the antigens.

Figure 10. Recognition of AD-4 mutants by human sera. The serum panel (80 specimens) was tested in an ELISA against GST fusion proteins containing AD-4 or the mutant peptides AD-4G

(K378K379), AD-4H (Q380E381), AD-4E (E359D362), AD-4I (N383S385) and AD-4L (N405T406), respectively. For each serum a ratio was calculated between the highest and lowest OD value and plotted as fold difference. The dotted line represents the mean difference of all serum samples.

Figure 11. This figure shows the results of a capture-ELISA determining antibody recognition of gB protein antigenic domains and subdomains. 293T cells were transfected with Control (pcUL132SigHA), AD-4 + AD-5, Subdomain 1 of AD-5 (AD-5-S1) or Subdomain 2 of AD-5 (AD-5-S2). Antibodies Ab-47 to Ab-50 were used for detection. Results were detected by indirect immunofluorescence.

Figure 12. Competitive neutralisation assay with the neutralising antibody Ab-28 and different human sera. The data on the left side of the graph represent triplicates of different hCMV-positive sera and hCMV-negative sera (on the x-axis) applied at a constant concentration around their 50% neutralising activity. The curves on the right side of the graph represent a combination of titrated Ab-28 with hCMV-positive or hMCV-negative sera added at a constant concentration identical with that concentration represented by the data on the left side of the diagram. All curves reflect the IgG-concentration of Ab-28 alone without adding the constant IgG-concentration of the sera. All samples were analysed in triplicate. Legend: ● constant concentration of hCMV-negative serum; ▲ constant concentration of hCMV-positive serum; ■ constant concentration of Intratec®; ○ titrated Ab-28 with a constant concentration of hCMV-negative serum; ▽ titrated Ab-28 with a constant concentration of hCMV-positive serum; □ titrated Ab-28 with a constant concentration of Intratect; ◇ titrated Ab-28 alone.

Figure 13. Post-adsorption assay with Ab-02, Ab-04 and Ab-28. hCMV virus was allowed to adsorb but not to penetrate into human foreskin fibroblasts for 1h at 4°C and then antibodies Ab-02, Ab-04 or Ab-28 were added and allowed to incubate for a period of either 30, 80 or 120mins at 4°C. 1:2 serial dilutions from 150 to 5µg/ml were performed for each antibody in triplicate. C23 (AD-2 specific antibody) was used as a control antibody for inhibition of virus penetration into cells. The x-axis shows IgG concentration (µg/ml) and the y-axis shows %-neutralisation. Legend: ● 30min incubation period; ■ 80min incubation period; ▲ 120min incubation period.

Figure 14. Representative competitive neutralisation assay between AD-1 and AD-2-specific antibodies and Ab-28.
As shown in Figures 12a and 12b, ITC52 (an AD-1 specific antibody) or ITC88 (an AD-2 specific antibody) were titrated in the absence (●) or presence (■) of Ab-28, respectively. Ab-28 was added to the titrated antibody at a constant concentration of 0.5µg/ml (▲). Figure 12c shows ITC52 titrated in the absence (●) or presence (■) of ITC88, which was added to the titrated antibody at a constant concentration of 3µg/ml (▲). All results represent triplicate analysis.

Figure 15. Competitive neutralisation assay between AD-4-specific and AD-5-specific antibodies.
For this assay one representative AD-4- (Dom II) specific antibody (Ab-28) and two representative AD-5- (Dom I) specific antibodies (Ab-49 and Ab-50) were used. The x-axis shows IgG concentration (µg/ml) and the y-axis shows %-neutralisation. Legend: ● AD-5 antibody (Ab-49 or Ab-50)

alone; ▲ AD-4 antibody alone; ■ AD-5 and AD-4 antibodies mixed. The starting concentration of the mixture was 3µg/ml, as 1.5µg/ml of each antibody was applied in the first well.

Detailed Description of the Invention

As noted above, a binding member in accordance with the present invention modulates and may neutralise a biological activity of hCMV. A high potency binding member may be obtained directly from an initial screen e.g. a biological hCMV neutralisation assay. Assays and potencies are described in more detail elsewhere herein.

Epstein-Barr Virus (EBV) transformation is a reliable method to immortalize mammalian cells and numerous EBV transformation protocols have been developed (Rosen *et al.*, 1977; Steinitz *et al.*, 1977; Steinitz *et al.*, 1980; Kozbor & Roder, 1981; Lundgren *et al.*, 1983; Rosen *et al.*, 1983; Steinitz *et al.*, 1984; Lanzavecchia, 1985; Bernasconi *et al.*, 2002; Jung *et al.*, 2002; Traggiai *et al.*, 2004). The technique is most often used to obtain cell lines from human lymphocytes that serve as a permanent source for DNA and protein isolation and has found widespread use in clinical trials as the principal method of generating a permanent source of patient DNA for genotyping. EBV is a Herpes virus and its genome consists of a 172kb linear double stranded DNA which has been completely sequenced. EBV molecular biology and pathogenesis are extensively studied and the roles of many crucial EBV and host cell genes in pathogenesis are known. EBV infects only certain mammalian epithelial cells and B lymphocytes. *In vitro* EBV immortalizes B cells by activating a number of cell cycle regulating genes as well as B cell specific genes including immunoglobulin genes. Growing clones secreting specific antibodies can then be selected for

analysis. The antibodies of interest can then be cloned and their sequence determined by conventional methods.

An antibody V_H variable domain with the amino acid sequence of a said selected binding member may be provided in isolated form, as may a binding member comprising such a V_H domain.

The ability to bind hCMV may be further tested, also the ability to compete with e.g. any antibody molecule of Ab-01 to Ab-50 of the present invention (e.g. in scFv format and/or IgG format, e.g. IgG_1) for binding to hCMV. Ability to neutralise hCMV may be tested, as discussed further elsewhere herein.

Binding affinity and neutralisation potency of different binding members can be compared under appropriate conditions.

Variants of the V_H and V_L domains and CDRs of the present invention, including those for which amino acid sequences are set out herein, and which can be employed in binding members of the invention can be obtained by means of methods of sequence alteration or mutation and screening for antigen binding members with desired characteristics. Examples of desired characteristics include but are not limited to:

- Increased binding affinity for antigen relative to known antibodies which are specific for the antigen
- Increased neutralisation of an antigen activity relative to known antibodies which are specific for the antigen if the activity is known
- Specified competitive ability with a known antibody or ligand to the antigen at a specific molar ratio
- Ability to immunoprecipitate complex

• Ability to bind to a specified epitope such as a linear epitope, e.g. using peptides screened in linear and/or constrained conformation or conformational epitope, formed by non-continuous residues

• Ability to modulate a new biological activity of hCMV, or a downstream molecule. Such methods are also provided herein.

An antibody antigen-binding site composed of a V_H domain and a V_L domain is typically formed by six loops of polypeptide: three from the light chain variable domain (V_L) and three from the heavy chain variable domain (V_H). Analysis of antibodies of known atomic structure has elucidated relationships between the sequence and three-dimensional structure of antibody combining sites. These relationships imply that, except for the third region (loop) in V_H domains, binding site loops have one of a small number of main-chain conformations or canonical structures. The canonical structure formed in a particular loop has been shown to be determined by its size and the presence of certain residues at key sites in both the loop and in framework regions (Chothia *et al.*, 1992; Al-Lazikani *et al.*, 1997).

This study of sequence-structure relationship can be used for prediction of those residues in an antibody of known sequence, but of an unknown three-dimensional structure, which are important in maintaining the three-dimensional structure of its CDR loops and hence maintain binding specificity. In a structural approach, a model can be created of the antibody molecule (Chothia *et al.*, 1986) using any freely available or commercial package, such as WAM (Whitelegg & Rees, 2000). A protein visualisation and analysis software package, such as Insight II (Accelrys, Inc.) or Deep View (Guex & Peitsch, 1997) may then be used to

evaluate possible substitutions at each position in the CDR. This information may then be used to make substitutions likely to have a minimal or beneficial effect on activity.

The techniques required to make substitutions within amino acid sequences of CDRs, antibody V_H or V_L domains and binding members generally are available in the art. Variant sequences may be made, with substitutions that may or may not be predicted to have a minimal or beneficial effect on activity, and tested for ability to bind and/or neutralise hCMV and/or for any other desired property.

Variable domain amino acid sequence variants of any of the V_H and V_L domains whose sequences are specifically disclosed herein may be employed in accordance with the present invention, as discussed.

A further aspect of the invention is an antibody molecule comprising a V_H domain that has at least 60, 70, 80, 85, 90, 95, 98 or 99 % amino acid sequence identity with a V_H domain of any of antibodies Ab-01 to Ab-50 shown in the appended sequence listing, and/or comprising a V_L domain that has at least 60, 70, 80, 85, 90, 95, 98 or 99 % amino acid sequence identity with a V_L domain of any of antibodies Ab-01 to Ab-50 shown in the appended sequence listing. Algorithms that can be used to calculate % identity of two amino acid sequences include e.g. BLAST (Altschul *et al.*, 1990), FASTA (Pearson & Lipman, 1988), or the Smith-Waterman algorithm (Smith & Waterman, 1981), e.g. employing default parameters. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue).

Alterations may be made in one or more framework regions and/or one or more CDRs. The alterations normally do not result in loss of function, so a binding member comprising a thus-altered amino acid sequence may retain an ability to bind and/or neutralise hCMV. It may retain the same quantitative binding and/or neutralising ability as a binding member in which the alteration is not made, e.g. as measured in an assay described herein. The binding member comprising a thus-altered amino acid sequence may have an improved ability to bind and/or neutralise hCMV infectivity.

Alteration may comprise replacing one or more amino acid residue with a non-naturally occurring or non-standard amino acid, modifying one or more amino acid residue into a non-naturally occurring or non-standard form, or inserting one or more non- naturally occurring or non-standard amino acid into the sequence. Examples of numbers and locations of alterations in sequences of the invention are described elsewhere herein. Naturally occurring amino acids include the 20 'standard' L-amino acids identified as G, A, V, L, I, M, P, F, W, S, T, N, Q, Y, C, K, R, H, D, E by their standard single-letter codes. Non-standard amino acids include any other residue that may be incorporated into a polypeptide backbone or result from modification of an existing amino acid residue. Non-standard amino acids may be naturally occurring or non-naturally occurring. Several naturally occurring non-standard amino acids are known in the art, such as 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine, N-acetylserine (Voet & Voet, 2004). Those amino acid residues that are derivatised at their N-alpha position will only be located at the N-terminus of an amino-acid sequence. Normally in the present invention an amino acid is an L-amino acid, but it may be a D-amino acid. Alteration may therefore

comprise modifying an L-amino acid into, or replacing it with, a D-amino acid. Methylated, acetylated and/or phosphorylated forms of amino acids are also known, and amino acids in the present invention may be subject to such modification.

Amino acid sequences in antibody domains and binding members of the invention may comprise non-natural or non-standard amino acids described above. Non-standard amino acids (e.g. D-amino acids) may be incorporated into an amino acid sequence during synthesis, or by modification or replacement of the 'original' standard amino acids after synthesis of the amino acid sequence.

Use of non-standard and/or non-naturally occurring amino acids increases structural and functional diversity, and can thus increase the potential for achieving desired hCMV-binding and neutralising properties in a binding member of the invention. Additionally, D-amino acids and analogues have been shown to have different pharmacokinetic profiles compared with standard L-amino acids, owing to *in vivo* degradation of polypeptides having L-amino acids after administration to an animal e.g. a human, meaning that D-amino acids are advantageous for some *in vivo* applications.

Novel V_H or V_L regions carrying CDR-derived sequences of the invention may be generated using random mutagenesis of one or more selected V_H and/or V_L genes to generate mutations within the entire variable domain. Such a technique is described by Gram *et al.*, (1992), who used error-prone PCR. In some embodiments one or two amino acid substitutions are made within an entire variable domain or set of CDRs. Another method that may be used is to direct mutagenesis to CDR

regions of V_H or V_L genes (Barbas *et al.*, 1994; Schier *et al.*, 1996).

All the above-described techniques are known as such in the art and the skilled person will be able to use such techniques to provide binding members of the invention using routine methodology in the art.

A further aspect of the invention provides a method for obtaining an antibody antigen-binding site for hCMV, the method comprising providing by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a V_H domain set out herein a V_H domain which is an amino acid sequence variant of the V_H domain, optionally combining the V_H domain thus provided with one or more V_L domains, and testing the V_H domain or V_H/V_L combination or combinations to identify a binding member or an antibody antigen-binding site for hCMV and optionally with one or more desired properties, e.g. ability to neutralise hCMV activity. Said V_L domain may have an amino acid sequence which is substantially as set out herein. An analogous method may be employed in which one or more sequence variants of a V_L domain disclosed herein are combined with one or more V_H domains. As noted above, a CDR amino acid sequence substantially as set out herein may be carried as a CDR in a human antibody variable domain or a substantial portion thereof. The HCDR3 sequences substantially as set out herein represent embodiments of the present invention and each of these may be carried as a HCDR3 in a human heavy chain variable domain or a substantial portion thereof.

Variable domains employed in the invention may be obtained or derived from any germline or rearranged human variable

domain, or may be a synthetic variable domain based on consensus or actual sequences of known human variable domains. A variable domain can be derived from a non-human antibody. A CDR sequence of the invention (e.g. CDR3) may be introduced into a repertoire of variable domains lacking a CDR (e.g. CDR3), using recombinant DNA technology. For example, Marks *et al.*, (1992) describe methods of producing repertoires of antibody variable domains in which consensus primers directed at or adjacent to the 5' end of the variable domain area are used in conjunction with consensus primers to the third framework region of human VH genes to provide a repertoire of V_H variable domains lacking a CDR3. Marks *et al.* further describe how this repertoire may be combined with a CDR3 of a particular antibody. Using analogous techniques, the CDR3- derived sequences of the present invention may be shuffled with repertoires of V_H or V_L domains lacking a CDR3, and the shuffled complete V_H or V_L domains combined with a cognate V_L or V_H domain to provide binding members of the invention. The repertoire may then be displayed in a suitable host system, such as a phage display, yeast display, bacterial display, T7 display, viral display, cell display, ribosome display or covalent display system.

Similarly, one or more, or all three CDRs may be grafted into a repertoire of V_H or V_L domains that are then screened for a binding member or binding members for hCMV.

For example, one or more of antibody Ab-01 to Ab-50 HCDR1, HCDR2 and HCDR3 or set of HCDRs may be employed, and/or one or more of antibody Ab-01 to Ab-50 LCDR1, LCDR2 and LCDR3 or set of LCDRs may be employed. Similarly, other V_H and V_L domains, sets of CDRs and sets of HCDRs and/or sets of LCDRs disclosed herein may be employed.

A substantial portion of an immunoglobulin variable domain may comprise at least the three CDR regions, together with their intervening framework regions. The portion may also include at least about 50% of either or both of the first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of binding members of the present invention made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps.

Other manipulation steps include the introduction of linkers to join variable domains of the invention to further protein sequences including antibody constant regions, other variable domains or detectable/functional labels as discussed in more detail elsewhere herein.

Although in some aspects of the invention, binding members comprise a pair of V_H and V_L domains, single binding domains based on either V_H or V_L domain sequences form further aspects of the invention. It is known that single immunoglobulin domains, especially V_H domains, are capable of binding target antigens in a specific manner. In the case of either of the single binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain binding member able to bind hCMV. This may be achieved by phage display screening methods using the so-called

hierarchical dual combinatorial approach as disclosed in WO92/01047 (McCafferty *et al*) and in Marks *et al.*, ibid.

Binding members of the present invention may further comprise antibody constant regions or parts thereof, e.g. human antibody constant regions or parts thereof. For example, a V_L domain may be attached at its C-terminal end to antibody light chain constant domains including human Cκ or Cλ chains. Similarly, a binding member based on a V_H domain may be attached at its C-terminal end to all or part (e.g. a C_H1 domain) of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, IgE and IgM and any of the isotype sub-classes, particularly IgG_1 and IgG_4. IgG_1 is advantageous, due to its effector function and ease of manufacture. Any synthetic or other constant region variant that has these properties and stabilizes variable regions may also be useful in the present invention.

Binding members of the invention may also comprise more than a pair of V_H and V_L domains, such as a bispecific or multispecific antibody, which forms a further aspect of the invention. In the case of a bispecific antibody, having two pairs of V_H and V_L domains, one of the pairs of V_H and V_L domains may be from any of antibodies Ab-01 to Ab-50 as described in the present invention. The second pair of V_H and V_L domains may be the same as the first pair or may be different. For example, the V_H and V_L domain pair may be selected from any of antibodies Ab-01 to Ab-50 or from a different antibody. In a preferred embodiment, a first V_H and V_L domain pair is selected from any of antibodies Ab-01 to Ab-50 and the second V_H and V_L domain pair is also selected from antibodies Ab-01 to Ab-50, but is different to the first domain pair, such that the bispecific antibody binds to hMCV.

Furthermore, a first V_H and V_L domain pair is selected from any of antibodies Ab-01 to Ab-50 and the second V_H and V_L domain pair is selected from a different antibody. The bispecific antibody may therefore bind to hCMV and to a different antigen or to a different epitope on hCMV. Preferably, the bispecific antibody comprises a first V_H and V_L domain pair that binds to AD-4 or AD-5 of hCMV (i.e. a first V_H and V_L domain pair from any of antibodies Ab-01 to Ab-50) and a second V_H and V_L domain pair selected from the hCMV binding antibodies described in the following: US5,043,281 (Mashuho *et al*), US5,750,106 (Ostberg), WO93/021952 A1 (Borrebaeck *et al*), WO08/084410 A2, WO10/007463 A1 and WO10/007533 A2 (Lanzavecchia & Macagno), WO08/071806 A1, WO09/003975 A1 and WO09/024445 A1 (Funaro *et al*), WO09/114560 A2 (Olsen), WO10/114105 A1 and WO10/114106 A1 (Takada *et al*).

Mixtures of antibodies such as mixtures of recombinant human monoclonal antibodies known in the art as Oligoclonics™ (Merus Biopharmaceutical BV; WO 04/106375) may be generated for use in neutralisation of hMCV. These mixtures may comprise binding members derived from any of antibodies Ab-01 to Ab-50. The mixture of antibodies may also comprise binding members from any of antibodies Ab-01 to Ab-50 in combination with a binding member for hCMV that recognises a different antigenic domain on gB protein such as AD-1 or AD-2, or recognises gH or recognises the hCMV proteins gpUL130, gpUL131A, gp128 etc. For example, the antibody mixture may comprise a V_L domain from any of antibodies Ab-01 to Ab-50 and a V_H domain selected from any of antibodies Ab-01 to Ab-50 and/or a V_H domain selected from any of the hCMV binding antibodies described in the following: US5,043,281 (Mashuho *et al*), US5,750,106 (Ostberg), WO93/021952 A1 (Borrebaeck *et*

al), WO08/084410 A2, WO10/007463 A1 and WO10/007533 A2 (Lanzavecchia & Macagno), WO08/071806 A1, WO09/003975 A1 and WO09/024445 A1 (Funaro *et al*), WO09/114560 A2 (Olsen), WO10/114105 A1 and WO10/114106 A1 (Takada *et al*). In the alternative, the antibody mixture may comprise a V_L domain selected from any of the hCMV binding antibodies described in the following: US5,043,281 (Mashuho *et al*), US5,750,106 (Ostberg), WO93/021952 A1 (Borrebaeck *et al*), WO08/084410 A2, WO10/007463 A1 and WO10/007533 A2 (Lanzavecchia & Macagno), WO08/071806 A1, WO09/003975 A1 and WO09/024445 A1 (Funaro *et al*), WO09/114560 A2 (Olsen), WO10/114105 A1 and WO10/114106 A1 (Takada *et al*), together with a V_H domain selected from any of the hCMV binding antibodies described in the afore mentioned list and/or a V_H domain selected from any of antibodies Ab-01 to Ab-50.

Binding members of the present invention may also include antibodies or fragments comprising a modified Fc region, wherein the modified Fc region comprises at least one amino acid modification relative to a wild-type Fc region. The variant Fc region may be designed, relative to a comparable molecule comprising the wild-type Fc region, so as to bind Fc receptors with a greater or lesser affinity. Fc region refers to naturally occurring or synthetic polypeptides homologous to the IgG C-terminal domain that is produced upon papain digestion of IgG. IgG Fc has a molecular weight of approximately 50kD. For antibodies and/or fragments of the present invention, an entire Fc region can be used, or only a half-life enhancing portion.

The Fc region can be mutated, if desired, to inhibit its ability to fix complement and bind the Fc receptor with high affinity. In the present invention, antibodies or fragments

may be provided with a modified Fc region where a naturally occurring Fc region is modified to increase the half-life of the antibody or fragment in a biological environment, for example, the serum half-life or a half-life measured by an *in vitro* assay. Methods for altering the original form of a Fc region of an IgG also are described in US6,998,253 (Presta & Snedecor). Effector functions that can be altered (e.g., enhanced) by making modifications to the Fc region, either by modifying glycosylation patterns or by modifying the amino acid sequence of the Fc region, include but are not limited to: increased Fc-mediated cellular cytotoxicity including increased antibody-dependent cellular cytotoxicity and increased complement-mediated lysis (e.g., of hCMV-infected cells), increased binding of antibody to Fc receptors, natural killer (NK) cells, macrophages, monocytes, and/or polymorphonuclear cells; increased dendritic cell maturation, and increased priming of T cells. Potential modifications include insertion, deletion or substitution of one or more amino acid residues, including substitution with alanine, a conservative substitution, a non-conservative substitution, or replacement with a corresponding amino acid residue at the same position from a different IgG subclass (e.g. replacing an IgG_1 residue with a corresponding IgG_2 residue at that position).

In other embodiments, an Fc polypeptide variant of the present invention may comprise one or more engineered glycoforms i.e. a carbohydrate composition that is covalently attached to a molecule comprising an Fc region. The Fc region of IgG-type antibodies contains a conserved N-linked glycosylation site at residue Asn297 of the CH2 domain. It has been shown that modification of the glycosylation pattern of oligosaccharides linked to this residue can increase

effector functions mediated by the Fc region in interactions with Fc receptors. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example β (1,4) - N- acetylglucosaminyl transferase III, by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed.

Methods for generating engineered glycoforms are known in the art, and include but are not limited to, those described in US6,602,684 (Umaña *et al*); US20030157108 (Presta *et al*); Umaña *et al*., (1999); Davies *et al*., (2001); Shields *et al*., (2002); Shinkawa *et al*., (2003); and patents and applications relating to Potelligent™ technology (Biowa, Inc., Princeton, NJ, U.S.) and GlycoMAb™ glycosylation engineering technology (GLYCART Biotechnology AG, Schlieren, CH).

Thus, in a further aspect, the present invention encompasses an hCMV binding member as described elsewhere herein, wherein said binding member comprises an Fc region or an equivalent region that comprises at least an IgG CH2 region, that has been modified to increase one or more effector functions. In one embodiment, the binding member is modified to alter the glycosylation pattern of the N-linked oligosaccharides at Asn 297 such that the activity of one or more effector functions is increased. In another embodiment, the binding member is modified to alter the amino acid sequence of the Fc region such that the activity of one or more effector functions is increased. Methods of measuring effector function activity

and determining whether or not they are increased are well known in the art.

Binding members of the invention may be labelled with a detectable or functional label. Thus, a binding member or antibody molecule can be present in the form of an immunoconjugate so as to obtain a detectable and/or quantifiable signal. An immunoconjugate may comprise an antibody molecule of the invention, for example any of antibodies Ab-01 to Ab-50, conjugated with detectable or functional label. A label can be any molecule that produces or can be induced to produce a signal, including but not limited to fluorochromes, radiolabels, enzymes, chemiluminescers or photosensitizers. Thus, binding may be detected and/or measured by detecting fluorescence or luminescence, radioactivity, enzyme activity or light absorbance.

Suitable labels include, by way of illustration and not limitation, enzymes, such as alkaline phosphatase, glucose-6-phosphate dehydrogenase ('G6PDH'), alpha-D-galactosidase, glucose oxydase, glucose amylase, carbonic anhydrase, acetylcholinesterase, lysozyme, malate dehydrogenase and peroxidises, e.g. horseradish peroxidase; dyes; fluorescent labels or fluorochromes, such as fluorescein and its derivatives, rhodamine compounds and derivatives, green/yellow fluorescent protein (G/YFP), red fluorescent protein (RFP), blue fluorescent protein (BFP), dansyl, umbelliferone, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, and fluorescamine; fluorophores such as lanthanide cryptates and chelates e.g. Europium etc (Perkin Elmer and Cis Biointernational), chemoluminescent labels or chemiluminescers, such as isoluminol, luminol and the

dioxetanes; bio-luminescent labels, such as luciferase and luciferin; sensitizers; coenzymes; enzyme substrates; radiolabels including but not limited to bromine77, carbon14, cobalt57, fluorine8, gallium67, gallium 68, hydrogen3 (tritium), indium111, indium113m, iodine123m, iodine125, iodine126, iodine131, iodine133, mercury107, mercury203, phosphorous32, rhenium99m, rhenium101, rhenium105, ruthenium95, ruthenium97, ruthenium103 , ruthenium105, scandium47, selenium75, sulphur35, technetium99, technetium99m, tellurium121m, tellurium122m, tellurium125m, thulium165, thulium167, thulium168, yttrium199 and other radiolabels mentioned herein; particles, such as latex or carbon particles; metal sol; crystallite; liposomes; cells, etc., which may be further labelled with a dye, catalyst or other detectable group; molecules such as biotin, digoxygenin or 5-bromodeoxyuridine; toxin moieties, such as for example a toxin moiety selected from a group of Pseudomonas exotoxin (PE or a cytotoxic fragment or mutant thereof), Diptheria toxin or a cytotoxic fragment or mutant thereof, a botulinum toxin A, B, C, D, E or F, ricin or a cytotoxic fragment thereof e.g. ricin A, abrin or a cytotoxic fragment thereof, saporin or a cytotoxic fragment thereof, pokeweed antiviral toxin or a cytotoxic fragment thereof and bryodin 1 or a cytotoxic fragment thereof.

Suitable enzymes and coenzymes are disclosed in US4,275,149 (Litman *et al*) and US4,318,980 (Boguslaski *et al*) and suitable fluorescers and chemiluminescers are disclosed in US4,275,149, which are incorporated herein by reference in their entirety. Labels further include chemical moieties, such as biotin that may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin or streptavidin, or genetically engineered streptavidin, like

streptactin (IBA GmbH, Göttingen, DE). Detectable labels may be attached to antibodies of the invention using conventional chemistry known in the art.

Immunoconjugates or their functional fragments can be prepared by methods known to the person skilled in the art. They can be coupled to enzymes or to fluorescent labels directly or by the intermediary of a spacer group or of a linking group, such as a polyaldehyde, like glutaraldehyde, ethylenediaminetetraacetic acid (EDTA), diethylene-triaminepentaacetic acid (DPTA), or in the presence of coupling agents, such as those mentioned above for the therapeutic conjugates. Conjugates containing labels of fluorescein type can be prepared by reaction with an isothiocyanate.

The methods known to the person skilled in the art existing for coupling the therapeutic radioisotopes to the antibodies either directly or via a chelating agent, such as EDTA, DTPA, mentioned above, can be used for the radio-elements which can be used in diagnosis. It is likewise possible to perform labelling with sodiuml25 by the chloramine T method (Hunter & Greenwood, 1962) or else with technetium-99m (Tc-99m) by the technique described in US4,424,200 (Crockford & Rhodes) or attached via DTPA as described in US4,479,930 (Hnatowich), both of which are herein incorporated by reference in their entirety.

There are numerous methods by which the label can produce a signal detectable by external means, for example, by visual examination, electromagnetic radiation, heat and chemical reagents. The label can also be bound to another binding

member that binds the binding member of the invention, or to a support.

The label can directly produce a signal, and therefore, additional components are not required to produce a signal. Numerous organic molecules, for example fluorescers, are able to absorb ultraviolet and visible light, where the light absorption transfers energy to these molecules and elevates them to an excited energy state. This absorbed energy is then dissipated by emission of light at a second wavelength. This second wavelength emission may also transfer energy to a labelled acceptor molecule, and the resultant energy dissipated from the acceptor molecule by emission of light for example fluorescence resonance energy transfer (FRET). Other labels that directly produce a signal include radioactive isotopes and dyes.

Alternately, the label may need other components to produce a signal, and the signal producing system would then include all the components required to produce a measurable signal, which may include substrates, coenzymes, enhancers, additional enzymes, substances that react with enzymatic products, catalysts, activators, co-factors, inhibitors, scavengers, metal ions, and a specific binding substance required for binding of signal generating substances. A detailed discussion of suitable signal producing systems can be found in US5,185,243 (Ullman *et al*). The present invention provides a method comprising causing or allowing binding of a binding member as provided herein specific for hCMV. As noted, such binding may take place *in vivo*, e.g. following administration of a binding member, or nucleic acid encoding a binding member, or it may take place *in vitro*, for example in ELISA, Western blotting, affinity chromatography,

immunocytochemistry, immunoprecipitation, neutralisation and biochemical or cell-based assays.

The present invention also provides methods for measuring levels of antigen directly, by employing a binding member according to the invention, e.g. in a biosensor system. For instance, the present invention comprises a method of detecting and/or measuring binding to hCMV, comprising, (i) exposing said binding member to hCMV and (ii) detecting binding of said binding member to hCMV, wherein binding is detected using any method or detectable label described herein. This, and any other binding detection method described herein, may be interpreted directly by the person performing the method, for instance, by visually observing a detectable label. Alternatively, this method, or any other binding detection method described herein, may produce a report in the form of an autoradiograph, a photograph, a computer printout, a flow cytometry report, a graph, a chart, a test tube or container or well containing the result, or any other visual or physical representation of a result of the method.

The amount of binding of a binding member to hCMV may be determined. Quantitation may be related to the amount of the antigen in a test sample, which may be of diagnostic interest. Screening for hCMV binding and/or the quantitation thereof may be useful, for instance, in screening patients for diseases or disorders referred to herein and/or any other disease or disorder involving aberrant hCMV expression and/or activity.

A diagnostic method of the invention may comprise (i) obtaining a tissue or fluid sample from a subject, (ii)

exposing said tissue or fluid sample to one or more binding members of the present invention; and (iii) detecting bound hCMV as compared with a control sample, wherein an increase in the amount of hCMV binding as compared with the control may indicate hCMV expression and/or activity. Tissue or fluid samples to be tested include blood, serum, saliva, urine, sputum, a biopsy material or any tissue suspected of containing hCMV. Subjects testing positive for hCMV may also benefit from the treatment methods disclosed later herein. Those skilled in the art are able to choose a suitable mode of determining binding of the binding member to an antigen according to their preference and general knowledge, in light of the methods disclosed herein.

The reactivities of binding members in a sample may be determined by any appropriate means. A competitive binding assay may be used with radioactive antigen, for example an isotope label such as ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, ^{3}H, ^{32}P or ^{35}S, or nonradioactive antigen using antigen or an analogue linked to a reporter molecule. The reporter molecule may be a fluorochrome, phosphor or laser dye with spectrally isolated absorption or emission characteristics. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red, and lanthanide chelates or cryptates. Suitable chromogenic dyes include diaminobenzidine.

Other reporters include macromolecular colloidal particles or particulate material, such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes, which catalyze reactions that develop, or change

colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase or horseradish peroxidase detection systems may be employed.

The signals generated by individual binding member-reporter conjugates may be used to derive quantifiable absolute or relative data of the relevant binding member binding in samples (normal and test).

A kit comprising a binding member according to any aspect or embodiment of the present invention is also provided. In the kit, the binding member may be labelled to allow its reactivity in a sample to be determined, e.g. as described further below. Further the binding member may or may not be attached to a solid support. Components of a kit are generally sterile and in sealed vials or other containers. Kits may be employed in diagnostic analyses or other methods for which binding members are useful. A kit may contain instructions for use of the components in a method, e.g. a method in accordance with the present invention. Ancillary materials to assist in or to enable performing such a method may be included within a kit of the invention. The ancillary materials include a second, different binding member, which binds to the first binding member and is conjugated to a detectable label (e.g., a fluorescent label, radioactive isotope or enzyme). Antibody-based kits may also comprise beads for conducting immunoprecipitation. Each component of the kits is generally in its own suitable container. Thus,

these kits generally comprise distinct containers suitable for each binding member. Further, the kits may comprise instructions for performing the assay and methods for interpreting and analysing the data resulting from the performance of the assay.

The present invention also provides the use of a binding member as above for measuring antigen levels in a competition assay, that is to say a method of measuring the level of antigen in a sample by employing a binding member as provided by the present invention in a competition assay. This may be where the physical separation of bound from unbound antigen is not required. Linking a reporter molecule to the binding member so that a physical or optical change occurs on binding is one possibility. The reporter molecule may directly or indirectly generate detectable signals, which may be quantifiable. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule.

In various aspects and embodiments, the present invention extends to a binding member that competes for binding to hCMV with any binding member defined herein, e.g. any of antibodies Ab-01 to Ab-50, e.g. in IgG format. Competition between binding members may be assayed *in vitro*, for example by tagging a specific reporter molecule to one binding member which can be detected in the presence of other untagged binding member(s), to enable identification of binding members which bind the same epitope or an overlapping epitope. Competition may be determined for example using ELISA or by surface plasmon resonance, in which hCMV is

immobilized to a solid phase and a first tagged or labelled binding member along with one or more other untagged or unlabelled binding members is added to the solid phase. Presence of an untagged binding member that competes with the tagged binding member is observed by a decrease in the signal emitted by the tagged binding member.

For example, the present invention includes a method of identifying a hCMV binding compound, comprising (i) immobilising gB protein to a support, (ii) contacting said immobilized gB simultaneously or in a step-wise manner with at least one tagged or labelled binding member according to the invention and one or more untagged or unlabelled test binding compounds, and (iii) identifying a new hCMV binding compound by observing a decrease in the amount of bound tag from the tagged binding member. Such methods can be performed in a high-throughput manner using a multiwell or array format. Such assays may be also be performed in solution. See, for instance, US5,814,468 (Sliman *et al*), which is herein incorporated by reference in its entirety. As described above, detection of binding may be interpreted directly by the person performing the method, for instance, by visually observing a detectable label, or a decrease in the presence thereof. Alternatively, the binding methods of the invention may produce a report in the form of an autoradiograph, a photograph, a computer printout, a flow cytometry report or any other visual or physical representation of a result of the method.

Competition assays can also be used in epitope characterisation. In one instance epitope characterisation may be used to identify the epitope bound by a hCMV binding member which optionally may have optimized neutralising

and/or modulating characteristics. Such an epitope can be linear or conformational. A conformational epitope can comprise at least two different domains of hCMV, wherein said domains are positioned in proximity to each other when hCMV proteins are folded in its tertiary or quaternary structure to form a conformational epitope which is recognised by an inhibitor of hCMV, such as an hCMV-binding member provided in this specification. In testing for competition a peptide fragment of the antigen may be employed, especially a peptide including or consisting of an epitope of interest. A peptide having the epitope sequence plus one or more amino acids at either end may be used. Binding members according to the present invention may be such that their binding for antigen is inhibited by a peptide with or including the sequence given.

The present invention further provides an isolated nucleic acid encoding a binding member of the present invention. Nucleic acid may include DNA and/or RNA. In one, the present invention provides a nucleic acid that codes for a CDR or set of CDRs or V_H domain or V_L domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG_1, of the invention as defined above.

The present invention also provides constructs in the form of plasmids, vectors, transcription or expression cassettes which comprise at least one polynucleotide as above.

The present invention also provides a recombinant host cell that comprises one or more constructs as above. A nucleic acid encoding any CDR or set of CDRs or V_H domain or V_L domain or antibody antigen-binding site or antibody molecule, e.g. scFv or IgG_1 as provided, itself forms an aspect of the

present invention, as does a method of production of the encoded product, which method comprises expression from encoding nucleic acid. Expression may conveniently be achieved by culturing said recombinant host cell containing the nucleic acid under appropriate conditions. Following production by expression a binding member comprising V_H or V_L domain as disclosed herein, the binding member may be isolated and/or purified using any suitable technique known in the art and deemed as appropriate.

Nucleic acid according to the present invention may comprise DNA or RNA and may be wholly or partially synthetic. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

A yet further aspect provides a method of production of a binding member comprising V_H and/or V_L variable domain of the present invention, the method including causing expression from encoding nucleic acid. Such a method may comprise culturing recombinant host cells under conditions for production of said antibody V_H and/or V_L variable domain.

A method of production may comprise a step of isolation and/or purification of the product. A method of production may comprise formulating the product into a composition including at least one additional component, such as a pharmaceutically active excipient.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host

cells include bacteria, mammalian cells, plant cells, filamentous fungi, yeast and insect cells and transgenic plants and animals. The expression of antibodies and antibody fragments in prokaryotic cells is well established in the art. For a review, see for example Pluckthun (1991). A common bacterial host is *E. coli*.

Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a binding member (Chadd & Chamow, 2001; Andersen & Krummen, 2002; Larrick & Thomas, 2001). Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, NS0 mouse melanoma cells, YB2/0 rat myeloma cells, human embryonic kidney (HEK) cells, human embryonic retina cells and many others.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, phagemids, or viral vectors, e.g. retroviral vectors, as appropriate (Sambrook & Russell, 2001). Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, as well as analysis of proteins, are described in detail in Ausubel *et al.*, (1999).

A further aspect of the present invention provides a host cell containing nucleic acid as disclosed herein. Such a host cell may be maintained *in vitro* and may be propagated in

tissue culture. Such a host cell may also be maintained *in vivo*, e.g. in order to produce binding members in ascites. *In vivo* presence of the host cell may allow intra-cellular expression of the binding members of the present invention as 'intrabodies' or intra-cellular antibodies. Intrabodies may be used for gene therapy.

A still further aspect provides a method comprising introducing nucleic acid of the invention into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus, or any combination thereof. Introducing nucleic acid in the host cell, in particular a eukaryotic cell may use a viral or a plasmid based system. The plasmid system may be maintained episomally or may be incorporated into the host cell genome or into an artificial chromosome. Incorporation may be either by random or targeted integration of one or more copies at single or multiple loci. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the binding member. The purification of the expressed product may be achieved by methods known to one of skill in the art.

Nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be

promoted by inclusion of sequences that promote recombination with the genome, in accordance with standard techniques.

The present invention also provides a method that comprises using a construct as stated above in an expression system in order to express a binding member or polypeptide as above.

There is evidence for involvement of hCMV infection in a variety of disorders, as discussed elsewhere herein. The binding members of the present invention may therefore be used in a method of diagnosis or treatment of a disorder associated with hCMV infection. Such a disorder may affect immunocompromised patients such as allograft recipients and HIV infected individuals, and may include for example: fever, hepatitis, retinitis, pneumonitis, myelosuppression, encephalopathy, polyradiculopathy, immunosuppression, rejection/graft-versus-host disease or atherosclerosis. A binding member of the present invention may also be used to treat intra-uterine infection in neonates. Frequently, neonates are born without signs or symptoms of the disorders listed above, but without treatment may develop progressive symptoms of CNS dysfunction and impairment, e.g. but not limited to hearing loss, loss of vision, and/or mental retardation.

Accordingly, the invention provides a method of treating an hCMV infection related disorder, comprising administering to a patient in need thereof an effective amount of one or more binding members of the present invention alone or in a combined therapeutic regimen with another appropriate medicament known in the art or described herein.

Evidence for involvement of hCMV infection in certain disorders is summarised elsewhere herein. In addition, the data presented herein further indicates that binding members of the invention can be used to treat such disorders, including preventative treatment and reduction of severity of the disorders. Accordingly, the invention provides a method of treating or reducing the severity of at least one symptom of any of the disorders mentioned herein, comprising administering to a patient in need thereof an effective amount of one or more binding members of the present invention alone or in a combined therapeutic regimen with another appropriate medicament known in the art or described herein such that the severity of at least one symptom of any of the above disorders is reduced.

Thus, the binding members of the present invention are useful as therapeutic agents in the treatment of diseases or disorders involving hCMV infection and/or activity, especially resulting from high viral load in a patient. A method of treatment may comprise administering an effective amount of a binding member of the invention to a patient in need thereof, wherein aberrant infection and/or activity of hCMV is decreased. A method of treatment may comprise (i) identifying a patient demonstrating hCMV infection levels or activity, for instance using the diagnostic methods described above, and (ii) administering an effective amount of a binding member of the invention to the patient, wherein expression and/or activity of hCMV is decreased. An effective amount according to the invention is an amount that decreases the expression and/or activity of hCMV so as to decrease or lessen the severity of at least one symptom of the hCMV infection or particular disease or disorder being treated, but not necessarily cure the disease or disorder.

The invention also provides a method of antagonising at least one effect of hCMV infection, comprising contacting with or administering an effective amount of one or more binding members of the present invention such that said at least one effect of hCMV infection is antagonised. Accordingly, further aspects of the invention provide methods of treatment comprising administration of a binding member as provided, pharmaceutical compositions comprising such a binding member, and use of such a binding member in the manufacture of a medicament for administration, for example in a method of making a medicament or pharmaceutical composition comprising formulating the binding member with a pharmaceutically active excipient. A pharmaceutically active excipient may be a compound or a combination of compounds entering into a pharmaceutical composition not provoking secondary reactions and which allows, for example, facilitation of the administration of the active compound(s), an increase in its lifespan and/or in its efficacy in the body, an increase in its solubility in solution or else an improvement in its conservation. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the nature and of the mode of administration of the active compound(s) chosen.

Binding members of the present invention will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the binding member. Thus, pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically active excipient, carrier, buffer, stabilizer or other materials well known to those

skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, inhaled, intra-tracheal, topical, intra-vesicular or by injection, as discussed below.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder, liquid or semisolid form. A tablet may comprise a solid carrier, such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier, such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols, such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intra-venous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection.

Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl

parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3'-pentanol; and m-cresol); low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, histidine, arginine, or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose or dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter- ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Binding members of the present invention may be formulated in liquid, semi-solid or solid forms depending on the physicochemical properties of the molecule and the route of delivery. Formulations may include excipients or combinations of excipients, for example: sugars, amino acids and surfactants. Liquid formulations may include a wide range of antibody concentrations and pH. Solid formulations may be produced by lyophilisation, spray drying, or drying by supercritical fluid technology, for example. Formulations of binding members will depend upon the intended route of delivery. A binding member may be prepared with a carrier that will protect the binding member against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known to those skilled in the art (Robinson, 1978).

Treatment may be given orally, or by injection (i.e. subcutaneously, intra-articular, intra-venously, intra-peritoneal, intra-arterial or intra-muscularly), by inhalation, intra-tracheal, by the intra-vesicular route (instillation into the urinary bladder), or topically (for example intra-ocular, intra-nasal, rectal, into wounds, on skin). The treatment may be administered by pulse infusion, particularly with declining doses of the binding member. The route of administration can be determined by the physicochemical characteristics of the treatment, by special considerations for the disease or by the requirement to optimize efficacy or to minimize side-effects. One particular route of administration is intra-venous. Another route of administering pharmaceutical compositions of the present invention is subcutaneously. It is envisaged that treatment will not be restricted to use in the clinic. Therefore, subcutaneous injection using a needle-free device is also advantageous.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

A binding member of the invention may be used as part of a combination therapy in conjunction with an additional medicinal component. Combination treatments may be used to provide significant synergistic effects, particularly the combination of a binding member of the invention with one or more other antibodies such as antibodies Ab-01 to Ab-50 as disclosed herein or any of the hCMV antibodies described in the following publications: US5,043,281 (Mashuho *et al*), US5,750,106 (Ostberg), WO93/021952 A1 (Borrebaeck *et al*),

WO08/084410 A2, WO10/007463 A1 and WO10/007533 A2 (Lanzavecchia & Macagno), WO08/071806 A1, WO09/003975 A1 and WO09/024445 A1 (Funaro *et al*), WO09/114560 A2 (Olsen), WO10/114105 A1 and WO10/114106 A1 (Takada *et al*) or any other drug. A binding member of the invention may be administered concurrently or sequentially or as a combined preparation with another therapeutic agent or agents, for the treatment of one or more of the conditions listed herein.

A binding member of the invention may be used as a chemosensitiser, whereby it can increase therapeutic efficacy of anti-viral agents, and may thus be provided for administration in combination with one or more anti-viral agents, either simultaneously or sequentially.

A binding member according to the present invention may be provided in combination or addition with one or more of the following antiviral agents, e.g. acyclovir, famciclovir, valganciclovir, ganciclovir, cidofovir, amantadine, rimantadine, ribavirin, zanamavir and/or oseltamavir.

A binding member of the invention and one or more of the above additional medicinal components may be used in the manufacture of a medicament. The medicament may be for separate or combined administration to an individual, and accordingly may comprise the binding member and the additional component as a combined preparation or as separate preparations. Separate preparations may be used to facilitate separate and sequential or simultaneous administration, and allow administration of the components by different routes e.g. oral and parenteral administration.

In accordance with the present invention, compositions provided may be administered to mammals. Administration is normally in a 'therapeutically effective amount', this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the type of binding member, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody are well known in the art (Ledermann *et al.*, 1991; Bagshawe *et al.*, 1991). Specific dosages indicated herein or in the Physician's Desk Reference (2009) as appropriate for the type of medicament being administered may be used. A therapeutically effective amount or suitable dose of a binding member of the invention can be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the antibody is for diagnosis, prevention or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody or fragment) and the nature of any detectable label or other molecule attached to the antibody. A typical antibody dose will be in the range 100µg to 1g for systemic applications,

and 1µg to 1mg for topical applications. An initial higher loading dose, followed by one or more lower doses, may be administered. Typically, the antibody will be a whole antibody, e.g. the IgG_1 isotype. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children, infants and neonates, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. Treatments may be every two to four weeks for subcutaneous administration and every four to eight weeks for intra-venous administration. Treatment may be periodic, and the period between administrations is about two weeks or more, e.g. about three weeks or more, about four weeks or more, or about once a month. Treatment may be given before, and/or after transplantation surgery, and/or may be administered or applied directly at the anatomical site of surgical treatment.

hCMV binding members of the invention may offer advantages in terms of dosage and administration requirements, compared with antibodies to hCMV disclosed previously in the art, as discussed above. For example, if the dose of an anti-hCMV therapeutic is lower there may be significant advantages in that the low dose facilitates sub-cutaneous injections as well as intra-venous injections. It is well known to those skilled in the art that sub-cutaneous dosing may be limited by the amount of binding member, e.g. antibody molecule, required per dose. This is due to the sub-cutaneous injections being limited by the volume that can be injected at one site in the skin. Sub-cutaneous injection volumes of 1.2ml or less are typically utilised. As it may be increasingly difficult to formulate a binding member for sub-

cutaneous injection at concentrations greater than 50mg/ml, doses above 100mg via this route usually require multiple injections and more discomfort for the patient. Thus, a lower dose of e.g., a more potent hCMV binding member, is advantageous because it expands the routes of administration.

Examples

Example 1: FACS sorting of hCMV specific memory B cells and EBV transformation of IgG-positive memory B cells

After informed donor consent was obtained, peripheral blood (300ml) was collected from healthy hCMV-seropositive blood donors, whose serum had been pre-screened for high gB-binding titers and efficient hCMV neutralising activity. Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll-density gradient centrifugation (Lymphoflot, Biotest, Dreieich, Germany). After B cell enrichment using anti-human CD22-microbeads (Mitenyi Biotec, Bergisch Gladbach, Germany), B cells were labelled with the following reagents: a. Anti-human CD19-FITC (Miltenyi Biotec, Germany); b. Anti-human CD27-PE (BD Bioscience Pharmigen, Basel, Switzerland); c. anti-human IgG-bio (Jackson Immuno-Research, West Grove, PA, USA); d. streptavidin, Alexa Fluor® 350 conjugate (Molecular Probes Inc, Eugene, OR, USA) and e. Cy5-labeled glycoprotein B (100ng per 1 x 10^6 B cells). gB-specific, IgG-positive memory B cells were isolated by sorting cells that fulfilled the following four criteria: FITC+/PE+/Alexa Fluor® 350+ and Cy5+ (see Figure 2). Alternatively, B cells were labeled with the following reagents: a. Anti-human CD19-PerCP (Dianova, Hamburg, Germany); b. Anti-human CD27-PE (BD Bioscience Pharmigen, Basel, Switzerland); c. anti-human IgG-FITC (Dianova, Hamburg, Germany); d. Cy5-labeled glycoprotein B (100ng per 1 x 10^6 B cells). These gB-specific, IgG-positive memory B cells were either analyzed using FACSCalibur® (Becton

Dickinson, Heidelberg, Germany) or isolated by sorting cells that fulfilled the criteria PerCP+/PE+/FITC+ and Cy5+.

Cells were sorted at a concentration of 5 or 10 cells/well, in 96-well flat-bottom microplates containing a confluent layer of irradiated feeder cells (human foreskin fibroblasts, HFFs), using a MoFlo™ cell sorter (Cytomation, Freiburg, Germany). Sorted cells were grown in complete RPMI-1640 medium supplemented with 2mM glutamine, 100IU/ml penicillin, 100µg/ml streptomycin, 50µM 2-mercaptoethanol and 10% fetal calf serum (heat-inactivated) (PAN-Biotech, Aidenbach, Germany) in the presence of EBV containing cell culture supernatant (30% supernatant of EBV producing cell line B95-8) and CpG ODN 2006 (2,5µg/ml) as previously described (Rosén *et al.*, 1977; Steinitz *et al.*, 1977; Bernasconi *et al.*, 2002; Jung *et al.*, 2002; Traggiai *et al.*, 2004). After three weeks, culture supernatants from the generated cell lines were screened for gB-specificity using enzyme-linked immunosorbent assay (ELISA). In brief, ELISA plates (Nunc) were coated with 0.5µg/ml glycoprotein B in carbonate buffer, pH 9.6, for 16h at 4°C. The gB-coated plates were washed six times with phosphate buffered saline (PBS) supplemented with 0.05% Tween (ELISA washing buffer) and blocked for 2h with PBS supplemented with 0.05% Tween and 2% fetal calf serum (ELISA buffer). 50µl of culture supernatant per well was incubated for 1h at room temperature and, after another washing step, the antibody bound was revealed using Fcγ fragment-specific secondary antibodies coupled with peroxidase (Jackson ImmunoResearch, USA). After a 1h incubation period unbound secondary antibody was removed by washing and the enzymatic activity was determined using 50µl/well o-phenyldiamine at a concentration of 0.04mg/ml in 0.05M phosphate-citrate buffer (pH 5.0), 0.05% H_2O_2. After incubation for 10min at room

temperature, the reaction was stopped by addition of 50µl/well 2M H_2SO_4 and the optical density (OD) was measured at 492nm with a SPECTRAmax™ 190 ELISA photometer (Molecular Devices, Sunnyvale, CA, USA). Software Softmax Pro 3.0 (Molecular Devices, USA) was used for analysis.

Example 2: *In vitro* hCMV neutralisation assay

The gB-specific culture supernatants were screened for neutralising activity using the hCMV recombinant strain AD169 (HB15-UL84prluc) containing a luciferase reporter gene expression cassette in the hCMV genome, and which results in the expression of luciferase enzyme upon infection of target cells (kindly provided by Prof. Dr. Thomas Stamminger, Institute of Clinical and Molecular Virology, University Hospital Erlangen, Germany). The infectious titer in viral supernatants was determined by TCID50 assays in primary fibroblast cells (either HFF or MRC-5 cells) on 96-well plates as described in Mahy & Kangro (1996). For the luciferase-based neutralisation assay, an equal volume of gB-specific culture supernatant and titered AD169ΔLuc supernatant (300pfu) were incubated at 37°C for 1h in 96 U-bottom microplates. The antibody-virus mixtures were transferred onto previously seeded HFF monolayers. After an additional incubation at 37°C for 4h, the antibody-virus mixtures were replaced by complete medium. Following another 42h incubation at 37°C, the cells were lysed with 100µl Glo Lysis Buffer (Promega, Madison, WI) per well. 30µl of each lysed well was placed into white 96-well LIA plates (Greiner Bio-one, Frickenhausen, Germany). Per well, 50µL assay buffer (15mM KH_2PO_4, 25mM glycylglycine, 1M $MgSO_4$, 0.5M EGTA, 5mM ATP, 1mM DTT) was added. Injection of 50µL D-luciferin (P.J.K., Kleinbittersdorf, Germany) solution per well (in 25mM glycylglycine, 1M $MgSO_4$, 0.5M EGTA, 2mM DTT, and 0.05mM D-

Luciferin) and detection of chemiluminescence were performed by a Centro LB 960 Luminometer (Berthold Technologies, Bad Wildbad, Germany). MicroWin2000 Software (Mikrotek Laborsysteme, Overath, Germany) was used for analysis. The relative light units (RLU) measured by the luminometer were expressed in percent neutralisation using the following calculation:

%-neutralisation = 100 x $(V_0 - V_n)/V_0$, where V_n represents the RLU in the wells containing virus and antibody, and V_0 represents the RLU in the wells that contained virus alone. The first screening revealed nine gB-specific culture supernatants that neutralised hCMV infectivity and a second screening revealed three further gB-specific culture supernatants that neutralised hCMV infectivity. The nine EBV-immortalised memory B cell lines and the neutralising antibodies produced by them were named SM1, SM3, SM4, SM5, SM6, SM7, SM9, SM10 and SM11 (see Table 1 below). The three additional EBV-immortalised memory B cell lines and the neutralising antibodies produced by them were named SM12, 2C2 and 1G2 (see Table 1 below).

A 50%-neutralising titre (IC_{50}) is indicated as the concentration of antibody that results in a 50% reduction of hCMV infection. Similarly, a 90%-neutralising titre (IC_{90}) is indicated as the concentration of antibody that results in a 90% reduction of hCMV infection. To calculate the neutralising activities of the antibodies, the IgG-concentrations of the culture supernatants were determined by ELISA. For this purpose, ELISA plates were coated with anti-human IgG, Fcγ fragment-specific catching antibody (Jackson ImmunoResearch, USA). Two-fold serial dilutions of SM-antibody culture supernatants in ELISA buffer were compared to polyclonal IgG standard of known concentration (11.1mg/ml

stock concentration; Cat No: 009-000-003, Jackson ImmunoResearch, USA). The IgG-concentrations of samples were calculated using the ELISA software Softmax Pro 3.0 (Molecular Devices, Sunnyvale, CA, USA). The neutralisation activities relative to IgG concentration of the EBV line cell culture supernatant are shown in Table 1 below:

Table 1: Properties of EBV-immortalised memory B cell lines

EBV line	IgG conc. of supernatant (µg/ml)	No of cells in pellet	50% neutralisation activity (µg/ml)	90% neutralisation activity (µg/ml)
SM1	10.8	3.6×10^5	0.8	2.0
SM3	14.7	3.5×10^5	0.5	1.0
SM4	19.5	6.0×10^5	0.3	0.6
SM5	18.5	3.3×10^5	0.4	0.9
SM6	8.7	2.2×10^5	0.3	1.0
SM7	13.4	3.8×10^5	0.4	1.0
SM9	21.5	1.0×10^6	1.0	9.0
SM10	6.9	5.0×10^5	0.3	1.0
SM10	28.5	n.d.	0.3	1.0
SM11	18.2	7.0×10^5	1.0	6.0
SM12	22.5	n.d.	1.6	8.0
2C2	11.9	n.d.	0.3	1.3
1G2	25.5	n.d.	0.1	0.4

Having determined the IgG-concentrations of the EBV line supernatants, a further neutralisation assay for six of the cell line supernatants was performed; these supernatants showed IC_{50} values between 0.5 and 2.3µg/ml (Table 2; see below). The neutralisation assay was performed as described in the paragraph above with the modification that two-fold serial dilutions of antibody supernatants in complete medium were prepared, in triplicate, prior to the addition of virus.

Table 2: hCMV neutralising activities of gB-specific antibodies produced by EBV-immortalised memory B cell lines

EBV line	**50% Neutralisation activity (µg/ml)**
SM1	1.3
SM3	0.8
SM4	0.6
SM5	0.6
SM6	0.5
SM11	2.3
ITC88*	1.5

Results reflect mean values of three independent assays. Variations between assays were in the range 10-20%.

*ITC88 was used as a positive control (Ohlin *et al.*, 1993).

The EBV-immortalised memory B cell lines producing neutralising antibodies were pelleted and frozen at -80°C, until required for further processing.

Example 3: Cloning of antibody variable regions from anti-hCMV EBV cell lines

In the Examples 3.1 to 3.4 the variable regions of anti-hCMV neutralising antibodies from nine EBV transformed human B cell lines (SM1, 3-7, 9-11) were amplified by semi-nested PCR and cloned in pCDNA3 vectors (Invitrogen) containing the appropriate immunoglobulin constant region. These constructs were subsequently used to transfect CHO cells and the expressed antibodies were tested using suspension array technology and surface plasmon resonance (Biacore®, GE Healthcare)(Example 5) and neutralisation assays (Example 4), for first screenings. In Examples 3.5-3.7, the variable regions of anti-hCMV neutralising antibodies from four EBV transformed human B cell lines (SM10, SM12, 2C2 and 1G2) were amplified by nested PCR and cloned in expression vectors

containing the appropriate immunoglobulin constant region according to the method described in Tiller *et al.*, 2008. These constructs were subsequently used to transfect HEK 293T cells and the expressed antibodies were tested in neutralisation assays (Example 4) as part of the initial screenings.

The term 'variable region' means VDJ rearranged genes for heavy chains and VJ rearranged genes for light chains.

3.1 RNA purification and first-strand cDNA synthesis

Frozen cell pellets of EBV transformed memory B cell lines SM1, 3-7 and 9-11 were subjected to total RNA purification with TRIZOL® reagent (Invitrogen). The cell pellets were taken out of the -80°C freezer and immediately lysed with TRIZOL®. After 5min incubation at room temperature, 0.2ml of chloroform (Roth, Germany) per 1ml of TRIZOL® was added and the tubes were mixed gently for 1min. Lysates were incubated for 3min on ice and centrifuged at 14000rpm for 15min at 4°C. The aqueous upper phase was transferred to a fresh tube and 0.5ml of isopropanol (Roth, Germany) per 1ml of TRIZOL® was added. After incubation for 10min at room temperature, the tubes were centrifuged at 14000 rpm for 10min at 4°C. Supernatants were discarded and the RNA pellets were washed with 1ml of 70% ethanol (Roth, Germany). Pellets were air-dried for 10 to 15min at room temperature and were dissolved by adding 30 to 50µl of RNAse-free double-distilled water (Fermentas Life Sciences) and by incubation for 10min at 55°C. RNA concentration was measured by UV spectrophotometry and the RNA samples were stored at -80°C. First-strand cDNA synthesis was performed using a RevertAid™ first-strand cDNA synthesis kit (Fermentas Life Sciences) following the manufacture's manual.

3.2 Semi-nested PCR

Variable coding regions of human antibodies were amplified by semi-nested PCR. The semi-nested PCR was performed by running two successive PCRs (PCR parameters are shown in Table 3 below), both with the same program (TDNPCR1; Table 4a below), with different 5' forward primers and the same 3' reverse primer mix (Table 5 below; all J-segment primers together). As a template for the 1st PCR 1µl cDNA was used. For the 2nd PCR 1µl of the PCR product from the 1st PCR was used (undiluted or diluted 1:10 - 1:100, depending on the DNA yield after the 1st PCR). The cDNA of all EBV-lines were amplified using all V_H, Vκ and Vλ primer combinations. Five different forward primers were used in combination with five reverse primers to amplify the kappa light chain variable regions (Table 5a). Three different forward primers were used in combination with 4 different reverse primers to amplify the lambda light chain variable regions (Table 5b). Six different forward primers were used in combination with four different reverse primers to amplify the heavy chain variable regions (Table 5c).

In all EBV lines more than one heavy and one light chain variable region were amplified. Amplified variable regions were digested with HindIII/Eco47III (heavy chains and kappa light chains) or with HindIII/AvrII (lambda light chains) and cloned into pCDNA3 (Invitrogen) already containing the matching constant coding regions for human γ1, κ or λ, as described in Example 3.4.

The resulting PCR products of predicted length were blunt end-ligated into PCR4Blunt-TOPO (Invitrogen) and after sequence analysis, variable regions were further sub-cloned into pCDNA3 (Invitrogen) as described in Example 3.4.

However, to further increase the yield of amplified λ light chain variable regions, the PCR conditions were further optimized (PCR Programme FWUWPCR, see Table 4b below).

Table 3: PCR parameters

Volume per reaction: 30µl	**Final concentration**
Polymerase: High fidelity PCR Enzyme Mix 5u/µl, Fermentas Cat No K0192	0,75u/30µl (0,15µl)
dNTP Mix 10mM each	300µM (0,9µl)
$MgCl_2$ 25mM	2mM (2,4µl)
5' forward primer 10pmol/µl	0,5µM (1,5µl)
3' reverse primer mix (all J-segment primers together)	J_H1-6: 2µM, or Jκ1-5: 1µM, or Jλ1-7: 0,5µM

Table 4: PCR Programs

a) TDNPCR1

1. Initial denaturation	95°C	2min	10 cycles
2. Denaturation	95°C	20s	
3. Annealing	60°C	30s	
4. Elongation	72°C	1min	
10 cycles with decreasing annealing temp. (-0,5°C each cycle)			
5. Denaturation	95°C	20s	10 cycles
6. Annealing	55°C	30s	
7. Elongation	72°C	1min	
10 cycles with 55°C annealing temp.			
8. Denaturation	95°C	20s	15 cycles
9. Annealing	53°C	30s	
10. Elongation	72°C	1min	
15 cycles with 53°C annealing temp.			
11. Final elongation	72°C	10min	
12. Cooling	10°C	hold	

b) FWUWPCR

1. Initial denaturation	94°C	5min	
2. Annealing	55°C	40s	
3. Elongation	72°C	1min	
4. Denaturation	94°C	40s	30 cycles
5. Annealing	55°C	40s	
6. Elongation	72°C	1min	
7. Final elongation	72°C	3min	
8. Cooling	10°C	hold	

Table 5a: Primers to amplify the κ light chain variable region

1. PCR forward Primers (5')			
Name	Number	Sequence	SEQ ID
nes Vκ1	074-Je	5' GTC AGW CCC AGT CAG GAC ACA GC 3'	No:198
nes Vκ2	075-Je	5' ACT CCT CAG TTC ACC TTC TCA CM 3'	No:199
nes Vκ3	076-Je	5' TCA GTT AGG ACC CAG ASG GAA 3'	No:200
nes Vκ4	150-Je	5' CAA CAG GCA GGC AGG GGC AGC AAG 3'	No:201
nes Vκ5	151-Je	5' CAC CTG CAG GTC AGG GCC AAG GTT 3'	No:202

2. PCR forward Primers (5')			
Name	Number	Sequence	SEQ ID
Vκ1 5'-forward neu	077-Je	5' CTG AAG CTT CCA TGG ACA TGA GGG TCC CCG CTC AGC TCC 3'	No:203
Vκ2 5'-forward	078-Je	5' CTG AGG CTT CCA TGA GGC TCC CTG CTC AGC TCC TGG GGC TG 3'	No:204
Vκ3 5' forward	007-Je	5' CTG AAG CTT CCA TGG AAG CCC CAG CGC AGC TTC TCT TCC TC 3'	No:205
Vκ4 5' forward	152-Je	5' CTG AAG CTT CCA TGG TGT TGC AGA CCC AGG TCT TCA TTT CTC 3'	No:206
Vκ5 5' forward	153-Je	5' CTG AAG CTT CCA TGG GGT CCC AGG TTC ACC TCC TCA GCT TCC 3'	No:207

1.+ 2. PCR reverse primers (3′) Mix „Jκ1-5": 66-Je + 67-Je + 68-Je + 69-Je + 70-Je			
Name	Number	Sequence	SEQ ID
Jκ1 HindIII/ Eco47III	066-Je	5' TAG AGC GCT TGA TTT CCA CCT TGG TCC CTT GG 3'	No:208
Jκ2 HindIII/ Eco47III	067-Je	5' TAG AGC GCT TGA TCT CCA GCT TGG TCC CCT GG 3'	No:209
Jκ3 HindIII/ Eco47III	068-Je	5' TAG AGC GCT TGA TAT CCA CTT TGG TCC CAG GG 3'	No:210
Jκ4 HindIII/ Eco47III	069-Je	5' TAG AGC GCT TGA TCT CCA CCT TGG TCC CTC CG 3'	No:211
Jκ5 HindIII/ Eco47III	070-Je	5' TAG AGC GCT TAA TCT CCA GTC GTG TCC CTT GG 3'	No:212

Table 5b: Primers to amplify the lambda λ chain variable region

PCR forward Primers (5')			
Name	Number	Sequence	SEQ ID
Vλ1-upstream	258-Je	5' CAG GAC TCA GGA CAA TCT CCA GC 3'	No:213
Vλ2-upstream	259-Je	5' YYY CSG GAC GTC YYC ACC 3'	No:214
Vλ3-upstream	260-Je	5' ATC TGG GGG KCT YYC RCC 3'	No:215

PCR forward Primers (5')			
Name	Number	Sequence	SEQ ID
Vλ1-leader	261-Je	5' GAT AAG CTT CCA TGG CCT GST CCC CTC TCC TCC TCA C 3'	No:216
Vλ2-leader	262-Je	5' GAT AAG CTT CCA TGG CCT GGG CTC TGC TCC TCC TC 3'	No:217
Vλ3-leader	263-Je	5' GAT AAG CTT CCA TGG CCT GGA CCC CTC TCC TSC TC 3'	No:218

5

1. + 2. PCR reverse Primers (3'), Mix "Jλ1-7": 264-Je + 265-Je + 266-Je + 267-Je			
Name	Number	Sequence	SEQ ID
Jλ1,2,3,6	264-Je	5' GAG CCT AGG ACG GTG ACC TTG GTC CC 3'	No:219
Jλ4	265-Je	5' GAG CCT AGG ATG ATC AGC TGG GTT CCT CC 3'	No:220

Jλ5	266-Je	5' GAG CCT AGG ACG GTC AGC TCG CTC CCC TC 3'	No:221
Jλ7	267-Je	5' GAG CCT AGG GCG GTC AGC TGG GTG CCT CC 3'	No:222

Table 5c: Primers to amplify the heavy chain variable region

1. PCR forward Primers (5')			
name	number	sequence	SEQ No
nes V_H1	84-B	5' CCC TGA GAG CAC AGY TCC TCA CC 3'	No:223
nes V_H2	155-Je	5' AGT GAC TCC TGT GCM CCA CC 3'	No:224
nes V_H3	85-B	5' GCA CTG AAC ACA GAG GCA TCA CC 3'	No:225
nes V_H4 v1	161-Je	5' CMT GGA YCT CMT GYR CRA GAA C 3'	No:226
nes V_H5	162-Je	5' AGG GCT TCA TTT TCT GTC CTC CAC CAT C 3'	No:227
nes V_H6	154-Je	5' GGG CAG TCA CCA GAG CTC CAG ACA 3'	No:228

2. PCR forward Primers (5')			
Name	Number	Sequence	SEQ ID
V_H1 5' forward	001-Je	5' CTG AAG CTT CCA TGG ACT GGA CCT GGA GGA TCC TCT TCT TG 3'	No:229
V_H2 5' forward neu	158-Je	5' CTG AAG CTT CCA TGG ACA CAC TTT GCT CCA CGC TCC TG 3'	No:230
V_H3 5' forward	003-Je	5' CTG AAG CTT CCA TGG AGT TTG GGC TGA GCT GGG TTT TCC TTG 3'	No:231
V_H4 5' forward neu	159-Je	5' CTG AAG CTT CCA TGA AAC ACC TGT GGT TCT TCC TCC TSC TGG 3'	No:232
V_H5 5' forward	156-Je	5' CTG AAG CTT CCA TGG GGT CAA CCG CCA TCC TCG CCC TCC TCC 3'	No:233
V_H6 5' forward	157-Je	5' CTG AAG CTT CCA TGT CTG TCT CCT TCC TCA TCT TCC TGC CCG 3'	No:234

1. + 2. PCR reverse Primers (3'), Mix "J_H1-6": 62-Je + 63-Je + 65-Je + 64-Je			
Name	Number	Sequence	SEQ ID
J_H 1,4,5 HindIII/ Eco47III	062-Je	5' TAG AGC GCT GGA GAC GGT GAC CAG GGT TCC CTG G 3'	No:235
J_H2 HindIII/ Eco47III	063-Je	5' TAG AGC GCT GGA GAC AGT GAC CAG GGT GCC ACG 3'	No:236
J_H3 HindIII/ Eco47III	065-Je	5' TAG AGC GCT AGA GAC GGT GAC CAT TGT CCC TTGG 3'	No:237
J_H6 HindIII/ Eco47III	064-Je	5' TAG AGC GCT GGA GAC GGT GAC CGT GGT GCC TTT TT 3'	No:238

3.3 Vector Backbone Preparation

Amplified variable regions were cloned into a pCDNA3 vector (Invitrogen) containing the appropriate immunoglobulin heavy and light chain constant regions. For the cloning of heavy chain variable regions, construct pd1612-Je (pCDNA3-EGFP-Cγ) was digested with HindIII/Eco47III (generates two bands of 6505bp and 727bp), dephosphorylated with CIP and the 6505bp fragment was gel-purified. For the cloning of lambda light chain variable regions, construct pd1864-Je (pCDNA3-2-4 Vλ2-AvrII(-) was digested with HindIII/AvrII (generates two bands of 5858bp and 394bp), dephosphorylated with CIP and the 5858bp fragment was gel-purified. For cloning the kappa light chain variable regions, construct pd703-Je (pCDNA3-ITC88 Vκ) was digested with HindIII/Eco47III (generates two bands of 6050bp and 392bp), dephosphorylated with CIP and the 6050 bp band was gel-purified.

3.4 Insert preparation

The PCR products of the amplified antibody variable regions were gel purified, digested with either HindIII/Eco47III (heavy chain and kappa light chain variable regions) or with HindIII/ AvrII (lambda light chain variable regions) and were then ligated in-frame into a pCDNA3 vector containing the appropriate immunoglobulin constant region using T4 DNA ligase as recommended by the enzyme manufacturer. DNA-ligation was performed overnight at 16°C. As an exception to this method, the PCR products of the antibody variable regions SM1 Vλ1, SM4 Vλ1 and SM9 Vλ2 were first blunt end-ligated into PCR4Blunt-TOPO (Invitrogen) following the user manual of the blunt-end ligation kit (Invitrogen). After analysing the sequences of various minipreps, unique clones containing bona fide Vλ sequences were digested with HindIII/ AvrII and the variable regions were gel-purified and sub-

cloned into the appropriate pCDNA3 vector (pd 1864-Je; see below). 1µl of each ligation was electroporated into DH10B cells (1900V/5ms). Then 200µl of the electroporated bacteria were plated onto LB-agar + 100µg/ml ampicillin plates. From each construct about 10 colonies were picked, grown overnight and minipreps were performed (each colony was also streaked onto LB-agar + 100µg/ml ampicillin plate). A control digestion to identify positive clones was performed with HindIII/Eco47III (H/E) for constructs with a heavy chain or a kappa light chain and with HindIII/AvrII (H/A) for constructs with a lambda light chain. Positive clones were analysed by DNA-sequencing with primer 179-Je (Sequence: 5' AGA GAA CCC ACT GCT TAC TG 3'; SEQ ID No: 196).

As mentioned above, a minority of variable regions were cloned first into a pCR4Blunt-TOPO (Invitrogen) vector backbone. After sequence analysis, positive clones were sub-cloned into pCDNA3 vectors as described above. For the insert preparation, Pd1887-Je (pCR4Blunt-TOPO-SM9 Vλ1_J_L7 #2) and pd1888-Je (pCR4Blunt-TOPO-SM1 Vl1_Jl7 #4) were digested with HindIII/AvrII. The variable regions (394 bp) were gel-purified and ligated into pCDNA3. 1µl of each ligation was electroporated into DH10B cells (1900V/5ms). 250µl of the electroporated bacteria were plated onto LB-agar + 100µg/ml ampicillin plates. Five colonies from each ligation were picked, minipreps were performed and DNA was digested with HindIII/AvrII to identify positive clones.

A summary of the number of antibody heavy chain (HC) variable regions and light chain (LC) variable regions cloned from EBV transformed human B cells is presented in Table 6 below. The 46 neutralising antibodies (final column) are the result of different combinations of 18 unique heavy chains and 18

Table 6: Summary of anti-CMV antibodies cloned from nine EBV lines

EBV line	HC (amino acids)	No. of HCs	LC kappa (amino acids)	LC lambda (amino acids)	No. of LCs	Theoretical combinations of H and L	Expressed antibodies	Neutralizing antibodies
SM 1	**Vh1 (151)**	2	Vk1 (130)		1	5	2	
				Vl1 (130)	1	5		
	Vh3 (142)	3		**Vl1 (128)***	2	10	9	3
SM 3	**Vh1 (151)**	3		**Vl1 (128)**	8	24	6	4
SM 4	**Vh1 (151)**	2		**Vl1 (128*, 129,** *133)*	10	20	16	13
			Vk1 (131)		2	4	1	
			Vk3 (129)		1	2	1	
SM 5	**Vh1 (151)**	3		**Vl1 (128)**	4	12	9	9
SM 6	**Vh1 (151)**	3		**Vl1 (128,** 129, 130, 131), Vl2 (130, 131)	15	75	25	12
	Vh5 (148)	2				28	9	
SM 7	Vh1 (151)	4	Vk1 (130)		2	8	2	0
SM 9	Vh1 (142)	2		Vl1 (128), Vl2 (130)	5	10	4	0
SM 10	Vh1 (138)	3	Vk2 (133)		2	6	2	0
SM 11	**Vh1 (151)**	10	Vk1 (130)		2	20	9	
				Vl1 (128)*	6	60	5	5
				Vl1 (130)	1	10		
		37			62	295	100	46

* Indicates chain cloned from 3 different SM lines.

unique lambda light chains. None of the combinations of heavy chains with kappa light chains resulted in hCMV neutralising antibodies. All heavy chains (V_H1-family; 151 amino acids, including leader sequence) are derived from one V-germline gene: IGHV1-1. All lambda light chains (VΛ1 family; 128 amino acids, including leader sequence) are derived from one V-germline gene: IGVΛ1-51. These variable regions of the hCMV neutralising antibodies are marked in bold in Table 6. Heavy and light chains derived from other V-germline genes were also recovered but did not result in the generation of neutralising antibodies. An overview of all heavy and lambda light chain combinations resulting in neutralising antibodies is given in Table 7 below. Table 19 on pages 149-150 summarises the SEQ ID numbers of the accompanying Sequence Listing, for the heavy and light chain CDRs of the neutralising antibodies shown in Table 7 below.

Table 7: Heavy and Light chain antibody combinations

	V_L					
V_H	**SM5-1**	**SM4-10**	**SM6-5**	**SM1-6**	**SM3-1**	**SM5-6**
SM5-1	Ab-28					Ab-03
SM4-10		Ab-02				
SM6-5			Ab-04			
SM1-6				Ab-11		
SM3-1					Ab-14	
SM11-17				Ab-42		
SM11-21				Ab-46		
SM6-11			Ab-01			
SM4-3		Ab-20		Ab-27		
SM5-9	Ab-10					Ab-31
SM1-7				Ab-12		
SM1-8				Ab-13		
SM3-4						

SM6-23			Ab-35			
SM11-18				Ab-43		
SM11-19				Ab-44		
SM5-3	Ab-29					
SM11-20				Ab-45		

cont.	**V_L**					
V_H	**SM6-48**	**SM4-3**	**SM5-9**	**SM4-12**	**SM5-5**	**SM3-2**
SM5-1			Ab-32		Ab-30	
SM4-10		Ab-26		Ab-08		
SM6-5	Ab-37					
SM3-1						Ab-15
SM6-11	Ab-05					
SM4-3		Ab-06		Ab-22		
SM5-9			Ab-07		Ab-09	
SM3-4						Ab-17
SM6-23	Ab-38					

cont.	**V_L**					
V_H	**SM3-4**	**SM4-1**	**SM4-5**	**SM4-7**	**SM6-6**	**SM6-51**
SM4-10		Ab-25	Ab-23	Ab-24		
SM6-5					Ab-33	Ab-39
SM6-11					Ab-34	Ab-40
SM4-3		Ab-18	Ab-19	Ab-21		
SM3-4	Ab-16					
SM6-23					Ab-36	Ab-41

3.5 RNA purification and first-strand cDNA synthesis

RNA purification of frozen cell pellets from EBV transformed memory B cell lines SM10, SM12, 2C2, 1G2 was performed using the RNeasy® Mini Kit (Qiagen) according to the manufacturer's manual. cDNA synthesis was performed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche) according to the manufacturer's instructions.

3.6 Nested PCR

From the cDNA synthesised in Example 3.5 above, variable coding regions of human antibodies were amplified by nested PCR by running two successive PCRs (PCR parameters are shown in Table 8 below) starting from 1-2µl of cDNA as template. Both PCR reactions were performed with the same program (Table 9) with different 5' forward primers and 3' reverse primers (Table 10). As a template, for the first PCR round 50ng of template cDNA was used and for the second PCR round 1-2µl of the PCR product (purified using QIAGEN PCR Purification Kit) from the first PCR round was used. The cDNA of all EBV-lines was amplified using V_H, Vκ and Vλ primer combinations for the first PCR round as shown in Table 10a and the V_H, Vκ and Vλ primer combinations for the second PCR round as shown in Table 10b. For the second round of PCR, a 'best fit' primer was selected (Table 2, Tiller *et al., ibid.*) for the sequence obtained from the first round of PCR.

Table 8: PCR parameters

Expand™ High Fidelity PCR System dNTPack
1x Expand™ High Fidelity buffer (with 15mM $MgCl_2$)
10mM/dNTP Expand™ High Fidelity dNTPs
20pmol of each primer
50ng template DNA or 1-2µl cDNA
2.6U Expand™ High Fidelity Enzyme Mix

Table 9: PCR Program

1. Initial denaturation	94°C	5min	1 cycle
2. Denaturation	94°C	10s	40 cycles
3. Annealing	50°C	20s	
4. Elongation	72°C	55s	
5. Final elongation	72°C	7min	
6. End	18°C	∞	1 cycle

Table 10a: Primers for the first round of PCR amplification

1st round PCR forward Primers (5′)			
EBV line	Primer Name	Sequence	SEQ ID No:
SM10 VH	5′ Ig L VH chain 4/6	5′ CCC AGA TGG GTC CTG TCC CAG GTG CAG 3′	296
SM10 Vκ	5′ Ig L Vκ chain 1/2	5′ ATG AGG STC CCY GCT CAG CTG CTG G 3′	297
SM12 VH	as SM10 VH	as SM10 VH	296
SM12 Vκ	as SM10 Vκ	as SM10 Vκ	297
SM12 Vλ	5′ Ig L Vλ chain 3	5′ GCT CTG TGA CCT CCT ATG AGC TG 3′	298
2C2 VH	as SM10 VH	as SM10 VH	296
2C2 Vκ	as SM10 Vκ	as SM10 Vκ	297
1G2 VH	as SM10 VH	as SM10 VH	296
1G2 Vλ	5′ Ig L Vλ chain 1	5′ GGT CCT GGG CCC AGT CTG TGC TG 3′	299

1st round PCR reverse Primers (3′)			
EBV line	Primer Name	Sequence	SEQ ID No:
SM10 VH	3′ Ig Cγ CH chain 1	5′ GGA AGG TGT GCA CGC CGC TGG TC 3′	300
SM10 Vκ	3′ Ig Cκ chain 543	5′ GTT TCT CGT AGT CTG CTT TGC TCA 3′	301
SM12 VH	as SM10 VH	as SM10 VH	300
SM12 Vκ	as SM10 Vκ	as SM10 Vκ	301
SM12 Vλ	as 1G2 Vλ	as 1G2 Vλ	302
2C2 VH	as SM10 VH	as SM10 VH	300
2C2 Vκ	as SM10 Vκ	as SM10 Vκ	301
1G2 VH	as SM10 VH	as SM10 VH	300
1G2 Vλ	3′ Ig Cλ chain	5′ CAC CAG TGT GGC CTT GTT GGC TTG 3′	302

Table 10b: Primers for the second round of PCR amplification

2nd round PCR forward Primers (5′)			
EBV line	Primer Name	Sequence	SEQ ID No:
SM10 VH	5′ Ig AgeI VH 4	5′ CTG CAA CCG GTG TAC ATT CCC AGG TGC AGC TGC AGG AG 3′	303
SM10 Vκ	5′ Ig AgeI Vκ 2-24	5′ CTG CAA CCG GTG TAC ATG GGG ATA TTG TGA TGA CCC AGA C 3′	304
SM12 VH	as SM10 VH	as SM10 Vκ	304
SM12 Vκ	5′ Ig AgeI Vκ 2-28	5′ CTG CAA CCG GTG TAC ATG GGG ATA TTG TGA TGA CTC AGT C 3′	305
SM12 Vλ	5′ Ig AgeI Vλ 3	5′ CTG CTA CCG GTT CTG TGA CCT CCT ATG AGC TGA CWC AG 3′	306

2C2 VH	as SM10 VH	same as SM10 VH	303
2C2 Vκ	5' Ig AgeI Vκ 1-5	5' CTG CAA CCG GTG TAC ATT CTG ACA TCC AGA TGA CCC AGT C 3'	307
1G2 VH	5' Ig AgeI VH 4-39	5' CTG CAA CCG GTG TAC ATT CCC AGC TGC AGC TGC AGG AG 3'	308
1G2 Vλ	5' Ig AgeI Vλ 1	5' CTG CTA CCG GTT CCT GGG CCC AGT CTG TGC TGA CKC AG 3'	309

2nd round PCR reverse Primers (3')			
EBV line	Primer Name	Sequence	SEQ ID No:
SM10 VH	3' Ig SalI JH 1/2/4/5	5' TGC GAA GTC GAC GCT GAG GAG ACG GTG ACC AG 3'	310
SM10 Vκ	3' Ig BsiWI Jκ 2	5' GCC ACC GTA CGT TTG ATC TCC AGC TTG GTC 3'	311
SM12 VH	3' Ig SalI JH 6	5' TGC GAA GTC GAC GCT GAG GAG ACG GTG ACC GTG 3'	312
SM12 Vκ	as SM10 Vκ	as SM10 Vκ	311
SM12 Vλ	as 1G2 Vλ	as 1G2 Vλ	314
2C2 VH	as SM12 VH	as SM12 VH	312
2C2 Vκ	3' Ig Bsi WI Jκ 1/4	5' GCC ACC GTA CGT TTG ATY TCC ACC TTG GTC 3'	313
1G2 VH	as SM10 VH	as SM10 VH	310
1G2 Vλ	3' Ig XhoI Cλ	5' CTC CTC ACT CGA GGG YGG GAA CAG AGT G 3'	314

Restriction enzyme cutting sites are underlined

3.7 Expression vector cloning

Before cloning, aliquots of the V_H, Vκ and Vλ chain second PCR products were purified with QUIAGEN PCR Purification Kit according to the manufacturer's instructions and sequenced with the respective forward or reverse primer (Table 10). Sequences were analysed by IgBLAST (GenBank http://www.ncbi.nlm.nih.gov/igblast/) to identify germline V(D)J gene segments with highest identity.

Amplified variable regions from the EBV lines were digested with AgeI/SalI (γ1 heavy chain), AgeI/BsiWI (κ light chain) or AgeI/XhoI (λ light chain) and cloned into human Igγ1, Igκ and Igλ expression vectors containing a murine Ig gene signal peptide sequence (GenBank Accession No: DQ407610) and a multiple cloning site upstream of the human Igγ1, Igκ or Igλ

constant regions. Also present in the expression vector is a human CMV promoter to drive transcription and an ampicillin resistance gene for selection. Ligation was performed in a total volume of 20µl with 1U T4 DNA-Ligase (Invitrogen), 7.5µl of digested and purified PCR product and 25ng linearised vector. Competent *E. coli* DH10B bacteria (Invitrogen) were transformed at 42°C with 2µl of the ligation product in 96-well plates by either electroporation or by heat-shock transformation. Colonies were screened by PCR using the 5'Absense forward primer (5'-GCT TCG TTA GAA CGC GGC TAC-3'; SEQ ID No: 315) and the 3'IgG internal reverse primer (5'-GTT CGG GGA AGT AGT CCT TGA C-3'; SEQ ID No: 316), the 3'Cκ494 reverse primer (5' GTG CTG TCC TTG CTG TCC TGC T 3'; SEQ ID No: 317) or the 3'Cλ reverse primer (5' CAC CAG TGT GGC CTT GTT GGC TTG 3'; SEQ ID No: 318), respectively. PCR products of the expected size were sequenced to confirm identity with the original PCR products.

A summary of the number of antibody heavy chain (HC) variable regions and light chain (LC) variable regions cloned from EBV transformed human B cells is presented in Table 11 below. The four neutralising antibodies (final column) are the result of combination of 4 unique heavy chains and unique lambda or kappa light chains. The heavy chains are derived from two V-germline genes: IGHV4-39 and IGHV4-59. The kappa light chains are derived from two V-germline genes: IGKV2D-28 and IGKV1D-33 and the lambda light chain is derived from the V-germline gene: IGLV1-47. The lambda light chain derived from the V-germline gene IGLV3-10 did not result in antigen recognition when paired with a heavy chain. The combinations of the neutralising antibody heavy and light chains are described in Table 12 below.

Table 11: Summary of anti-hCMV antibodies cloned from four EBV lines

EBV line	HC (amino acids)	No of HCs	LC kappa (amino acids)	LC lambda (amino acids)	No of LCs	Theoretical combinations of H and L	Expressed antibodies	Neutralizing antibodies
SM 10	Vh4-39 (125)	1	Vk2-24 (112)		1	1	1	1
SM 12	Vh4-59 (125)	1	Vk2-28 (112)	Vl3-10 (109)	2	2	1	1
2C2	Vh4-59 (130)	1	VkID-33 (107)		1	1	1	1
1G2	Vh4-39 (120)	1		Vl1-47 (108)	1	1	1	1
		4			5	5	4	4

Table 12: Heavy and Light chain antibody combinations

Antibody	Heavy chain	Light chain
Ab-47	SM10 Vh	SM10 Vκ
Ab-48	SM12 Vh	SM12 Vκ
Ab-49	2C2 Vh	2C2 Vκ
Ab-50	1G2 Vh	1G2 Vλ

Table 19 on page 149 summarises the SEQ ID numbers of the accompanying Sequence Listing, for the heavy and light chain CDRs of the neutralising antibodies shown in Table 12 above.

Example 4: Expression and Purification of recombinant antibodies

Further characterisation of the cloned anti-hCMV antibodies required their expression and subsequent purification. Forty-six recombinant antibodies (Ab-01 to Ab-46) were expressed by transient transfection of CHO cells (DSMZ, Braunschweig, DE). Briefly, cells were seeded into cell culture dishes (diameter 10 cm; Greiner Bio-One, GmbH) at a density of 2.2 x 10^6 cells per dish in SF-IMDM medium (Invitrogen) containing 2% FCS (Sigma). After 24h, the transfection mixture was prepared by mixing 1ml MEM (PAA Laboratories) with 6.45µg of expression plasmid for heavy chain, 6.45µg of expression vector for light chain, and 12.9µl of MATra transfection reagent (IBA GmbH, Göttingen, DE) per dish. This transfection mixture was incubated for 20min at room temperature. The medium of the seeded cells was exchanged for 10ml of MEM and the transfection mixture was added to cells dropwise. Subsequently, the culture dish was incubated on a magnetic plate (IBA GmbH) for 15min. The medium was then aspirated and 10ml of SF-IMDM medium with 2% FCS containing ultra low levels of bovine IgG (Lonza) was added to the cells. 24h later, the medium was renewed. After

two additional days, the medium was renewed and the supernatant containing recombinant antibody was harvested by centrifugation at 244g for 5min. Three days later, conditioned cell culture supernatant was harvested again by centrifugation and the cleared, antibody-containing supernatants were pooled.

A Vivacell 70 ultrafiltration device (MWCO 10kDa; Sartorius Stedim Biotech) was used to concentrate the conditioned cell culture supernatant 100-fold by centrifugation at 1,000g and 20°C for 1h. For purification of recombinant antibody, a Protein A HP Spin Trap column (GE Healthcare) was equilibrated with binding buffer (50mM Tris-HCl, 150mM NaCl, pH7.5) and 300µl of concentrate were loaded. The column was sealed with a lid and was incubated on an end-over-end mixer at room temperature. After 1h the spin column was centrifuged for 1min at 150g. After washing the column with 400µl of binding buffer and centrifugation at 150g for 1min, the loading process was repeated for an additional three times until the whole concentrate had been loaded. After the final loading step, the column was washed four times by application of 400µl binding buffer and subsequent centrifugation at 150g for 1min. Bound recombinant antibody was eluted from the spin column twice by adding 200µl elution buffer (100mM glycine/HCl, pH 2.5) and centrifugation at 150g for 1min. Eluates were immediately neutralised with 30µl of neutralisation buffer (1M Tris-HCl, pH9.0). The buffer was exchanged by loading the combined eluates onto a Zeba Desalt Spin column (Pierce) pre-equilibrated with PBS and subsequent centrifugation at 150g for 2min. Purified recombinant antibody was stored in protein LoBind tubes (Eppendorf) at 4°C until further processing for characterisation studies.

The produced antibody culture supernatants were analysed for gB recognition either by ELISA or by Biacore, as described in Examples 1 and 5. After IgG-concentrations were determined, as explained in Examples 2 and 5, gB-specific culture supernatants were analysed for neutralising activity, using the luciferase assay as described in Example 2 (luciferase assay) using primary fibroblast cells HFF. Seven anti-hCMV antibodies displaying efficient 50%-neutralising activities were chosen for further experiments (see Table 13 below).

Table 13: 50% neutralising activities of monoclonal SM-antibodies produced by CHO cells

Monoclonal antibody	**50% Neutralising activity (µg/ml)**
Ab-02	0.3
Ab-04	0.5
Ab-11	1.3
Ab-14	0.4
Ab-19	0.6
Ab-28	0.3
Ab-42	1.0
ITC88*	1.5
Cytotect❖	200

Results reflect mean values of three independent assays. Variations between assays were in the range 10-20%. *Ohlin *et al.*, (1993); ❖Cytotect (Biotest) is a pool of HCMV hyperimmune IgG.

For the expression of the four recombinant antibodies Ab-47 to Ab-50, the method according to Tiller *et al.*, *ibid* was followed. Briefly, HEK 293T cells (DSMZ, Braunschweig, DE) were cultured in 75cm^2 flasks (Greiner Bio-One, GmbH) under standard conditions in DMEM medium (GibcoBRL) supplemented

with 10% heat-inactivated FCS (PAN Biotech GmbH), 350µg/ml L-glutamine (Merck) and 100µg/ml gentamycine (SERVA Electrophoresis GmbH). Transient transfections of exponentially growing 293T cells were performed by $CaPO_4$ precipitation at 80% cell confluency. Equal amounts (12.5-20µg each) of heavy and corresponding light chain expression vector DNA were mixed in 1ml sterile water and 2.5M $CaCl_2$ was added drop-wise to a concentration of 250mM. An equal volume of 2xHEPES-buffered saline was mixed with the calcium-DNA solution under slow vortexing and incubated at room temperature for 1min (1min RT + 1min 37°C) to allow formation of precipitates. The precipitation mixture was distributed evenly to the culture dish. The cells were washed with 10ml PBS after 6-8h and cultured for 6-7 days in 15ml DMEM before supernatants were harvested.

The culture supernatants were analysed for gB recognition by ELISA as described in Example 1. After IgG-concentrations were determined, as explained in Example 2, gB-specific culture supernatants were analysed for neutralising activity, using the luciferase assay (as described in Example 2) using primary fibroblast cells HFF. The 50% neutralising activities of the monoclonal antibodies produced by HEK-293T cells are shown in Table 14 below:

Table 14: 50% neutralising activities of monoclonal antibodies produced by HEK-293T cells

Monoclonal antibody	**50% Neutralising activity (µg/ml)**
Ab-47	0.14
Ab-48	0.33
Ab-49	0.53
Ab-50	0.23

C23*	0.23

Results reflect mean values of two independent assays.
*C23 is a gB specific antibody used as control (T123; a kind gift from Teijin Pharma Limited, Japan)

Example 5: Characterisation of anti-hCMV antibodies

5.1: Quantification of hCMV antibodies using suspension array technology (Luminex®)

Cell culture supernatants containing human IgG were diluted in assay buffer (Roche, Cat# 1112589) and dilutions were assessed in duplicate in a 96-half well plate (Corning, Cat#3884). Briefly, 25µl samples were incubated in the dark (20°C, 650rpm) for 1h with 5µl containing 1200 Luminex-COOH-beads loaded by amine coupling with anti-human IgG-Fc-specific (Caltag, Cat#H10500). Standard Curves were generated using duplicates of 25µl of a 1:3 dilution series (0.08 - 60ng/ml) of ChromPure human IgG whole molecule (Jackson Immuno-Research, USA Cat# 009-000-003). Detection was done by the addition of 30µl anti-human IgG-Fc-specific labelled with R-PE (5µg/ml; JIR Cat# 109-116-098) and further incubation for 1h. Plates were then read and analysed using a Luminex® 200 instrument (Millipore) using the following settings: 100 beads, 50µl sample size.

5.2: Quantification of gB protein (hCMV) using suspension array technology

Cell culture supernatants containing gB (hCMV) were diluted in assay buffer (Roche Cat# 1112589) and dilutions were assessed in triplicate in a 96-well filter plate (Millipore Cat# MABVN 1250). Briefly, 25µl samples were incubated in the dark (20°C, 650rpm) for 1.5h with 5µl containing 1500 Luminex-COOH-beads loaded by amine coupling with the human-anti-hCMV-IgG antibody $V_H3/65$-Vκ1/19, a non-neutralising, but

very high affinity hCMV specific antibody previously identified in-house. Standard curves were generated using triplicates of 25µl of a 1:3 dilution series (6-1458ng/ml) of gB. Plates were washed twice (100µl PBS per well) using a vacuum manifold and for detection 50µl biotinylated anti-hCMV-IgG antibody ITC52 (5µg/ml; generated in-house; Ohlin *et al.*, 1993) was added for further 1.5h. After two wash steps (with 100µl PBS each) 50µl 1.2µg/ml Neutravidin labelled with R-PE (Invitrogen, Cat# A2660) was added for 30min before plates were read and analysed using the Luminex® 200 instrument (settings: 100 beads, 40µl sample size).

5.3: Biacore

Protein-protein interactions were analysed by surface plasmon resonance technique using a Biacore® T100 instrument (Biacore, GE Healthcare, Munich) with Biacore® T100 control software v 2.0.1. All interactions were analysed at 25°C in 1 x DPBS with P20 (0.05%). Each binding interaction was assayed at least twice. The hCMV gB protein was coupled to the flowcells of a CM5 sensor chip (carboxymethylated dextran matrix, GE Healthcare) via standard amine-coupling procedure. The carboxymethylated dextran matrix was activated with 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 0.1 M N-hydroxysuccinimide (NHS) according to the instructions of the manufacturer (GE Healthcare). Two flowcells were activated and gB protein was diluted with 10mM sodium acetate, pH 5.0, to 50µg/ml and injected at a flow rate of 5µl/min until an appropriate level of coupling for the binding experiments (5,000 resonance units) or for the kinetics experiments (2,000 resonance units) was reached. Unreactive groups were inactivated by injection of 1M ethanolamine-HCl, pH 8.5. A control flowcell was prepared accordingly with ovalbumine (Imject, Pierce, Thermo Fisher Scientific, Schwerte, lot.

JF124260) at pH 4.0. Before the binding experiments, the flowcells were washed thoroughly with running buffer.

For binding analyses cell culture supernatants containing anti-gB-specific antibodies were adjusted to 2.5µg/ml IgG by diluting in SF-IMDM 2% ultra low IgG and injected for 90sec in PBS with 0.02% BSA and 0.05% Tween 20 at 10µl/min. After a dissociation time of 90sec the binding stability was plotted using Biacore® T100 Evaluation Software version 2.0.1.

For kinetic analysis, Fab fragments of Antibodies Ab-02, Ab-04, Ab-11, Ab-14, Ab-28, Ab-42 and ITC88, as positive control, were prepared from protein A-purified human IgG using immobilised papain according to standard protocol of the manufacturer (Pierce, Thermo Fisher Scientific). The cleavage products were confirmed by SDS-PAGE with silver staining and by Luminex® bead array Fab/Fc detection. For kinetic analyses, the flow rate was raised to 70µl/min and three blank curves (zero concentrations) were introduced into each run. The surface was regenerated with 10mM glycine/HCl at pH 2.0. The binding curves were evaluated using Biacore® T100 Evaluation Software version 2.0.1 applying a Langmuir 1:1 model with global fit of R_{max}.

Table 15: Summary of on- and off-rates and calculated K_D

anti-gB Fab	k_a (1/Ms)	k_d (1/s)	K_D (M)
Ab-02	$3.8(\pm0.3)\times10^{5}$	$1.3(\pm0.1)\times10^{-4}$	$3.5(\pm0.1)\times10^{-10}$
Ab-04	$7.7(\pm0.3)\times10^{4}$	$1.2(\pm0.1)\times10^{-4}$	$1.5(\pm0.1)\times10^{-9}$
Ab-11	$7.6(\pm0.4)\times10^{4}$	$5.1(\pm0.1)\times10^{-4}$	$6.8(\pm0.4)\times10^{-9}$
Ab-14	$5.6(\pm0.3)\times10^{4}$	$8.4(\pm0.1)\times10^{-4}$	$1.5(\pm0.1)\times10^{-8}$

Ab-28*	$4.0(\pm0.6)x10^{5}$	$2.4(\pm0.8)x10^{-5}$	$5.7(\pm1.2)x10^{-11}$
Ab-42	$5.7(\pm0.1)x10^{4}$	$3.2(\pm0.01)x10^{-4}$	$5.6(\pm0.1)x10^{-9}$
ITC88**[$]	$4.5(\pm0.04)x10^{4}$	$4.7(\pm0.1)x10^{-5}$	$1.0(\pm0.01)x10^{-9}$

based on three measurements on two Biacore chip surfaces

* reaches limitation of the method

** based on two measurements on two Biacore chip surfaces

[$] published affinity ($K_D$) for ITC88 on pHM90-5 antigen is 2nM (Ohlin *et al.*, 1993)

Example 6: Neutralisation of different hCMV gB-genotypes

HCMV lab strains and clinical isolates were classified in several gB genotypes (Chou and Dennison, 1991). 50%-neutralising activities of anti-HCMV antibodies were determined using additional different hCMV gB-genotypes using indirect immunofluorescence as a read-out. The virus strains Towne, AD169 and the clinical isolate Altu are classified as gB-genotype 1, gB-genotype 2 and gB-genotype 3, respectively. All virus strains were propagated on human foreskin fibroblasts (HFFs) by standard procedures and titres of infectivity in viral supernatants were determined by the method as described in Mahy & Kangro (1996). An indirect immunofluorescence assay was carried out as described in Andreoni *et al.* (1989). In brief, two-fold serial dilutions of six monoclonal antibodies (Antibodies Ab-04, Ab-11, Ab-14, Ab-19, Ab-28 and Ab-42) were incubated with a titered amount of the respective HCMV gB-genotype (300 pfu) for 1h at 37°C. After incubation, virus-antibody mixtures were added to HFF cultures grown to confluence in 96-well microplates. All samples were tested in triplicate. The viral supernatants were removed from the HFFs, after an incubation of 4h at 37°C and replaced by complete medium. After another 16h to 20h incubation period, the cells were washed and fixed with

ethanol. Infected cells were stained using the monoclonal antibody p63-27, which is specific for the major immediate-early (IE) protein, UL123, of hCMV and Cy3-conjugated anti-mouse IgG secondary antibodies (Jackson ImmunoResearch, USA). Following extensive washing, the IE-positive nuclei were counted under a fluorescence microscope and percent neutralisation was calculated as follows: %-neutralisation = 100 x $(V_0 - V_n)/V_0$, where V_n is the number of IE-positive nuclei in the wells containing virus and antibody, and V_0 is the number of IE-positive nuclei in the wells that were incubated with virus alone. In general, the infectious dose was adjusted to produce 1000 infected cells per well. Table 16 below summarizes the 50%-neutralising activities of various recombinant antibodies on hCMV representing different gB-genotypes. The monoclonal antibodies tested were found to neutralise hCMV gB-genotypes 1, 2 and 3 with comparable efficiency.

Table 16: 50%-neutralising activities of monoclonal recombinant antibodies against 3 different hCMV gB-genotypes

Monoclonal antibody	**50% neutralising activity (µg/ml)**		
	gB-genotype 1 (Towne strain)	gB-genotype 2 (AD169strain)	gB-genotype 3 (Altu isolate)
Ab-04	0.5	0.7	0.3
Ab-11	1.0	1.9	0.7
Ab-14	1.3	1.5	0.6
Ab-19	0.6	1.4	0.4
Ab-28	0.4	0.5	0.1
Ab-42	2.0	2.9	0.5
ITC88*	1.0	1.9	0.4

*(Ohlin *et al.*, 1993)

Example 7: Neutralisation of hCMV entry into endothelial, epithelial and dendritic cells of recombinant antibodies

The neutralisation assays described in the previous examples were all carried out using fibroblasts as target cells. To investigate whether the previously identified neutralising recombinant antibodies are capable of also neutralising the infection of endothelial, epithelial and dendritic cells, an endothelio- and epitheliotropic HCMV isolate TB40E (a generous gift from Dr. Christian Sinzger, Institute of Medical Virology and Epidemiology of Viral Diseases, University of Tübingen, Germany) was utilised. TB40E was propagated in HFFs and titers of infectivity in viral supernatants were determined as described by Mahy & Kangro (1996). Human umbilical vein endothelial cells (HUVEC) were cultured in endothelial cell basal medium EBM-2 supplemented with EGM-2MV-kit (Lonza, Verviers, Belgium) and were used for experiments at passage 4-7. Human ARPE-19 retinal pigment epithelial cells (ATCC CRL-2302) were propagated in Dulbecco´s modified Eagle medium: Nutrient Mixture F12, 1:1 mixture, supplemented with 2.5mM glutamine, 15mM Hepes buffer, pyridoxine HCl, 55mg/l sodium pyruvate, 10% fetal calf serum (heat-inactivated), 100IU/ml penicillin, 100µg/ml streptomycin (PAN-Biotech, Aidenbach, Germany). Primary dendritic cells (DC) were isolated as follows: Purified peripheral blood mononuclear cells (PBMCs) of HCMV-seronegative blood donors were incubated in RPMI-1640 medium supplemented with 2mM glutamine, 10mM Hepes buffer, 100IU/ml penicillin, 100µg/ml streptomycin in the presence of autologous serum (2% v/v, heat-inactivated) for 2h at 37°C. Following the incubation period, non-adhering cells were removed by washing with cell culture medium and adhering monocytes differentiated into DCs after the addition of IL-4 (25U/ml) and GM-CSF (800U/ml) (CellGenix Technologie Transfer GmbH, Freiburg, Germany) day two and four after isolation. On day six, neutralisation assays were performed by incubating

antibody and virus for 1h at 37°C as described above. The infection of DCs required a 500-fold higher amount of virus particles. The antibody-virus mixtures were added to the DCs followed by another incubation period of 12h. After fixation and permeabilization with ice-cold Methanol infected cells were stained using the monoclonal antibody E13 (Morphosys AbD GmbH, Duesseldorf, Germany) which is specific for the major immediate-early (IE) protein, UL123, of HCMV and FITC-conjugated anti-mouse IgG secondary antibodies (Jackson ImmunoResearch, West Grove, PA). Fluorescence activated cell sorting (FACS) was used as read-out. Both FlowJo 5.7.2. (Tree Star Inc., Ashland, OR) and Graph Pad Prism 4 (GraphPad Software, Inc., La Jolla, CA) were used for analysis. Results are summarized in Tables 17a and 17b.

Neutralisation assays with endothelial and epithelial cells were carried out as described in Example 6 using indirect immunofluorescence assay as a read-out; however the following modifications to that method were made: HUVECs were incubated in EBM-2 supplemented with EGM-2MV-kit without hFGF-B for 1h at 37°C prior to infection. This was necessary to remove FGF-associated heparin, which showed an inhibitory effect on infection. Furthermore, a 10-fold higher amount of viral particles (3000 pfu) was applied both for the infection of HUVECs and ARPE.

All antibodies tested were observed to neutralise the infection of endothelial, epithelial and dendritic cells with comparable efficiency to the neutralisation of HFFs (see Tables 17a and 17b below).

Table 17a: Comparison of 50% neutralising activities of the tested monoclonal antibodies in different cell types

mAb	50% neutralising activity (µg/ml)			
	Fibroblast cells (HFFs)	**Endothelial cells (HUVECs)**	**Epithelial cells (ARPE-19)**	**Dendritic cells (donor dependent°)**
Ab-02	0.3	0.3	0.3	0.2/0.4
Ab-04	0.5	0.4	0.3	0.3/0.5
Ab-11	1.3	1.3	0.3	0.2/1.0
Ab-14	0.4	1.0	0.4	0.2/1.0
Ab-19	0.6	0.6	0.9	n.d.
Ab-28	0.3	0.2	0.2	0.3/0.5
Ab-42	1.0	1.0	0.3	0.6/1.0
ITC88*	1.2	0.6	0.4	0.3/0.9

*(Ohlin *et al.*, 1993)

° Dendritic cells of six different HCMV seronegative donors were analysed. The lowest and highest IgG-concentrations for 50%-neutralisation are shown.

n.d.: not determined

Table 17b: Comparison of 50% neutralising activities of the tested monoclonal antibodies in different cell types

mAb	50% neutralising activity (µg/ml)			
	Fibroblast cells (HFFs)	**Endothelial cells (HUVECs)**	**Epithelial cells (ARPE-19)**	**Dendritic cells (donor dependent)**
Ab-47	0.1	0.4	1.0	n.d.
Ab-48	0.3	0.7	n.d.	n.d.
Ab-49	0.5	1.8	n.d.	n.d.
Ab-50	0.2	0.5	1.5	n.d.
C23*	0.2	0.6	0.8	n.d.

*C23 control antibody; n.d.: not determined

Example 8: Epitope characterisation of hCMV neutralising antibodies

8.1 Epitope-binding competition assay by Biacore of novel anti-hCMV antibodies

For epitope competition analysis, antibodies Ab-02 (18'900 RU), Ab-04 (16'800 RU) and Ab-28 (18'300 RU) were coupled to the flowcells of a CM5 sensor chip via standard amine-coupling. A control flowcell was prepared accordingly using an irrelevant human IgG_1 antibody (12'500 RU). Prior to the binding experiments, the flowcells were washed thoroughly with running buffer. The hCMV gB protein was captured onto the immobilized anti-gB antibodies for 180s at a flow rate of 5µl/min from the supernatant of a stable gB-producing CHO cell line 6-H5 (lot 080527 KS), which contained approximately 13µg/ml gB. Secondary reference antibodies ITC48 (recognising gB epitope AD-1), ITC52 (recognising gB epitope AD-1) and ITC88 (recognising epitope gB AD-2) were applied at a concentration of 1000nM. Binding was analysed in PBS with 0.02% BSA and 0.05% Tween 20 at a flow rate of 30µl/min. The surface was regenerated with 10mM glycine at pH 1.8. Binding curves were evaluated using Biacore® T100 Evaluation Software version 2.0.1. The results are summarised in Table 18 below where '+' indicates that the secondary binding antibody could bind to gB protein at the same time as the immobilised capture antibody.

The tested antibodies (immobilised capture antibody Ab-02, Ab-04 and Ab-28) all appear to bind gB epitopes that are outside of the gB epitopes AD-1 and AD-2 recognised by the ITC48, ITC52, ITC88 reference antibodies, since the reference antibodies were able to efficiently bind gB protein at the same time as the tested antibodies.

Table 18: Summary of antibody epitope mapping by Biacore®

Secondary binding Ab	ITC88	ITC52	ITC48
Immobilised capture Ab			
Ab-02	+	+	+
Ab-04	+	+	+
Ab-28	+	+	+

8.2 Analysis of the epitope recognised by the novel anti-hCMV antibodies

To further investigate whether or not the antibodies of the present invention recognise the known antigenic domains on gB, antigenic domain-1 (AD-1) and antigenic domain-2 (AD-2), an ELISA was performed. For this purpose, AD-1 (a procaryotically expressed fusion protein) and AD-2 (a synthetic peptide, pep90, amino acid sequence: NETIYNTTLKYGDVVGV (SEQ ID No: 197), Meyer *et al.*, 1992) were both coated at 1µg/ml on ELISA plates. 50µl of undiluted supernatant per well was incubated for 1h at room temperature. The ELISA was performed as described in Example 1 above. ELISA analysis revealed that the antibodies of the present invention are neither AD-1- nor AD-2-specific, thereby confirming the Biacore competition assay of Example 8.1.

In order to positively identify the epitopes recognised by the antibodies of the present invention, a mammalian expression vector encoding amino acids 100-447 of gB was constructed. This region comprises all amino acids in between the AD-1 and AD-2 gB epitopes. Transient transfection of Cos-7 cells with this expression construct was carried out with Lipofectamine™ 2000 (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions. At 48h post

transfection, the cells were washed twice with PBS, fixed and permeabilised with cold methanol. After washing with PBS, the cells were incubated with primary antibody (tested antibodies or control antibodies) for 45min at 37°C in a humid atmosphere. Following another washing step, slides were incubated with FITC-conjugated anti-human IgG or FITC-conjugated anti-mouse IgG (Jackson ImmunoResearch, USA) for 45min at 37°C in a humid atmosphere. After washing the slides with PBS, coverslips were applied using the DAPI containing mounting medium VECTASHIELD® (LINARIS GmbH, Wertheim-Bettingen, Germany).

Antibody binding was documented by fluorescence microscopy (Axioplan 2, Carl Zeiss MicroImaging GmbH, Jena). Recombinant, neutralising monoclonal antibodies Ab-02, Ab-04, Ab-28 were shown to specifically react with the truncated gB protein covering amino acids 100-447 (AD169; SEQ ID NO: 239). Therefore, the antibody-binding domain of the recombinant, neutralising monoclonal antibodies Ab-02, Ab-04, Ab-28 has positively been identified to be located within a region encoded by amino acids 100-447 of gB (numbering of gB strain AD169; SEQ ID NO: 239). This unequivocally demonstrates that the monoclonal antibodies Ab-01 to Ab-46 of the present invention react with a novel antigenic epitope of gB protein and not with known AD-1 and AD-2 epitopes of gB protein that are recognised by previously identified human monoclonal antibodies in the prior art.

8.3: Competition ELISA with anti-hCMV antibodies

To determine potential competition between a number of anti-hCMV antibodies versus the anti-hCMV antibody Ab-50 for binding to gB protein, an ELISA was performed using a method similar to that described in Example 1. In brief, a

sequential dilution of antibodies Ab-47, Ab-48, Ab-49, C23 (control), ITC52 (AD-1 specific), ITC88 (AD-2 specific), 89-104 (gH specific) or 89-109 (gH specific) was pre-incubated in PBS/2%FCS, with a constant concentration of Ab-50 (0.5ng per well; termed 'Antibody Mixture') in a 96-well plate to prevent premature binding of gB by any of the investigated antibodies. In addition, five wells of Ab-50 alone at a concentration of 5ng per well were prepared to determine the OD_{450} of Ab-50 without a potential competing antibody. 96-well ELISA-plates (Nunc) were coated with 25ng per well gB protein in carbonate buffer, pH9.6 for 16h at 4°C. The gB-coated plates were washed three times with PBS supplemented with 0.1% Tween (ELISA washing buffer) and blocked for 2h with PBS/2%FCS (ELISA buffer) and washed again three times with ELISA washing buffer. Plates were then incubated with 50µl Antibody Mixture diluted in PBS/2%FCS for 1h at 37°C. Following a further washing step, the binding of Ab-50 was revealed using anti-λ-specific secondary antibodies coupled with peroxidase (antibodies-online.com). After a 1h incubation period, unbound secondary antibody was removed by washing and the enzymatic activity was determined using tetramethylbenzidine (TMB) reagent at a concentration of 100µl per well (1:1 mix of TMB peroxidase substrate and peroxidase solution B, (KPL, Inc., USA). After incubation for 5min at room temperature, the reaction was stopped with 100µl 1M phosphoric acid per well. Absorption (optical density (OD)) was detected at 450nm using an Emax microplate reader and the software Softmax Pro 3.0 (Molecular Devices, USA) was used for analysis.

Where competition existed between the tested antibodies and Ab-50, a reduction of the OD_{450} signal in each Antibody Mixture when compared to Ab-50 alone was observed. Additionally, a gB ELISA was performed with identical IgG-

concentrations in order to visualise binding of all antibodies tested to gB. Here detection was performed using Fcγ fragment-specific secondary antibodies coupled with peroxidase (Jackson ImmunoResearch, USA). The results are shown in Figure 4 and demonstrate that Ab-50 competes for binding to gB protein with antibodies Ab-47 and Ab-49. No competition was observed between Ab-48 and Ab-50 (Figure 4a). In addition, no competition for binding to gB protein was observed between Ab-50 and antibodies ITC52 (AD-1 specific), ITC88 (AD-2 specific), 89-104 (gH specific), 89-109 (gH specific)(Figure 4b), indicating that Ab-50 recognises a different epitope on hCMV than these antibodies and therefore does not bind to AD-1 or AD-2 on gB protein.

Example 9: Structural model of hCMV gB

As there is currently no structural information available for the hCMV gB protein, a three dimensional model of the trimeric conformation of the ectodomain of HCMV gB strain AD169 (SEQ ID NO: 239) based on the crystal structure of HSV-1 gB was generated, which most likely represents the postfusion conformation (Heldwein *et al.*, 2006). This model of the hCMV gB postfusion structure was generated by standard homology modelling procedures using the program MODELLER (Eswar *et al.*, 2006), based on a sequence alignment with the template structure of HSV-1 gB (Heldwein *et al.*, *ibid*).

Glycoprotein B (gB) is the most conserved of all herpes virus envelope glycoproteins, and protein sequences of HSV-1 and HCMV gB share 28% identity and 40% similarity. The hCMV gB monomer consists of 906 amino acids (gB strain AD169; SEQ ID NO: 239), of which almost the entire ectodomain (residues Tyr_{89} to Val_{700}) is included in the model (Figure 3). The individual domains I to V, which were previously defined

based on the HSV-1 gB structure, can be clearly identified from the homology model of HCMV gB. Domain I (Dom I) (Ile_{133} to Thr_{343}) constitutes part of the trimer interface and is located proximal to the membrane. The discontinuous Dom II is composed of residues Leu_{121} to Asn_{132} and Cys_{344} to Ser_{438} (Figure 3). The flexible loops comprising residues Val_{306} to Glu_{317} and Leu_{439} to His_{468} are not included in the model, since they are not resolved in the template structure of HSV-1 gB. Dom III comprises three discontinuous segments, Ser_{95} to Cys_{111}, Asn_{477} to Ser_{549}, and Leu_{638} to Ser_{646} (Figure 3). Like in HSV-1 gB, its long α helix forms, together with the respective segments from the other monomers, form the interface of the trimer. Dom IV (Tyr_{89} to Cys_{94} and Cys_{550} to Asp_{637}) is located at the top of the molecule and contains the AD-1 epitope, while Dom V (Met_{647} to Asp_{698}) represents the bridging element between the extracellular part and the transmembrane helix. The overall structure of the HCMV gB monomer and also the arrangement of the subunits in the trimer are thus suggested to be highly similar to that of HSV-1 gB, as would be expected from the degree of sequence similarity.

The HSV-1 gB crystal structure was chosen as template for the modelling studies, as two crystal structures of the gB-proteins from HSV-1 (Heldwein *et al.*, *ibid*) and EBV (Backovic *et al.*, 2009) already exist. Both these proteins display a sequence identity of 30% and show a highly similar tertiary structure. The sequence identity of hCMV gB to these two proteins of known structure is 28 to 33%, strongly suggesting that hCMV gB also shares the same three-dimensional fold. HSV-1 gB was chosen as a modelling template because the resolution of the crystal structure is significantly better than that of EBV gB. The resulting model of HCMV gB was found

to exhibit a good local geometry and no steric clashes were detected. In addition, pairs of cysteines are located in disulfide-bonding distance indicating that not only the global structural features, but also local structural details were reflected correctly by the model. This model also provided the basis for the design of a construct that allowed the expression of Dom II as a single continuous peptide chain. For this domain, which is discontinuous in the primary amino acid sequence, a five-residue linker was designed to connect the two parts of the domain as described in Example 10 below.

Example 10: hCMV gB protein recognition by human sera

To investigate Dom I and Dom II for antibody binding, expression plasmids were constructed which allowed for the synthesis of either domain in eukaryotic cells. In both cases the cloning strategy involved the attachment of a HA-epitope tag at the amino terminus of the respective peptide in order to facilitate detection.

Example 10.1: Expression of hCMV gB protein Dom II and recognition by human sera

Based on the structural model of hCMV gB, it was analysed, whether recombinantly expressed gB Dom II would be immunogenic during natural infection. To this end a eukaryotic expression vector was constructed which allowed the expression of Dom II in mammalian cells. Dom II is a discontinuous epitope that is generated by amino acids 121-132 and amino acids 344-438 of gB strain AD169 (SEQ ID NO: 239). To express Dom II, the nucleotide sequences coding for the gB-specific residues 121-132 and 344-438 were joined by a nucleotide stretch encoding a flexible five amino acid linker (Ile-Ala-Gly-Ser-Gly; SEQ ID NO: 319). This nucleotide sequence was inserted into the expression vector

pcUL132sigHA, a pcDNA3.1-based vector containing the authentic signal sequence of the envelope glycoprotein gpUL132 of hCMV (amino acids 1-27; SEQ ID No: 320; Spaderna *et al.*, 2005), followed by an influenza hemagglutinin (HA)-epitope tag (YPYDVPDYA; SEQ ID NO: 321). The Dom II encoding nucleotide sequence was inserted downstream of the HA-tag sequence using the restriction sites *EcoRI* and *XbaI*. Correct protein expression from the plasmid gives rise to a HA-tagged Dom II fusion protein, which is transported through the endoplasmic reticulum and trans-golgi network, and thus properly modified by glycosylation. The coding region for the linker-coupled discontinuous Dom II was chemically synthesized (GeneArt, Regensburg, Germany).

To analyse the Dom II peptide for antibody recognition, Dom II was transiently expressed in Cos7 cells and analysed for reactivity in indirect immunofluorescence using 13 sera from randomly selected hCMV-seropositive donors. Cos7 cells grown on glass coverslips in 24-well plates were transfected with 0.8µg of the expression plasmid DNA encoding Dom II using Lipofectamine (Invitrogen, Karlsruhe, Germany). 48h after transfection the cells were fixed and permeabilised with ice-cold methanol. Patient sera were then added as primary antibody. Unbound serum antibodies were removed by three washing steps using PBS. Binding of the primary antibody from human sera was detected with the appropriate secondary antibody conjugated with FITC (fluorescein isothiocyanate) (Dako, Hamburg, Germany). Counterstaining of cell nuclei was done with DAPI (4',6-diamidino-2-phenylindole). Images were collected using a Zeiss Axioplan 2 fluorescence microscope fitted with a Visitron Systems charge-coupled device camera (Puchheim, Germany). Images were processed using MetaView software and Adobe Photoshop. Antibodies: Controls: gB-

specific human monoclonal antibody C23 (TI-23; Meyer *et al.*, 1990), gN-specific murine monoclonal antibody 14-16A (Mach *et al.*, 2000), and gH-specific murine monoclonal antibody SA4 (Urban *et al.*, 1992), murine anti-HA (Sigma Aldrich, Steinheim, Germany), and murine anti-GST (BIOZOL, Eching, Germany). A plasmid expressing total gB (amino acids 1-906) served as additional control. While all of the sera showed a positive reaction with whole gB, only four out of 13 sera stained positive for Dom II. This demonstrates that Dom II is responsible for inducing antibodies during natural hCMV infection.

To test the frequency of patient sera containing antibodies directed against gB Dom II on a larger panel of human sera and to compare it to the frequency of sera containing antibodies against the known antigenic domains of gB, AD-1 and AD-2, the Dom II coding sequence was bacterially expressed as a GST-fusion protein, purified and used in ELISA. Plasmids for the expression of Dom II-GST (Glutathione-S-transferase) fusion proteins in *E. coli* were generated using the expression vector pGEX-6P-1 (Pharmacia Biotech, Freiburg, Germany). Plasmid DNA was used to transform *E. coli* DH10B for expression of GST fusion proteins. The respective fusion proteins were induced and the soluble form of the protein was purified from *E. coli* lysates according to the manufacturer's instructions. To prepare an affinity matrix, 2.6mg of purified Dom II-GST fusion protein was dialysed against coupling buffer and conjugated to AminoLink Plus Coupling Resin (Thermo Fisher Scientific, Rockford, USA), according to the manufacturer's instructions. 4ml of an hCMV hyperimmune globulin preparation, diluted 1:3 (v/v) with PBS, was passed over 2ml antigen-coupled beads, followed by extensive washing with PBS. Bound IgG was eluted

with 0.2M Glycine-HCl, pH 3.0, in 1ml fractions and fractions were dialysed against PBS. Total IgG concentration was determined by an ELISA. In brief, polystyrene 96-well plates were coated with 100ng AffiniPure goat anti-human IgG, Fcγ-specific (Jackson Immuno Research, West Grove, USA) in 0.5M carbonate buffer, pH 9.6, overnight at 4°C. Serial log_2 dilutions of the eluted fractions in a volume of 50µl were added and bound IgG was detected by using a polyclonal peroxidase-conjugated goat $F(ab)_2$-fragment anti-human IgG, Fcγ-specific (Jackson Immuno Research, West Grove, USA). A human IgG preparation (Jackson Immuno Research, West Grove, USA) with known concentration was used as standard.

The purity of the Dom II-GST fusion protein was >90% as estimated from Coomassie staining of the protein following PAGE. A total of 80 randomly selected sera from hCMV seropositive individuals, as determined by a commercially available test, were analysed. Ten sera from hCMV negative donors served as negative controls. Within the serum panel from hCMV-seropositive individuals reactivity for gB was 100%, highlighting the high immunogenicity of this protein (Figure 5). In accordance with our previous observations, positive reaction with AD-1 of gB was also 100%. 57% of the sera contained antibodies against AD-2 (Schoppel *et al.*, 1996). The Dom II fusion protein was recognised by 94% of the sera. Thus, Dom II represents another highly immunogenic domain of gB. Since a procaryotically expressed fusion protein could be used as antigen, this would suggest that protein glycosylation is not essential for antibody binding. The differences in recognition frequency between the immunofluorescence analysis that was initially used for analysis of Dom II binding antibodies and the ELISA may be due to the different sensitivities of the assays.

For the sake of consistency in nomemclature of gB antigenic domains, Dom II was designated AD-4.

Example 10.2: Expression of hCMV gB protein Dom I with a HA tag and recognition by human sera

To express Dom I, the nucleotide sequence coding for amino acids 132-343 of gB strain AD169 (SEQ ID NO: 239) was inserted into expression vector pcUL132sigHA (described above) to generate the vector pcAD-5. To analyse the Dom I peptide for antibody recognition, Dom I was transiently expressed in Cos7 cells and analysed for reactivity by indirect immunofluorescence using the same method as that described in Example 10.1 above.

Dom I specific antibodies in human sera were measured in a capture ELISA. For recombinant antibody production, 293T cells in 75cm^2 flasks were transfected with 20µg of plasmid pcAD-5 DNA by calcium phosphate precipitation. The flasks were incubated for 6 days and then the cells and their supernatant were harvested. For the capture-ELISA, ELISA plates were coated with 125ng/well of mouse anti-HA monoclonal antibody (Sigma), washed, blocked and washed again, as described in Example 8.3 above, and then incubated with supernatant of transfected 293T cells (containing HA-tagged Dom I) for 2h at 37°C. Plates were then rinsed and incubated with human sera in a 1:50 dilution for 2h at 37°C. Unbound antibody was removed by washing and peroxidase-conjugated anti-human or anti-mouse IgG (Dako, Hamburg, Germany) was added at an appropriate dilution for 1h. The plate was then washed and 100µl TMB peroxidase substrate diluted 1:1 in peroxidase substrate solution B (KPL, Inc., USA) was added for 5min. The reaction was stopped by the

addition of 100µl 1M H_3PO_4 and the OD_{450} was determined using an Emax microplate reader (Eurofins MWG Operon, Ebersberg, Germany). The plates were washed and developed as described in Example 10.1 above. All antibodies were diluted in PBS with 2% FCS. Antibody binding analysed by indirect immunofluorescence confirmed that the four antibodies tested (Ab-47. Ab-48, Ab-49 and Ab-50) were reactive with the Dom I-specific peptide (results not shown).

Having identified Dom I as a new target to neutralising antibodies, clonal antibody supernatants from four individuals were re-tested to obtain information about the overall frequency of Dom I specific antibodies in hCMV-infected individuals. The frequency of Dom I specific memory B cells was variable among different donors; however 100% of Dom I antibodies (6/6) that were tested, showed neutralising activity (results not shown).

To obtain information on the frequency of recognition of Dom I antibodies, antibody reactivity was determined against Dom I in a larger serum panel of hCMV-infected individuals and compared to known antigenic domains. As described in Example 10.1 above, a total of 80 randomly selected sera from hCMV seropositive individuals were analysed (Figure 5). Dom I was recognised by 57% of the sera therefore indicating that this domain represents an antigenic domain on gB protein which induces antibodies with high frequency during infection.

For the sake of consistency in nomemclature of gB antigenic domains, Dom I was designated AD-5.

Example 11: Correlation between AD-4 (Dom II) antibody titer and neutralising capacity in human sera

Data in the literature support the assumption that gB is one of the dominant antigens with respect to induction of neutralising antibodies during natural infection and a correlation between anti-gB titer and neutralisation capacity has been reported (Marshall *et al.*, 1992). It is unclear whether this correlation resides on a variety of different antibody specificities directed against a number of different epitopes or whether a limited number of domains are responsible. To investigate whether Ad-4 (Dom II) specific antibodies contribute significantly to the overall neutralisation capacity of a given serum, we determined the neutralisation titer in the serum panel and correlated it to the ELISA titer against recombinant gB, AD-1, AD-2 and AD-4, respectively. Proteins were diluted between 25ng and 200ng (depending on antigen) in 0.5M sodium carbonate buffer, pH 9.6, or in 6M urea (AD-1) and 50µl was used to coat microtiter plates overnight at 4°C. All subsequent steps were carried out at room temperature. Reaction wells were rinsed with PBS supplemented with 0.1% Tween 20 and blocked for 2h with PBS containing 2% FCS. Plates were again rinsed with PBS supplemented with 0.1% Tween 20 and incubated with monoclonal antibodies, human serum, polyclonal eluted antibody fractions or mouse serum (50µl/well) for 2h. Unbound antibody was removed by washing and peroxidase-conjugated anti-human or anti-mouse IgG (Dako, Hamburg, Germany) was added at an appropriate dilution for 1h. The plate was washed and 100µl tetramethylbenzidine (TMB) peroxidase substrate, diluted 1:1 in peroxidase substrate solution B (KPL, Inc., USA), was added for 5min. The reaction was stopped by the addition of 100µl 1M H_3PO_4 and the OD_{450} was determined using Emax microplate reader (Eurofins MWG Operon, Ebersberg, Germany).

Dilution of all antibodies was done in PBS with 2% FCS. In all assays involving gB fusion proteins, the respective prokaryotic fusion partner was assayed in parallel and the optical density subtracted from values obtained with the gB-fusion protein.

As reported previously, there was a correlation between recognition of gB in ELISA and neutralisation capacity (Marshall *et al., ibid*). The analysis also showed statistically significant correlation between neutralisation capacity and antibody binding titer against AD-1 and AD-4 (Dom II) but not AD-2 (Figure 6).

Example 12: AD-4 (Dom II) induces virus neutralising antibodies during natural infection

To investigate in more detail the question of whether AD-4 induces virus neutralising antibodies during natural infection we used two approaches: First, we isolated polyclonal anti-AD-4 antibodies from a pooled human IgG preparation using the purified AD-4-GST (Dom II-GST) fusion protein as affinity matrix. As expected, the pooled human IgG preparation contained antibodies reactive with a number of different hCMV-specific envelope glycoproteins in indirect immunofluorescence analysis following transient expression of the respective glycoprotein complexes in Cos-7 cells. Second, we tested the gB-specific human monoclonal antibodies disclosed in this invention for binding to AD-4. All of the recombinant antibodies were found to bind to AD-4 in indirect immunofluorescence using the transiently expressed AD-4 protein in Cos7 cells. Therefore, AD-4 represents a conformational epitope that is recognised by the human monoclonal antibodies disclosed in this invention.

To prepare a matrix for the isolation of AD-4-specific antibodies, 2.6mg of the purified AD-4-GST fusion protein was covalently coupled to Sepharose and used to affinity purify AD-4-specific IgG from 4ml of the human IgG preparation. A total of 127µg IgG was obtained. ELISA tests verified that the affinity purified IgG fraction (E3) showed specific binding to AD-4 and gB but not to AD-1 and AD-2 (Figure 7a). To further exclude contamination of the affinity purified AD-4 antibodies with antibodies directed against additional neutralisation-relevant antigens on hCMV we performed indirect immunofluorescence analysis with Cos7 cells transiently expressing gH or the gM/gN complex; viral envelope proteins which are known to induce neutralising antibodies during natural infection (Shimamura *et al.*, 2006; Urban *et al.*, 1996). The purified polyclonal anti-AD-4 IgG fraction did not contain detectable antibodies specific against non-gB envelope complexes. Moreover, the IgG fraction was free of IgM (not shown). The affinity purified IgG preparation (E3) was then tested in neutralisation assays. 50% neutralisation of virus infectivity was achieved at IgG concentrations of approximately 0.2µg/ml, which is within the range of the potent gB-specific human monoclonal antibodies disclosed in this invention (Figure 7b). In comparison, the original serum pool from which the affinity purified IgG fraction was derived showed 50% neutralisation of input virus at approximately 200µg/ml IgG (Figure 7b). In summary, these data provide further evidence that AD-4 not only represents a highly immunogenic domain of gB, but it is also the target of virus neutralising antibodies.

Example 13: Specificity of human recombinant antibody binding

13.1: Fine specificity of human recombinant antibodies binding to AD-4

The size of AD-4 (>100 amino acids) is large enough to harbour several antibody binding epitopes. Close proximity of epitopes that can be bound by neutralising and non-neutralising antibodies have been found for AD-1 and AD-2 of gB and have been implicated as a mechanism to evade effective virus neutralisation. Thus, it was of interest to obtain more information on potential epitopes within AD-4. Initial attempts to shorten AD-4 by omission of amino acids 121-132 at the amino terminal end or the last five amino acids at the carboxy terminal end resulted in complete loss of antibody binding indicating that only the entire domain is capable of forming the antibody binding structure. To identify potentially critical antibody contact residues within AD-4, a number (n=17) of eukaryotic expression plasmids was constructed which expressed AD-4 mutant peptides, in each of which two adjacent surface exposed residues were changed to alanine (Figure 8). Surface exposure of residues was identified from the hCMV gB model as described in Example 9.

When the human monoclonal antibodies Ab-11, Ab-14, and Ab-28 were tested in indirect immunofluorescence, following transient expression of the respective mutant AD-4 proteins, two patterns of recognition were found. Antibody Ab-28 bound to all AD-4 mutant proteins, whereas the remaining antibodies did not recognise the mutants in which the lysine residues 378 and 379 were exchanged for alanine. This proves that AD-4 is recognised by the recombinant human monoclonal antibodies disclosed in this specification.

In addition, these data indicate that the di-lysine sequence (K378K379) within AD-4 represents a critical antibody binding site. However, the signals obtained in indirect immunofluorescence are difficult to quantify and there was a possibility that the lack of reaction with the AD-4G mutant peptide was just reflecting reduced binding of antibodies below the detection limit of the assay. Thus, to obtain more quantitative data and to confirm the results obtained by indirect immunofluorescence, five of the AD-4 mutant proteins were expressed as GST-fusion proteins, purified and used in an ELISA. Purity of the GST-fusion proteins was comparable for all peptides to the wild type AD-4 shown above in Figure 5. Besides the AD-4G (K378K379) mutant, the fusion proteins included AD-4H (Q380E381), AD-4E (E359D362) and AD-4I (N383S385), which affect sterically adjacent residues, as well as the more distantly located mutant AD-4L (N405T406). The results obtained by the ELISA were in accordance with the immunofluorescence data. With the exception of AD-4G, the mutant proteins were recognised by the monoclonal antibodies with comparable efficiency. AD-4G however, was only recognised by Antibody Ab-28 (Figure 9a). Interestingly, the affinity purified IgG fraction showed similar reactivity with all AD-4 fusion proteins indicating that the majority of antibodies bound independently of the AD-4G mutation (Figure 9b). The affinity purified IgG preparation was derived from a serum pool of an unknown number of donors and thus individual differences in the recognition of the AD-4 epitopes would expect to be averaged.

To obtain more information of potential AD-4 epitope specificity, we tested whether the two lysines at positions 378 and 379 are also important residues for reactivity with antibodies present in individual human sera. To this end, the

five AD-4 mutant peptides were tested with the same serum panel as above and a quotient was calculated between the lowest and highest absorbance value for each serum. As can be seen in Figure 10, most sera recognised the five AD-4 peptides with similar efficiency resulting in a low maximal difference of 1 to 5 fold. However, five sera showed maximal differences in the range of 10 to 100 fold. Interestingly, the difference was due to a reduced recognition of the mutant protein AD-4G with one exception. The serum with the highest maximal difference showed reduced binding to the mutant protein AD-4H (Q380E381), which in the 3D model of AD-4 is situated in close proximity to the di-lysine motif in AD-4G (K378K379). Thus, although binding of a few sera showed almost complete dependence on the original gB peptide sequence between 378 and 381, recognition of AD-4 by individual human sera was to a large extent independent of this sequence. In line with this result was the finding that IgG fractions that were affinity purified using the AD-4G mutant protein as antigen had a similar recognition pattern in ELISA of AD-4 mutant proteins and comparable neutralisation titer as IgG purified on AD-4 wild type.

13.2: Fine specificity of human recombinant antibodies binding to AD-5 (Dom I)

Similar experiments to those described in Example 13.1 above could not be performed with AD-5 (Dom I) because, in contrast to AD-4, AD-5 does not fold correctly after prokaryotic expression and therefore antigen for affinity purification could not be generated. However, it was possible to subdivide AD-5 into two domains, which were then tested for antibody recognition. Based on structural information, AD-5 was divided into Subdomain 1 (AD-5-S1), which comprised amino acids 133-144 and 251-343 of gB protein AD169 (SEQ ID No:

239) and Subdomain 2 (AD-5-S2), which comprised amino acids 140-255 of gB protein AD169 (SEQ ID No: 239).

In order to determine whether the AD-5 antibodies described in the present invention recognised these subdomains, a capture ELISA was performed as described in Example 10.2 wherein 293T cells were transfected with subdomains AD-5-S1 and AD-5-S2, as well as AD-4 + AD-5. Recombinant AD-5 antibodies Ab-47 to Ab-50 were used for detection rather than patient sera. Results of indirect immunofluorescence performed showed that antibodies Ab-47 and Ab-50 recognised AD-5-S1 (results not shown). Results from the capture ELISA are shown in Figure 11, and these show that all of antibodies Ab-47 to Ab-50 recognise gB AD-4 + AD-5 and that antibodies Ab-47, Ab-49 and Ab-50 recognise gB AD-5 and in particular AD-5-S1. None of antibodies Ab-47 to Ab-50 recognised gB AD-5 -S2. From these results it can be deduced therefore that antibodies Ab-47, Ab-49 and Ab-50 recognise an epitope on gB protein that is located in AD-5-S1, i.e. within amino acids 133-144 and 251-343 of gB protein (strain AD169; SEQ ID No: 239).

Example 14: Model of a prefusion conformation of hCMV gB

Within the structure of hCMV gB that was modelled according to HSV-1 gB, AD-4 is situated at a hump in the middle of the molecule. The di-lysine motif is easily accessible at the surface. In the absence of data on the orientation of AD-4 binding antibodies in the 3D space it can be predicted that antibody binding in this protein conformation may influence the interaction of gB with neighbouring proteins. For a number of herpes viruses, including hCMV, it is known that gB needs to interact with additional envelope glycoproteins in order to properly function during the fusion process

(Avitabile *et al.*, 2009; Patrone *et al.*, 2007). Most probably, however, the HSV-1 gB represents the postfusion conformation. This assumption is based on the structural homology of HSV-1 gB to VSV-G for which both post- and prefusion structures are available (Roche *et al.*, 2006 & 2007). It is thought that the prefusion form is prevalent on the virion whereas the postfusion form resides mainly in some, as yet unidentified, cellular compartment. AD-4-specific antibodies can apparently recognise both gB conformations since they bind to cellular as well as viral forms of gB. Since for VSV-G the pre- and postfusion forms show extensive structural rearrangements of individual protein domains we modelled the prefusion form of HCMV gB in order to gain more insight into the potential localisation of AD-4 within the prefusion trimer and the position of the residues which are important for antibody binding.

Single hCMV gB domains I, II, III and IV were taken from the postfusion model and superimposed on the prefusion structure of VSV-G (Roche *et al.*, 2007) using the MultiProt algorithm (Shatsky *et al.*, 2004). HCMV gB domain V and residues Leu469 to Arg496 of domain III were excluded, since they are not globular and there are no equivalent structures present in the VSV-G template. Connection loops between the individually fitted domains were modelled with ModLoop (Fiser *et al.*, 2003). The trimeric prefusion model was obtained by applying the VSV-G prefusion geometry.

While the postfusion conformation of hCMV gB can readily be modelled based on the homologous crystal structure of HSV-gB, there is no experimental structural information yet available for the prefusion conformation of this family of molecules. From the two conformations of VSV-G it is known that the

individual protein domains keep their folds but the relative arrangement of the domains drastically changes upon transition between pre-fusion and post-fusion state. In order to gain further insights into the potential localization of AD-4 within the prefusion trimer and the position of the residues, which are important for antibody binding, a hypothetical model of the hCMV gB prefusion conformation was created. To this end, the individual domain folds were obtained from the postfusion model and employed information about the domain arrangement from the prefusion structure of glycoprotein G from VSV-G (Roche *et al.*, ibid). The resulting model exhibits no steric clashes and the linking sequences are sufficiently long to connect the domains in this alternative geometry suggesting that this domain arrangement is structurally feasible in hCMV gB. The domain arrangement of the hCMV gB prefusion structure is highly similar to that of a previous model of the EBV gB pre-fusion conformation that was generated based on the respective post-fusion crystal structure (Backovic *et al.*, 2009).

A key difference between the pre-fusion and post-fusion conformation of hCMV gB is found in the composition of the apical part of the protein. In the post-fusion conformation this region is formed by domain IV, in which AD-1 is located. In contrast, in the pre-fusion model Dom II/AD-4 is situated on top of the molecule, with the di-lysine motif (Lys378, Lys379) being located in a central position on the apical surface of Dom II/AD-4. Thus, the spatial organisation of bound IgG molecules to the pre-fusion form of gB is likely to be different from the post-fusion form and may interfere with binding of gB to components of the target cell. Apart from blocking interactions with non-gB molecules, IgG molecules bound to Dom II/AD-4 may also be capable of constraining the

conformational changes within the protein that may be necessary for proper function.

Example 15: Neutralisation assays in the presence of human sera

In a clinical setting, the antibodies of the present invention may be administered prophylactically or therapeutically by means of intravenous infusion. Therefore the possibility that antibody function is impaired by the presence of antibodies in human serum, needs to be excluded. Three different types of sera were examined: serum negative for hCMV-specific antibodies, serum positive for hCMV-specific antibodies and Intratect® (Biotest AG), a hCMV serum preparation enriched with CMV-specific antibodies. First the IgG concentration and the 50% neutralising activity of each analysed serum was determined by titration. Briefly, ELISA plates were coated with anti human IgG, Fcγ fragment-specific catching antibody (Jackson ImmunoResearch, USA). Two-fold serial dilutions of sera in ELISA buffer were compared to an IgG standard of known concentration (Jackson ImmunoResearch, USA). The IgG-concentration was calculated using the ELISA software Softmax Pro 3.0 (Molecular Devices, Sunnyvale, CA, USA). The 50% neutralization activities of the sera were determined by performing a luciferase-based neutralization assay as described above in Example 2.

Next, in a competitive neutralisation assay as previously described in Example 2, antibody Ab-28 was titrated so that it would cross the 50% neutralisation mark, prior to the addition of serum at a constant concentration. Sera that were either negative or positive for hCMV-specific antibodies and Intratect® were added to the titrated Ab-28 at a constant concentration around their respective 50%-neutralising

activities. As is shown in Figure 12, there appeared to be no inhibition of Ab-28 neutralising potency by serum antibodies. After adding hCMV-negative serum to titrated Ab-28, the curve looked identical to the curve of Ab-28 alone. This result indicates that there is no unspecific reagent in human serum that might impair the neutralising capacity of Ab-28. When adding hCMV-positive serum or Intratect® to Ab-28, an enhancement of both Ab-28 and serum neutralising activity was observed. Serum neutralising activity was increased by about 20-40% in the presence of Ab-28 and the neutralising capacity of Ab-28 was increased by about 15% after the addition of hCMV positive sera or Intratect®.

Example 16: Post-adsorption neutralisation assay

To determine whether the antibodies of the present invention might block an early stage of virus penetration into cells, we performed a post-adsorption neutralisation assay. The method of the neutralisation assay was similar to that described in Example 2; however initially HFFs and the luciferase expressing hCMV were incubated for 1h at 4°C to allow only virus adsorption but not fusion of virus- and cell membrane. After this adsorption period, non-adsorbed virus was washed away with 1xPBS. The antibodies were titrated from very high IgG-concentrations of 150µg/ml to 5µg/ml in a separate plate and then added to the pre-adsorbed virus-cell mixtures. Antibodies Ab-02, Ab-28, Ab-04 and a control antibody C23 (T123; a kind gift from Teijin Pharma Limited, Japan) were used for this experiment and each antibody was incubated for 30, 80 or 120min at 4°C with the pre-adsorbed virus-cell mixtures. The AD-2-specific antibody C23 has been shown to inhibit virus penetration into cells (Ohizumi *et al.*, 1992). After the 30, 80 or 120min incubation periods, plates were washed once more and then incubated for 48h at

37°C. From this point on, the assay was continued as per Example 2. The results are shown in Figure 13 and are summarised as follows. After an incubation period of 30min, at least 100µg/ml of each antibody was required to achieve 50% reduction of virus infectivity. C23, on the other hand, appeared to inhibit virus penetration even at very low concentrations of 5µg/ml after an incubation of 30min. After 80min only 20µg/ml of Ab-02 and Ab-28 was needed for 50% neutralization. For Ab-04 however, 55µg/ml was required to neutralize virus infectivity by 50%. The 80min curve of the control antibody C23 did not show the expected result. It would be expected that this antibody would be at least as good, if not better after a longer incubation time. Also, the standard errors of the mean were very disperse for C23 at the 80 min incubation period and therefore this time point was excluded from the results. When incubating the pre-adsorbed virus with the antibodies for 120min only around 5-15µg/ml of each antibody or C23 is required for a 50% reduction of viral infectivity. In conclusion, the antibodies Ab-02, Ab-28 and Ab-04 are able to prevent penetration of already adsorbed virus into cells.

Example 17: Competitive neutralisation assays with other gB-specific antibodies

Competition for gB-epitope binding between neutralizing and non-neutralizing antibodies has been reported for AD-1-specific antibodies (Ohlin *et al.*, 1993). To investigate possible competitive or even synergistic effects between antibodies of the present invention and other gB-specific antibodies, competitive neutralization assays were performed to determine the effect of AD-1 (ITC52) and AD-2-specific (ITC88) antibodies on the neutralizing activity of the antibodies of the present invention. To do this, one antibody

was titrated and the other antibody added at a constant concentration around its 50%-neutralizing activity. These competitive neutralisation assays were conducted with each of the antibodies: Ab-11, Ab-14, Ab-19, Ab-28, Ab-04, Ab-42. Two different approaches were compared whereby the test antibody was titrated and ITC52 or ITC88 were added at a constant concentration or the ITC-antibodies were titrated and the test antibody was added at a constant concentration. Since ITC52 is a non-neutralizing antibody, it was added at a concentration of 3µg/ml, the same concentration at which the neutralizing antibody ITC88 was added. Only the data for Ab-28 are shown, since the other antibodies tested behaved similarly. Also, only one approach is shown, namely leaving Ab-28 at a constant concentration and titrating the ITC-antibody, because the alternative approach showed comparable results (Figure 14).

The results indicate that there appears to be a slight impairment of Ab-28´s neutralizing activity in the presence of a high concentration of the AD-1-specific antibody ITC52. This effect was observed for each antibody tested and has been reproduced in a further independent experiment (data not shown). However, a high concentration of ITC52 appears not to decrease the neutralization activity of ITC88. Ab-28 and ITC88 together resulted in an improved neutralization activity. This is particularly visible at the second data point of ITC88 alone as opposed when mixed. A 40% increase of ITC88´s neutralizing activity in the presence of Ab-28 is observed at this point with Ab-28 also showing an increase in neutralisation of around 15% with ITC88 than by itself.

In addition to the above experiment analyzing potential inhibitory, additive or synergistic effects between different

AD-4-specific antibodies and AD-1 or AD-2-specific antibodies, we also investigated whether similar effects could be observed between AD-4 (Dom II) and AD-5 (Dom I)-specific antibodies. Ab-28 (AD-4-specific) was mixed with either Ab-50 or Ab-49 (AD-5-specific) in a ratio of 50:50 in the first well and continued with 1:2 serial dilutions before adding luciferase-expressing hCMV. The neutralising activities of these antibody mixtures were compared to the single titrations of the respective antibodies (Figure 15). Only a slight additive effect between AD-4- and AD-5-specific antibodies was observed with no inhibitory or synergistic effects observed. This experiment was repeated on two further occasions with comparable results (data not shown).

Table 19: Summary of the SEQ ID numbers in the accompanying Sequence Listing, for the heavy and light chain CDRs of the neutralising antibodies shown in Tables 7 and 13 above.

	SEQ ID NO:					
Antibody	**CDRH1**	**CDRH2**	**CDRH3**	**CDRL1**	**CDRL2**	**CDRL3**
Ab-01	38	39	40	103	104	105
Ab-02	8	9	10	98	99	100
Ab-03	3	4	5	118	119	120
Ab-04	13	14	15	103	104	105
Ab-05	38	39	40	123	124	125
Ab-06	43	44	45	128	129	130
Ab-07	53	54	55	133	134	135
Ab-08	8	9	10	138	139	140
Ab-09	53	54	55	143	144	145
Ab-10	53	54	55	93	94	95
Ab-11	18	19	20	108	109	110
Ab-12	58	59	60	108	109	110
Ab-13	63	64	65	108	109	110
Ab-14	23	24	25	113	114	115
Ab-15	23	24	25	148	149	150
Ab-16	68	69	70	153	154	155
Ab-17	68	69	70	148	149	150
Ab-18	43	44	45	158	159	160
Ab-19	43	44	45	163	164	165
Ab-20	43	44	45	98	99	100
Ab-21	43	44	45	168	169	170
Ab-22	43	44	45	138	139	140
Ab-23	8	9	10	163	164	165
Ab-24	8	9	10	168	169	170
Ab-25	8	9	10	158	159	160
Ab-26	8	9	10	128	129	130
Ab-27	43	44	45	108	109	110

Ab-28	3	4	5	93	94	95
Ab-29	48	49	50	93	94	95
Ab-30	3	4	5	143	144	145
Ab-31	53	54	55	118	119	120
Ab-32	3	4	5	133	134	135
Ab-33	13	14	15	173	174	175
Ab-34	38	39	40	173	174	175
Ab-35	73	74	75	103	104	105
Ab-36	73	74	75	173	174	175
Ab-37	13	14	15	123	124	125
Ab-38	73	74	75	123	124	125
Ab-39	13	14	15	178	179	180
Ab-40	38	39	40	178	179	180
Ab-41	73	74	75	178	179	180
Ab-42	28	29	30	108	109	110
Ab-43	78	79	80	108	109	110
Ab-44	83	84	85	108	109	110
Ab-45	88	89	90	108	109	110
Ab-46	33	34	35	108	109	110
Ab-47	243	244	245	263	264	265
Ab-48	248	249	250	268	269	270
Ab-49	253	254	255	273	274	275
Ab-50	258	259	260	278	278	280

Table 20a

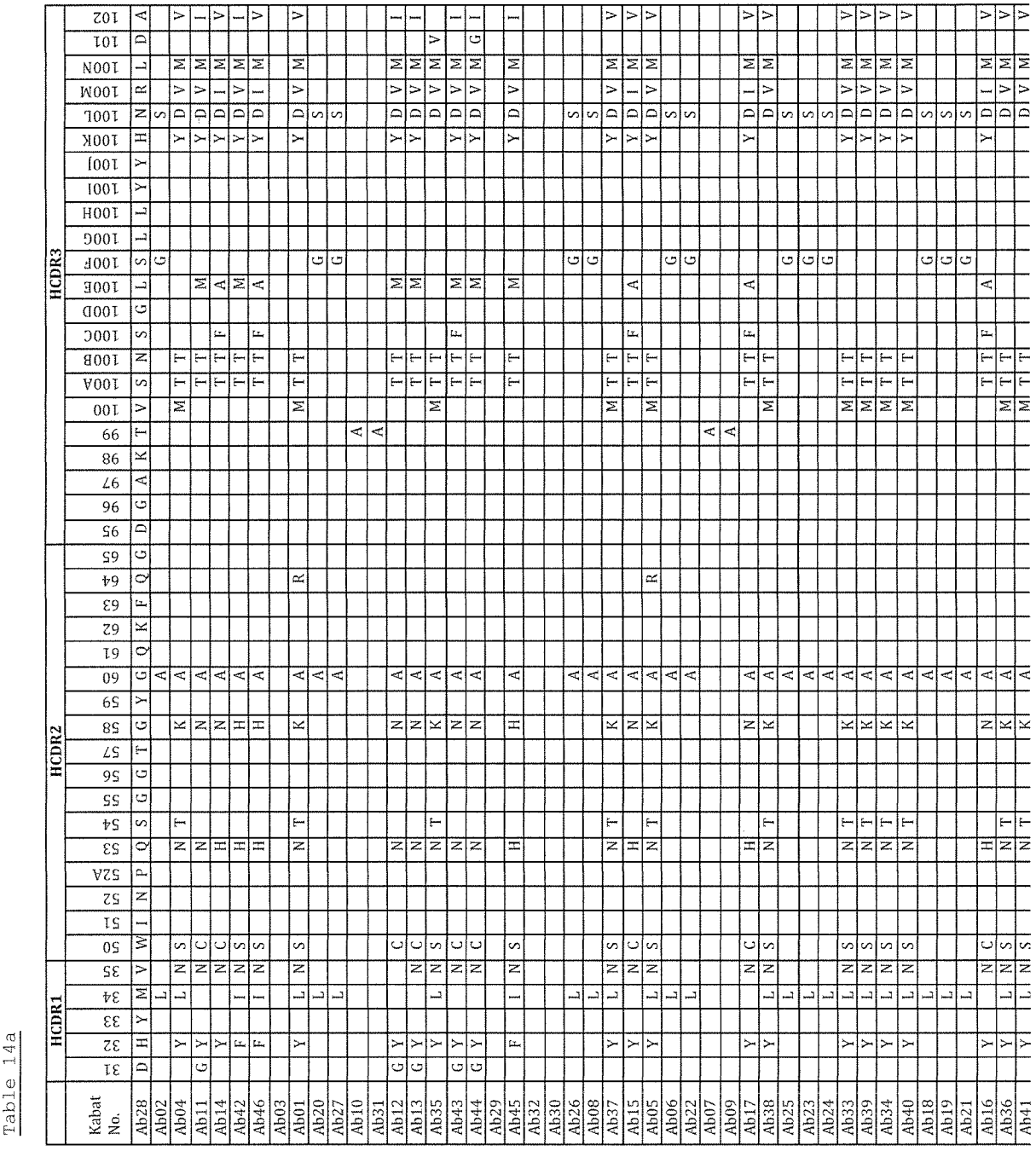


Table 14a

Table 20b

	LCDR1													LCDR2							LCDR3										
Kabat No.	24	25	26	27	27A	27B	28	29	30	31	32	33	34	50	51	52	53	54	55	56	89	90	91	92	93	94	95	95A	95B	96	97
Ab28	S	G	S	S	S	N	I	G	K	N	Y	V	S	D	N	N	K	R	P	S	G	T	P	D	R	S	L	S	-	V	I
Ab02				R																			W		S						V
Ab04																					A		W		S			G			V
Ab11																							W		S						V
Ab14																					A		W								V
Ab42																							W		S						V
Ab46																							W		S						V
Ab03																															V
Ab01																					A		W		S			G			V
Ab20				R																			W		S						V
Ab27																							W		S						V
Ab10																															
Ab31																															V
Ab12																							W		S						V
Ab13																							W		S						V
Ab35																					A		W		S			G			V
Ab43																							W		S						V
Ab44																							W		S						V
Ab29																															
Ab45																							W		S						V
Ab32																															V
Ab30																															V
Ab26				R																			W		S						V
Ab08				R																			W		S						V
Ab37																					A		W		S			G			V
Ab15																				P	A		W								V
Ab05																					A		W		S			G			V
Ab06				R																			W		S						V
Ab22				R																			W		S						V
Ab07																															V
Ab09																															V
Ab17																				P	A		W								V
Ab38																					A		W		S			G			V
Ab25				R																			W		S						V
Ab23				R																			W		S	D			A	Y	V
Ab24				R																			W		S						V
Ab33			N																		A		W		S			G			V
Ab39																					A		W		S			G			V
Ab34			N																		A		W		S			G			V
Ab40																					A		W		S			G			V
Ab18				R																			W		S						V
Ab19				R																			W		S	D			A	Y	V
Ab21				R																			W		S						V
Ab16																					A		W								V
Ab36			N																		A		W		S			G			V
Ab41																					A		W		S			G			V

Sequences

V_H domain, V_L domain and CDR sequences of binding members are shown in the appended sequence listing, in which SEQ ID NOS correspond as follows:

1	SM5-1 V_H nucleotide	26	SM11-17 VH nucleotide
2	SM5-1 V_H amino acid	27	SM11-17 VH amino acid
3	SM5-1 V_H CDR 1 aa	28	SM11-17 VH CDR 1 aa
4	SM5-1 V_H CDR 2 aa	29	SM11-17 VH CDR 2 aa
5	SM5-1 V_H CDR 3 aa	30	SM11-17 VH CDR 3 aa
6	SM4-10 V_H nucleotide	31	SM11-21 VH nucleotide
7	SM4-10 V_H amino acid	32	SM11-21 VH amino acid
8	SM4-10 V_H CDR 1 aa	33	SM11-21 VH CDR 1 aa
9	SM4-10 V_H CDR 2 aa	34	SM11-21 VH CDR 2 aa
10	SM4-10 V_H CDR 3 aa	35	SM11-21 VH CDR 3 aa
11	SM6-5 V_H nucleotide	36	SM6-11 VH nucleotide
12	SM6-5 V_H amino acid	37	SM6-11 VH amino acid
13	SM6-5 V_H CDR 1 aa	38	SM6-11 VH CDR 1 aa
14	SM6-5 V_H CDR 2 aa	39	SM6-11 VH CDR 2 aa
15	SM6-5 V_H CDR 3 aa	40	SM6-11 VH CDR 3 aa
16	SM1-6 VH nucleotide	41	SM4-3 VH nucleotide
17	SM1-6 VH amino acid	42	SM4-3 VH amino acid
18	SM1-6 VH CDR 1 aa	43	SM4-3 VH CDR 1 aa
19	SM1-6 VH CDR 2 aa	44	SM4-3 VH CDR 2 aa
20	SM1-6 VH CDR 3 aa	45	SM4-3 VH CDR 3 aa
21	SM3-1 VH nucleotide	46	SM5-3 VH nucleotide
22	SM3-1 VH amino acid	47	SM5-3 VH amino acid
23	SM3-1 VH CDR 1 aa	48	SM5-3 VH CDR 1 aa
24	SM3-1 VH CDR 2 aa	49	SM5-3 VH CDR 2 aa
25	SM3-1 VH CDR 3 aa	50	SM5-3 VH CDR 3 aa

	51	SM5-9 VH nucleotide		76	SM11-18 VH nucleotide
	52	SM5-9 VH amino acid		77	SM11-18 VH amino acid
	53	SM5-9 VH CDR 1 aa		78	SM11-18 VH CDR 1 aa
	54	SM5-9 VH CDR 2 aa		79	SM11-18 VH CDR 2 aa
	55	SM5-9 VH CDR 3 aa		80	SM11-18 VH CDR 3 aa
	56	SM1-7 VH nucleotide		81	SM11-19 VH nucleotide
	57	SM1-7 VH amino acid		82	SM11-19 VH amino acid
	58	SM1-7 VH CDR 1 aa		83	SM11-19 VH CDR 1 aa
	59	SM1-7 VH CDR 2 aa		84	SM11-19 VH CDR 2 aa
	60	SM1-7 VH CDR 3 aa		85	SM11-19 VH CDR 3 aa
	61	SM1-8 VH nucleotide		86	SM11-20 VH nucleotide
	62	SM1-8 VH amino acid		87	SM11-20 VH amino acid
	63	SM1-8 VH CDR 1 aa		88	SM11-20 VH CDR 1 aa
	64	SM1-8 VH CDR 2 aa		89	SM11-20 VH CDR 2 aa
	65	SM1-8 VH CDR 3 aa		90	SM11-20 VH CDR 3 aa
	66	SM3-4 VH nucleotide		91	SM5-1 VL nucleotide
	67	SM3-4 VH amino acid		92	SM5-1 VL amino acid
	68	SM3-4 VH CDR 1 aa		93	SM5-1 VL CDR 1 aa
	69	SM3-4 VH CDR 2 aa		94	SM5-1 VL CDR 2 aa
	70	SM3-4 VH CDR 3 aa		95	SM5-1 VL CDR 3 aa
	71	SM6-23 VH nucleotide		96	SM4-10 VL nucleotide
	72	SM6-23 VH amino acid		97	SM4-10 VL amino acid
	73	SM6-23 VH CDR 1 aa		98	SM4-10 VL CDR 1 aa
	74	SM6-23 VH CDR 2 aa		99	SM4-10 VL CDR 2 aa
	75	SM6-23 VH CDR 3 aa		100	SM4-10 VL CDR 3 aa

	101	SM6-5 VL nucleotide		126	SM4-3 VL nucleotide
	102	SM6-5 VL amino acid		127	SM4-3 VL amino acid
	103	SM6-5 VL CDR 1 aa		128	SM4-3 VL CDR 1 aa
	104	SM6-5 VL CDR 2 aa		129	SM4-3 VL CDR 2 aa
	105	SM6-5 VL CDR 3 aa		130	SM4-3 VL CDR 3 aa
	106	SM1-6 VL nucleotide		131	SM5-9 VL nucleotide
	107	SM1-6 VL amino acid		132	SM5-9 VL amino acid
	108	SM1-6 VL CDR 1 aa		133	SM5-9 VL CDR 1 aa
	109	SM1-6 VL CDR 2 aa		134	SM5-9 VL CDR 2 aa
	110	SM1-6 VL CDR 3 aa		135	SM5-9 VL CDR 3 aa
	111	SM3-1 VL nucleotide		136	SM4-12 VL nucleotide
	112	SM3-1 VL amino acid		137	SM4-12 VL amino acid
	113	SM3-1 VL CDR 1 aa		138	SM4-12 VL CDR 1 aa
	114	SM3-1 VL CDR 2 aa		139	SM4-12 VL CDR 2 aa
	115	SM3-1 VL CDR 3 aa		140	SM4-12 VL CDR 3 aa
	116	SM5-6 VL nucleotide		141	SM5-5 VL nucleotide
	117	SM5-6 VL amino acid		142	SM5-5 VL amino acid
	118	SM5-6 VL CDR 1 aa		143	SM5-5 VL CDR 1 aa
	119	SM5-6 VL CDR 2 aa		144	SM5-5 VL CDR 2 aa
	120	SM5-6 VL CDR 3 aa		145	SM5-5 VL CDR 3 aa
	121	SM6-48 VH nucleotide		146	SM3-2 VL nucleotide
	122	SM6-48 VH amino acid		147	SM3-2 VL amino acid
	123	SM6-48 VH CDR 1 aa		148	SM3-2 VL CDR 1 aa
	124	SM6-48 VH CDR 2 aa		149	SM3-2 VL CDR 2 aa
	125	SM6-48 VH CDR 3 aa		150	SM3-2 VL CDR 3 aa

151 SM3-4 VL nucleotide
152 SM3-4 VL amino acid
153 SM3-4 VL CDR 1 aa
154 SM3-4 VL CDR 2 aa
155 SM3-4 VL CDR 3 aa

156 SM4-1 VL nucleotide
157 SM4-1 VL amino acid
158 SM4-1 VL CDR 1 aa
159 SM4-1 VL CDR 2 aa
160 SM4-1 VL CDR 3 aa

161 SM4-5 VL nucleotide
162 SM4-5 VL amino acid
163 SM4-5 VL CDR 1 aa
164 SM4-5 VL CDR 2 aa
165 SM4-5 VL CDR 3 aa

166 SM4-7 VL nucleotide
167 SM4-7 VL amino acid
168 SM4-7 VL CDR 1 aa
169 SM4-7 VL CDR 2 aa
170 SM4-7 VL CDR 3 aa

171 SM6-6 VL nucleotide
172 SM6-6 VL amino acid
173 SM6-6 VL CDR 1 aa
174 SM6-6 VL CDR 2 aa
175 SM6-6 VL CDR 3 aa

176 SM6-51 VL nucleotide
177 SM6-51 VL amino acid
178 SM6-51 VL CDR 1 aa
179 SM6-51 VL CDR 2 aa
180 SM6-51 VL CDR 3 aa

181 VH FWR1
182 VH FWR3
183 VH FWR3 (*02,*03,*04)
184 VH FWR4 (*01)
185 VL FWR1

186 VL FWR2
187 VL FWR3
188 IGHJ6*02 aa
189 IGHJ3*02 aa
190 IGHJ5*01 aa

191 IGHJ5*02 aa
192 IGLJ1*01 aa
193 IGLJ2*01 aa
194 IGLJ3*01 aa
195 IGLG3*02 aa

196 Primer 179-Je
197 Synthetic peptide
198 Primer 074-Je
199 Primer 075-Je
200 Primer 076-Je

201 Primer 150-Je
202 Primer 151-Je
203 Primer 077-Je
204 Primer 078-Je
205 Primer 007-Je

206 Primer 152-Je
207 Primer 153-Je
208 Primer 066-Je
209 Primer 067-Je
210 Primer 068-Je

211 Primer 069-Je
212 Primer 070-Je
213 Primer 258-Je
214 Primer 259-Je
215 Primer 260-Je

216 Primer 261-Je
217 Primer 262-Je
218 Primer 263-Je
219 Primer 264-Je
220 Primer 265-Je

221 Primer 266-Je
222 Primer 267-Je
223 Primer 84-B
224 Primer 155-Je
225 Primer 85-B

226 Primer 161-Je
227 Primer 162-Je
228 Primer 154-Je
229 Primer 001-Je
230 Primer 158-Je

231 Primer 003-Je
232 Primer 159-Je
233 Primer 156-Je
234 Primer 157-Je
235 Primer 062-Je

236 Primer 063-Je
237 Primer 065-Je
238 Primer 064-Je
239 gB strain AD169
240 gB strain Towne

241 SM10 V_H nucleotide
242 SM10 V_H amino acid
243 SM10 V_H CDR 1 aa
244 SM10 V_H CDR 2 aa
245 SM10 V_H CDR 3 aa

246 SM12 V_H nucleotide
247 SM12 V_H amino acid
248 SM12 V_H CDR 1 aa
249 SM12 V_H CDR 2 aa
250 SM12 V_H CDR 3 aa

251 2C2 V_H nucleotide
252 2C2 V_H amino acid
253 2C2 V_H CDR 1 aa
254 2C2 V_H CDR 2 aa
255 2C2 V_H CDR 3 aa

256 1G2 V_H nucleotide
257 1G2 V_H amino acid
258 1G2 V_H CDR 1 aa
259 1G2 V_H CDR 2 aa
260 1G2 V_H CDR 3 aa

261 SM10 V_L nucleotide
262 SM10 V_L amino acid
263 SM10 V_L CDR 1 aa
264 SM10 V_L CDR 2 aa
265 SM10 V_L CDR 3 aa

266 SM12 V_L nucleotide
267 SM12 V_L amino acid
268 SM12 V_L CDR 1 aa
269 SM12 V_L CDR 2 aa
270 SM12 V_L CDR 3 aa

271 2C2 V_L nucleotide
272 2C2 V_L amino acid
273 2C2 V_L CDR 1 aa
274 2C2 V_L CDR 2 aa
275 2C2 V_L CDR 3 aa

276 1G2 V_L nucleotide
277 1G2 V_L amino acid
278 1G2 V_L CDR 1 aa
279 1G2 V_L CDR 2 aa
280 1G2 V_L CDR 3 aa

281 IGHV4-39 FWR1
282 IGHV4-39 FWR2
283 IGHV4-39 FWR3
284 IGHV4-59 FWR1
285 IGHV4-59 FWR2
286 IGHV4-59 FWR3
287 IGKV2D-28 FWR1
288 IGKV2D-28 FWR2
289 IGKV2D-28 FWR3
290 IGKV1D-33 FWR1
291 IGKV1D-33 FWR2
292 IGKV1D-33 FWR3
293 IGLV1-47 FW1
294 IGLV1-47 FW2
295 IGLV1-47 FW3

296 Primer 5'Ig L VH 4/6
297 Primer 5'Ig L Vκ 1/2
298 Primer 5'Ig L Vλ 3
299 Primer 5'Ig L Vλ 1
300 Primer 3'Ig Cγ CH 1

301 Primer 3'Ig Cκ 543
302 Primer 3'Ig Cλ chain
303 Primer 5'Ig AgeI VH4
304 Primer 5'Ig AgeI Vκ 2-24
305 Primer 5'Ig AgeI Vκ 2-28
306 Primer 5'Ig AgeI Vλ 3
307 Primer 5'Ig AgeI Vκ 1-5
308 Primer 5'Ig AgeI VH 4-39
309 Primer 5'Ig AgeI Vλ 1
310 Primer 3'Ig SalI JH

311 Primer 3'Ig BsiWI Jκ 2
312 Primer 3'Ig SalI JH 6
313 Primer 3'Ig Bsi WIJκ 1/4
314 Primer 3'Ig XhoI Cλ
315 Primer 5'Absense
316 Primer 3'IgG internal
317 Primer 3'Cκ494
318 Primer 3'Cλ
319 synthetic linker
320 gpUL132 signal sequence
321 HA epitope tag

References

All references cited anywhere in this specification, including those cited anywhere above, are incorporated herein by reference in their entirety and for all purposes.

- Al-Lazikani B, Lesk AM, Chothia C. (1997) J. Mol. Biol. 273(4): 927-948.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. (1990) J. Mol. Biol. 215(3): 403-10.
- Amit AG, Mariuzza RA, Phillips SE, Poljak RJ. (1986) Science 233(4765): 747-53.
- Andersen DC & Krummen L. (2002) Curr. Op. Biotech. 13: 117.
- Andreoni M, Faircloth M, Vugler L, Britt WJ. (1989) J. Virol. Methods 23(2): 157-67.
- Ausubel *et al.* eds. Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, John Wiley & Sons, 4th edition 1999.
- Avitabile E, Forghieri C, Campadelli-Fiume G. (2009) J. Virol. 83: 10752-10760.
- Backovic M, Longnecker R, Jardetzky TS. (2009) PNAS USA 106: 2880-5.
- Bagshaw SM, Crabtree T, Green F, Stewart DA. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922.
- Bankier AT, Beck S, Bohni R, Brown CM, Cerny R, *et al.* (1991) DNA Seq. 2(1): 1-12.
- Barbas CF 3rd, Hu D, Dunlop N, Sawyer L, Cababa D. (1994) PNAS USA 91: 3809-3813.
- Bernasconi NL, Traggiai E, Lanzavecchia A. (2002) Science 298: 2199-2202.
- Bird RE, Hardman KD, Jacobson JW, Johnson S, Kaufman BM, *et al.* (1998) Science 242(4877): 423-6.

- Boeckh M, Bowden RA, Storer B, Chao NJ, Spielberger R, Tierney DK, Gallez-Hawkins G, Cunningham T, Blume KG, Levitt D, Zaia JA. (2001) Biol. Blood Marrow Transplant. 7: 343-351.
- Britt WJ, Vugler L, Butfiloski EJ, Stephens EB. (1990) J. Virol. 64(3): 1079-85.
- Borucki MJ, Spritzler J, Asmuth DM, Gnann J, Hirsch MS, Nokta M, Aweeka F, Nadler PI, Sattler F, Alston B, Nevin TT, Owens S, Waterman K, Hubbard L, Caliendo A, Pollard RB; AACTG 266 Team. (2004) Antiviral Res. 64(2): 103-111.
- Casali P. (1986) Science 234: 476-9.
- Caton AJ, Herlyn D, Ross AH, Koprowski H. (1990) J. Immunol. 144(5): 1965-8.
- Chadd HE & Chamow SM. (2001) Curr. Op. Biotech. 12: 188-194.
- Chee MS, Bankier AT, Beck S, Bohni R, Brown CM, *et al.* (1990) Curr. Top Microbiol. Immunol. 154:125-69.
- Chothia C, Lesk AM, Levitt M, Amit AG, Mariuzza RA, *et al.* (1986) Science 223: 755-758.
- Chothia C, Lesk AM. (1987) J. Mol. Biol. 196(4): 901-17.
- Chothia C, Lesk AM, Tramontano A, Levitt M, Smith-Gill SJ, *et al.* (1989) Nature 342(6252): 877-83.
- Chothia C, Lesk AM, Gherardi E, Tomlinson IM, Walter G, *et al.* (1992) J. Molecular Biology 227, 799-817.
- Chou SW, Dennison KM. (1991) J. Infect. Dis. 163(6): 1229-34.
- Davies J, Jiang L, Pan LZ, LaBarre MJ, Anderson D, Reff M. (2001) Biotechnol. Bioeng. 74(4): 288-94.
- Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, Pieper U, Sali A. (2006) Curr. Protoc. Bioinformatics, Chapter 5: Unit.
- Fiser A & Sali A. (2003) Bioinformatics 19(18): 2500-1.

- Gram H, Marconi LA, Barbas CF 3rd, Collet TA, Lerner RA, Kang AS. (1992) PNAS USA 89(8): 3576-80.
- Guex N. & Peitsch MC. (1997) Electrophoresis 18: 2714-2723.
- Haan & Maggos (2004) BioCentury, 12(5): A1-A6.
- Hamilton AA, Manuel DM, Grundy JE, Turner AJ, King SI, Adair JR, White P, Carr FJ, Harris WJ. (1997) J. Infect. Dis. 176: 59-68.
- Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor N. Y., pp. 726, 1988.
- Heldwein EE, Lou H, Bender FC, Cohen GH, Eisenberg RJ, Harrison SC. (2006) Science 313: 217-220.
- Hieter PA, Maizel JV Jr, Leder P. (1982) J. Biol. Chem. 257(3): 1516-22.
- Holliger P, Prospero T, Winter G. (1993) PNAS USA. 90(14): 6444-8.
- Holliger P & Winter G. (1993) Curr. Op. Biotech. 4: 446-449.
- Holliger P & Bohlen H. (1999) Cancer & Metastasis Rev. 18: 411-419.
- Holliger P & Hudson PJ. (2005) Nature Biot. 23(9): 1126-1136.
- Holt LJ, Herring C, Jespers LS, Woolven BP, Tomlinson IM. (2003) Trends in Biotechnology 21: 484-490.
- Hu S, Shively L, Raubitschek A, Sherman M, Williams LE, *et al.* (1996) Cancer Res. 56: 3055-3061.
- Hunter WM & Greenwood FC. (1962) Nature 194: 495-6.
- Huston JS, Levinson D, Mudgett-Hunter M, Tai MS, Novotný J, *et al.* (1988) PNAS USA, 85: 5879-5883.
- Jung J, Yi AK, Zhang X, Jongseon C, Li L Choi YS. (2002) J. Immunol. 169: 2368-73.

- Kabat, EA, Wu TT, Perry HM, Gottesman KS, Foeller C. (1991) Sequences of Proteins of Immunological Interest, 5th Edition. US Department of Health and Human Services, Public Service, NIH, Washington.
- Kabatt EA & Wu TT. (1991) J. Immunol. 147: 1709-1719.
- Kari B, Gehrz R. (1991) J. Gen. Virol. 72: 1975-83.
- Kenneson A, Cannon MJ. (2007) Rev. Med. Virol. 17: 253-276.
- Kniess N, Mach M, Fay J, Britt WJ. (1991) J. Virol. 65: 138-46.
- Köhler G & Milstein C. (1975) Nature 256(5517): 495-7.
- Koide A, Bailey CW, Huang X, Koide S. (1998) J. Mol. Biol. 284: 1141- 1151.
- Kontermann R & Dubel S. (2001) Antibody Engineering, Springer-Verlag New York, LLC; ISBN: 3540413545.
- Kozbor D, Roder JC. (1981) J. Immunol. 127(4): 1275-80.
- Kufer P, Lutterbüse R, Baeuerle PA. (2004) TRENDS in Biotechnology 22(5): 238-244.
- Lanzavecchia A. (1985) Nature 314(6011): 537-9.
- Larrick JW & Thomas DW (2001) Curr. Op. Biotech. 12: 411-418.
- Ledermann JA, Begent RH, Massof C, Kelly AM, Adam T, Bagshawe KD. (1991) Int. J. Cancer 47: 659-664.
- Lundgren K, Wahlgren M, Troye-Blomberg M, Berzins K, Perlmann H, *et al*. (1983) J. Immunol. 131(4): 2000-3.
- Mach M, Kropff B, Dal-Monte P, Britt WJ. (2000) J. Virol. 74: 11881-11892.
- Mahy BWJ, Kangro HO. (1996) Virology Methods Manual, pages 35-37. San Diego: Academic Press.
- Marks JD, Griffiths AD, Malmqvist M, Clackson TP, Bye JM, Winter G. (1992) Biotechnology 10(7): 779-83.
- Marshall GS, Rabalais GP, Stout GG, Waldeyer SL. (1992) J. Infect. Dis. 165: 381-384.

- Martin, ACR. (1996) PROTEINS: Structure, Function and Genetics, 25, 130-133.
- McCafferty J, Griffiths AD, Winter G, Chiswell DJ. (1990) Nature 348(6301): 552-4.
- Mendez MJ, Green LL, Corvalan JR, Jia XC, Maynard-Currie CE (1997) Nature Genet. 15(2): 146-156.
- Meyer H, Masuho Y, Mach M. (1990) J. Gen. Virol. 71: 2443-50.
- Meyer H, Sundqvist VA, Pereira L, Mach M. (1992) J. Gen. Virol. 73(9): 2375-83.
- Milhavet O, Gary DS, Mattson MP (2003) Pharmacol. Rev. 55(4): 629-48.
- Moss P, Rickinson A. (2005) Nat. Rev. Immunol. 5: 9-20.
- Nigro G, Adler SP, La TR, Best AM. (2005) N. Engl. J. Med. 353: 1350-1362.
- Nygren PA, Uhlén M. (1997) Curr. Op. Struct. Biol. 7: 463-469.
- Ohizumi Y, Suzuki H, Matsumoto Y, Masuho Y, Numazaki Y. (1992) J. Gen. Virol. 73(10): 2705-7.
- Ohlin M, Sundqvist VA, Mach M, Wahren B, Borrebaeck CA. (1993) J. Virol. 67(2): 703-10.
- Opalinska JB & Gewirtz AM (2003) Sci. STKE 206: pe47
- Patrone M, Secchi M, Bonaparte E, Milanesi G, Gallina A. (2007) J. Virol. 81: 11479-11488.
- Pearson WR, Lipman DJ. (1988) PNAS USA 85(8): 2444-8.
- Plückthun A. (1991) BioTechnology 9: 545-551.
- Posner MR, Hideshima T, Cannon T, Mukherjee M, Mayer KH, Byrn RA. (1991) J. Immunol. 146(12): 4325-32.
- Qiu XQ, Wang H, Cai B, Wang LL, Yue ST. (2007) Nat. Biotechnol. 25: 921-929.
- Raanani P, Gafter-Gvili A, Paul M, Ben-Bassat I, Leibovici L, Shpilberg O. (2009) J. Clin. Oncol. 27: 770-781.

- Raff HV, Siscoe PJ, Wolff EA, Maloney G, Shuford W. (1988) J. Exp. Med. 168(3): 905-17.
- Ravetch JV, Siebenlist U, Korsmeyer S, Waldmann T, Leder P. (1981) Cell 3(2): 583-91.
- Razonable RR, Paya CV. (2003) Herpes 10: 60-65.
- Reiter Y, Brinkmann U, Lee B, Pastan I. (1996) Nature Biotech, 14: 1239-1245.
- Robinson JR. ed., (1978) Sustained and Controlled Release Drug Delivery Systems, Marcel Dekker, Inc., New York.
- Roche S, Bressanelli S, Rey FA, Gaudin Y. (2006) Science 313: 187-191.
- Roche S, Rey FA, Gaudin Y, Bressanelli S. (2007) Science 315: 843-8.
- Rosén A, Gergely P, Jondal M, Klein G, Britton S. (1977) Nature 267(5606): 52-4.
- Rosén A, Persson K, Klein G. (1983) J. Immunol. 130(6): 2899-902.
- Sambrook and Russell, Molecular Cloning: a Laboratory Manual: 3rd edition, 2001, Cold Spring Harbor Laboratory Press.
- Schier R, McCall A, Adams GP, Marshall KW, Merritt H, *et al*. (1996) J. Mol. Biol. 263: 551-567.
- Schoppel K, Kropff B, Schmidt C, Vornhagen R, Mach M. (1997) J. Infect. Dis. 175: 533-544.
- Schreiber A, Harter G, Schubert A, Bunjes D, Mertens T, Michel D. (2009) Expert. Opin. Pharmacother. 10: 191-209.
- Segal DM, Padlan EA, Cohen GH, Rudikoff S, Potter M, Davies DR. (1974) Proc Natl Acad Sci USA. 71(11): 4298-302.
- Sharon J. (1990a) PNAS USA. 87(12): 4814-7.
- Sharon J. (1990b) J. Immunol. 144: 4863-4869.
- Shatsky M, Nussinov R, Wolfson HJ. (2004) Proteins 56(1): 143-156.

- Shields RL, Lai J, Keck R, O'Connell LY, Hong K, *et al* (2002) J. Biol. Chem. 277(30): 26733-40.
- Shimamura M, Mach M, Britt WJ. (2006) J. Virol. 80: 4591-4600.
- Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, *et al* (2003) J. Biol. Chem. 278(5): 3466-73.
- Smith TF, & Waterman MS. (1981) J. Mol. Biol. 147(1): 195-7.
- Sokos DR, Berger M, Lazarus HM. (2002) Biol. Blood Marrow Transplant. 8: 117-130.
- Spaderna S, Kropff B, Kodel Y, Shen S, Coley S, Lu S, Britt W, Mach M. (2005) J Virol 79: 11837-11847.
- Steenbakkers PG, Van Wezenbeek PM, van Zanten J, The TH. (1993) Hum. Antibodies Hybridomas 4(4): 166-73.
- Steenbakkers PG, Hubers HA, Rijnders AW. (1994) Mol. Biol. Rep. 19(2):125-34.
- Steinitz M, Klein G, Koskimies S, Makel O. (1977) Nature 269(5627): 420-2.
- Steinitz M, Izak G, Cohen S, Ehrenfeld M, Flechner I. (1980) 287(5781): 443-5.
- Steinitz M, Tamir S, Goldfarb A. (1984) J. Immunol. 132(2): 877-82.
- Streblow DN, Orloff SL, Nelson JA. (2007) Curr. Opin. Immunol. 19: 577-582.
- Tiller T, Meffre E, Yurasov S, Tsuiji M, Nussenzweig MC, Wardemann H. (2008) J. Immunol. Methods 329: 112-24 and Corrigendum (2008) J. Immunol. Methods 334: 142.
- Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, Murphy BR, Rappuoli R, Lanzavecchia A. (2004) Nat. Med. 10(8): 871-5.
- Udey JA, Blomberg B. (1987) Immunogenetics 25(1): 63-70.

- Umaña P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE. (1999) Nat. Biotechnol. 17(2): 176-80.
- Urban M, Britt W, Mach M. (1992) J. Virol. 66: 1303-1311.
- Urban M, Klein M, Britt WJ, Hassfurther E, Mach M. (1996) J. Gen. Virol. 77(7): 1537-1547.
- Vasicek TJ, Leder P. J. Exp. Med. 172(2): 609-20.
- Voet & Voet, Biochemistry, 3rd Edition, (Wiley) 2004.
- Wagner B, Kropff B, Kalbacher H, Britt W, Sundqvist VA, Ostberg L, Mach M. (1992) J. Virol. 66: 5290-5297.
- Ward ES, Güssow D, Griffiths AD, Jones PT, Winter G. (1989) Nature 341(6242): 544-6.
- Wess L. (2004) In: BioCentury, The Bernstein Report on BioBusiness, 12(42), A1-A7.
- Whitelegg NRU & Rees AR. (2000) Prot. Eng. 12: 815-824.
- Yamaguchi H, Furukawa K, Fortunato SR, Livingston PO, Lloyd KO, Oettgen HF, Old LJ. (1987) Proc. Natl. Acad. Sci USA 84: 2416-20.

- EP0120694A: Boss MA *et al.*
- EP0125023A: Cabilly S *et al.*
- EP0184187A: Kudo A *et al.*
- EP0239400A: Winter GP.
- EP0664834B: Harris WJ *et al.*

- US4,275,149: Litman DJ *et al.*
- US4,318,980: Boguslaski RC *et al.*
- US4,424,200: Crockford DR, Rhodes BA
- US4,479,930: Hnatowich DJ.
- US5,043,281: Masuho Y *et al.*
- US5,750,106: Ostberg L.
- US5,814,468: Sliman O *et al.*

- US6,602,684: Umaña P et al.
- US6,998,253: Presta LG, Snedecor BR
- US20030157108: Presta LG *et al.*
- US2009004198: Nakajima K *et al.*

- WO 91/009967 A1: Adair JR *et al.*
- WO 92/001047 A1: McCafferty J *et al.*
- WO 93/011161 A1: Whitlow MD *et al.*
- WO 93/021952 A1: Borrebaeck C *et al.*
- WO 00/034784 A1: Lipovsek D.
- WO 03/068819 A1: Grawunder U & Melchers GF.
- WO 04/006955 A1: Foote J.
- WO 04/076677 A2: Lanzavecchia A.
- WO 04/106375 A1: Hoogenboom HRJM *et al.*
- WO 07/068758 A1: Funaro A *et al.*
- WO 08/084410 A2: Lanzavecchia A, Macagano A.
- WO 08/071806 A1: Funaro A *et al.*
- WO 09/003975 A1: Funaro A *et al.*
- WO 09/024445 A1: Funaro A *et al.*
- WO 09/114560 A2: Olsen O.
- WO 10/007463 A1: Lanzavecchia A, Macagano A.
- WO 10/007533 A2: Lanzavecchia A, Macagano A.
- WO 10/114105 A1: Takada K, Kurino R, Watanabe M.
- WO 10/114106 A1: Takada K, Kurino R, Torashima T.

Claims

1. An isolated binding member for human cytomegalovirus (hCMV) gB protein, which binds hCMV gB protein at a region within residues 100 to 447, the residue numbering being defined according to the full length gB strain AD169 amino acid sequence SEQ ID No: 239.

2. An isolated binding member for hCMV gB protein, which binds hCMV gB protein with a K_D of not more than 1nM as defined by surface plasmon resonance.

3. A binding member according to claim 2, wherein the K_D is not more than 0.5nM.

4. A binding member according to claim 2 or claim 3, wherein the K_D is not more than 0.1nM.

5. A binding member according to any of the preceding claims, wherein the binding member does not bind to antigenic domain 1 (AD-1) or antigenic domain 2 (AD-2) of hCMV gB protein.

6. An isolated binding member according to any of the preceding claims, wherein the concentration of binding member required for 50% neutralisation of a clinical isolate of hCMV is 10µg/ml or less in a neutralisation assay for neutralisation of hCMV infection of human foreskin fibroblasts.

7. An isolated binding member for hCMV, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the set of CDRs has 22 or fewer amino acid alterations from a set of CDRs in which:

HCDR1 has amino acid sequence SEQ ID No: 3;
HCDR2 has amino acid sequence SEQ ID No: 4;
HCDR3 has amino acid sequence SEQ ID No: 5;
LCDR1 has amino acid sequence SEQ ID No: 93;
LCDR2 has amino acid sequence SEQ ID No: 94; and
LCDR3 has amino acid sequence SEQ ID No: 95, and wherein the binding member has a K_D of not more than 50nM as defined by surface plasmon resonance.

8. An isolated binding member for hCMV according to any of claims 1 to 6, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein the set of CDRs has 22 or fewer amino acid alterations from a set of CDRs in which:
HCDR1 has amino acid sequence SEQ ID No: 3;
HCDR2 has amino acid sequence SEQ ID No: 4;
HCDR3 has amino acid sequence SEQ ID No: 5;
LCDR1 has amino acid sequence SEQ ID No: 93;
LCDR2 has amino acid sequence SEQ ID No: 94; and
LCDR3 has amino acid sequence SEQ ID No: 95.

9. A binding member according to any of claims 7 or 8, comprising an HCDR1 wherein:
Kabat residue 31 is Asp or Gly;
Kabat residue 32 is His, Phe or Tyr;
Kabat residue 33 is Tyr;
Kabat residue 34 is Met, Ile or Leu; and
Kabat residue 35 is Val or Asn.

10. A binding member according to claim 9, wherein HCDR1 is SEQ ID No: 3.

11. A binding member according to any of claims 7 to 10, comprising an HCDR2 wherein:

Kabat residue 50 is Trp, Ser or Cys;
Kabat residue 53 is Gln, Asn or His;
Kabat residue 54 is Ser or Thr;
Kabat residue 58 is Gly, Lys, Asn or His;
Kabat residue 60 is Gly or Ala; and
Kabat residue 64 is Gln or Arg.

12. A binding member according to claim 11, wherein HCDR2 has amino acid sequence SEQ ID No: 4.

13. A binding member according to any of the preceding claims, comprising an HCDR3 wherein:
Kabat residue 99 is Thr or Ala;
Kabat residue 100 is Val or Met;
Kabat residue 100A is Ser or Thr
Kabat residue 100B is Asn or Thr;
Kabat residue 100C is Ser or Phe;
Kabat residue 100E is Leu, Met or Ala;
Kabat residue 100F is Ser or Gly;
Kabat residue 100K is His or Tyr;
Kabat residue 100L is Asn, Ser or Asp;
Kabat residue 100M is Arg, Val or Ile;
Kabat residue 100N is Leu or Met;
Kabat residue 101 is Asp or Gly; and
Kabat residue 102 is Ala, Val or Ile.

14. A binding member according to claim 13, wherein HCDR3 has amino acid sequence SEQ ID No: 5.

15. A binding member according to any of claims 7 to 14, wherein Kabat residue 26 in LCDR1 is Ser or Asn or where Kabat residue 27 in LCDR1 is Ser or Arg.

16. A binding member according to claim 15, wherein LCDR1 is SEQ ID No: 93.

17. A binding member according to any of claims 7 to 16, wherein Kabat residue 56 in LCDR2 is Ser or Pro.

18. A binding member according to claim 17, wherein LCDR2 is SEQ ID No: 94.

19. A binding member according to any of the preceding claims, comprising an LCDR3 wherein:
Kabat residue 89 is Gly or Ala;
Kabat residue 91 is Pro or Trp;
Kabat residue 93 is Arg or Ser;
Kabat residue 94 is Ser or Asp;
Kabat residue 95a is Ser, Gly or Ala;
Kabat residue 96 is Val or Tyr; and
Kabat residue 97 is Ile or Val.

20. A binding member according to claim 19, comprising an LCDR3 having amino acid sequence SEQ ID No: 95.

21. An isolated binding member for hCMV gB protein, comprising an HCDR3, a LCDR3 and/or a set of CDRs of any of antibodies Ab-01 to Ab-46.

22. An isolated binding member according to claim 21, which binds hCMV gB protein at a region within residues 121 to 132 and 344 to 438, the residue numbering being defined according to the full length gB strain AD169 amino acid sequence SEQ ID No: 239.

23. A binding member according to any of the preceding claims, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein:
HCDR1 is SEQ ID NO: 3;
HCDR2 is SEQ ID NO: 4;
HCDR3 is SEQ ID NO: 5;
LCDR1 is SEQ ID NO: 93;
LCDR2 is SEQ ID NO: 94; and
LCDR3 is SEQ ID NO: 95.

24. A binding member according to any of the preceding claims, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_H domain, and wherein the V_H domain has the V_H domain amino acid sequence shown in SEQ ID No: 2.

25. A binding member according to any of the preceding claims, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_L domain wherein the V_L domain has the V_L domain amino acid sequence shown in SEQ ID No: 92.

26. An isolated antibody molecule comprising a heavy chain comprising amino acid sequence SEQ ID No: 2 and a light chain comprising amino acid sequence SEQ ID No: 92.

27. An isolated antibody molecule that binds hCMV gB protein, wherein the antibody molecule comprises a V_H domain amino acid sequence at least 90% identical to SEQ ID No: 2 and a V_L domain amino acid sequence at least 90% identical to SEQ ID No: 92.

28. An isolated binding member for hCMV gB protein, comprising an HCDR3, a LCDR3 and/or a set of CDRs of any of antibodies Ab-47 to Ab-50.

29. An isolated binding member according to claim 28, which binds hCMV gB protein at a region within residues 133 to 343, the residue numbering being defined according to the full length gB strain AD169 amino acid sequence SEQ ID No: 239.

30. A binding member according to any of claims 1 to 6, 28 and 29, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein:
HCDR1 is SEQ ID No: 243;
HCDR2 is SEQ ID No: 244;
HCDR3 is SEQ ID No: 245;
LCDR1 is SEQ ID No: 263;
LCDR2 is SEQ ID No: 264; and
LCDR3 is SEQ ID No: 265.

31. A binding member according to any of claims 1 to 6, 28 and 29, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein:
HCDR1 is SEQ ID No: 248;
HCDR2 is SEQ ID No: 249;
HCDR3 is SEQ ID No: 250;
LCDR1 is SEQ ID No: 268;
LCDR2 is SEQ ID No: 269; and
LCDR3 is SEQ ID No: 270.

32. A binding member according to any of claims 1 to 6, 28 and 29, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein:
HCDR1 is SEQ ID No: 253;

HCDR2 is SEQ ID No: 254;
HCDR3 is SEQ ID No: 255;
LCDR1 is SEQ ID No: 273;
LCDR2 is SEQ ID No: 274; and
LCDR3 is SEQ ID No: 275.

33. A binding member according to any of claims 1 to 6, 28 and 29, comprising a set of CDRs: HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, wherein:
HCDR1 is SEQ ID No: 258;
HCDR2 is SEQ ID No: 259;
HCDR3 is SEQ ID No: 260;
LCDR1 is SEQ ID No: 278;
LCDR2 is SEQ ID No: 279; and
LCDR3 is SEQ ID No: 280.

34. A binding member according to any of claims 1 to 6 and 28 to 30, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_H domain, and wherein the V_H domain has the V_H domain amino acid sequence shown in SEQ ID No: 242.

35. A binding member according to any of claims 1 to 6, 28, 29 and 31, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_H domain, and wherein the V_H domain has the V_H domain amino acid sequence shown in SEQ ID No: 247.

36. A binding member according to any of claims 1 to 6, 28, 29 and 32, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_H domain, and wherein the V_H domain has the V_H domain amino acid sequence shown in SEQ ID No: 252.

37. A binding member according to any of claims 1 to 6, 28, 29 and 33, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_H domain, and wherein the V_H domain has the V_H domain amino acid sequence shown in SEQ ID No: 257.

38. A binding member according to any of claims 1 to 6, 28 to 30 and 34, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_L domain wherein the V_L domain has the V_L domain amino acid sequence shown in SEQ ID No: 262.

39. A binding member according to any of claims 1 to 6, 28, 29, 31 and 35, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_L domain wherein the V_L domain has the V_L domain amino acid sequence shown in SEQ ID No: 267.

40. A binding member according to any of claims 1 to 6, 28, 29, 32 and 36, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_L domain wherein the V_L domain has the V_L domain amino acid sequence shown in SEQ ID No: 272.

41. A binding member according to any of claims 1 to 6, 28, 29, 33 and 37, wherein the binding member comprises an antibody molecule or functional fragment thereof comprising an antibody V_L domain wherein the V_L domain has the V_L domain amino acid sequence shown in SEQ ID No: 277.

42. An isolated antibody molecule comprising a heavy chain comprising amino acid sequence SEQ ID No: 242 and a light chain comprising amino acid sequence SEQ ID No: 262.

43. An isolated antibody molecule comprising a heavy chain comprising amino acid sequence SEQ ID No: 247 and a light chain comprising amino acid sequence SEQ ID No: 267.

44. An isolated antibody molecule comprising a heavy chain comprising amino acid sequence SEQ ID No: 252 and a light chain comprising amino acid sequence SEQ ID No: 272.

45. An isolated antibody molecule comprising a heavy chain comprising amino acid sequence SEQ ID No: 257 and a light chain comprising amino acid sequence SEQ ID No: 277.

46. An isolated antibody molecule that binds hCMV gB protein, wherein the antibody molecule comprises a V_H domain amino acid sequence at least 90% identical to SEQ ID No: 242 and a V_L domain amino acid sequence at least 90% identical to SEQ ID No: 262.

47. An isolated antibody molecule that binds hCMV gB protein, wherein the antibody molecule comprises a V_H domain amino acid sequence at least 90% identical to SEQ ID No: 247 and a V_L domain amino acid sequence at least 90% identical to SEQ ID No: 267.

48. An isolated antibody molecule that binds hCMV gB protein, wherein the antibody molecule comprises a V_H domain amino acid sequence at least 90% identical to SEQ ID No: 252 and a V_L domain amino acid sequence at least 90% identical to SEQ ID No: 272.

49. An isolated antibody molecule that binds hCMV gB protein, wherein the antibody molecule comprises a V_H domain amino acid sequence at least 90% identical to SEQ ID No: 257 and a V_L domain amino acid sequence at least 90% identical to SEQ ID No: 277.

50. A binding member or antibody molecule that competes for binding to hCMV gB protein with a binding member or isolated antibody molecule of any of the preceeding claims.

51. An antibody molecule according to any of claims 26, 27, 42 to 50, wherein the antibody molecule is an IgG.

52. An isolated VH domain of an antibody molecule according to any of claims 24, 26, 27, 34 to 37 and 42 to 51.

53. An isolated VL domain of an antibody molecule according to any of claims 25 to 27 and 38 to 51.

54. A composition comprising an isolated binding member according to any of claims 1 to 25 and 28 to 41, or an antibody molecule according to any of claims 26, 27 and 42 to 51, and a pharmaceutically acceptable excipient.

55. A composition comprising an isolated binding member according to any of claims 1 to 25 and 28 to 41, or an antibody molecule according to any of claims 26, 27 and 42 to 51, for use in a method of treatment of the human or animal body by therapy.

56. A composition according to claim 55 for use in treating a disorder associated with hCMV.

57. A composition comprising an isolated binding member according to any of claims 1 to 25 and 28 to 41, or an antibody molecule according to any of claims 26, 27 and 42 to 51, for use in the treatment of a disorder associated with hCMV.

58. A composition according to claim 56 or use according to claim 57, wherein the disorder is a hCMV infection

59. A composition according to any one of claims 55 to 58, further comprising an isolated binding member or antibody molecule that binds hCMV gB, gH, gL, UL128, UL130 and/or UL131A protein.

60. A method of treating a disorder associated with hCMV in an individual, comprising administering a binding member according to any of claims 1 to 25 and 28 to 41, or an antibody molecule according to any of claims 26, 27 and 42 to 51, to the individual, and preferably wherein the individual has a compromised immune system.

61. A method according to claim 60, wherein the individual is a pregnant woman, a newborn, a transplant recipient or an individual infected with HIV.

62. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a binding member according to any of claims 1 to 25 and 28 to 41, a V_H domain according to claim 52, a V_L domain according to claim 53, or an antibody molecule according to any of claims 26, 27 and 42 to 51.

63. A host cell *in vitro* transfected or transduced with a nucleic acid molecule according to claim 62.

64. A method of producing a binding member, an antibody molecule or an antibody V_H or V_L domain, comprising culturing a host cell according to claim 63 under conditions for production of the binding member, antibody molecule or antibody V_H or V_L domain.

65. A method according to claim 64, further comprising isolating and/or purifying the binding member, antibody molecule, V_H domain or V_L domain.

66. A method according to claim 64 or claim 65, further comprising formulating the binding member, antibody molecule, V_H domain or V_L domain into a composition comprising at least one additional component.

67. A method for producing an antibody antigen-binding domain for hCMV, the method comprising providing, by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent V_H domain comprising HCDR1, HCDR2 and HCDR3, wherein the parent V_H domain HCDR1, HCDR2 and HCDR3 are a set of HCDRs as shown in Table 19, a V_H domain which is an amino acid sequence variant of the parent V_H domain, and optionally combining the VH domain thus provided with one or more VL domains to provide one or more V_H/V_L combinations; and testing said V_H domain which is an amino acid sequence variant of the parent V_H domain or the V_H/V_L combination or combinations to identify an antibody antigen binding domain for hCMV.

68. A method according to claim 67, wherein said one or more V_L domains is provided by way of addition, deletion, substitution or insertion of one or more amino acids in the amino acid sequence of a parent V_L domain comprising LCDR1, LCDR2 and LCDR3, wherein the parent V_L domain LCDR1, LCDR2 and LCDR3 are a V_L set of CDRs as shown in Table 19, producing one or more V_L domains each of which is an amino acid sequence variant of the parent V_L domain.

69. A method according to claim 67 or claim 68, wherein said V_H domain which is an amino acid sequence variant of the parent V_H domain is provided by CDR mutagenesis.

70. A method according to any of claims 67 to 69 further comprising producing the antibody antigen-binding domain as a component of an IgG, scFv, Fab or bispecific antibody molecule or a multi-specific antibody.

71. A method for producing a binding member that binds hCMV, which method comprises: providing starting nucleic acid encoding a V_H domain or a starting repertoire of nucleic acids each encoding a V_H domain, wherein the V_H domain or V_H domains either comprise a HCDR1, HCDR2 and/or HCDR3 to be replaced or lack a HCDR1, HCDR2 and/or HCDR3 encoding region; combining said starting nucleic acid or starting repertoire with donor nucleic acid or donor nucleic acids encoding or produced by mutation of the amino acid sequence of an HCDR1, HCDR2, and/or HCDR3 shown in Table 19, such that said donor nucleic acid is or donor nucleic acids are inserted into the CDR1, CDR2 and/or CDR3 region in the starting nucleic acid or starting repertoire, so as to provide a product repertoire of nucleic acids encoding V_H domains; expressing the nucleic acids of said product repertoire to produce product V_H

domains; optionally combining said product V_H domains with one or more V_L domains; selecting a binding member for hCMV, which binding member comprises a product V_H domain and optionally a V_L domain; and recovering said binding member or nucleic acid encoding it.

72. A method according to claim 71 wherein the donor nucleic acids are produced by mutation of said HCDR1 and/or HCDR2 and/or HCDR3.

73. A method according to claim 72, comprising providing the donor nucleic acid by random mutation of nucleic acid.

74. A method according to any one of claims 67 to 73, further comprising attaching a product V_H domain that is comprised within the recovered binding member to an antibody constant region.

75. A method according to any one of claims 67 to 74 comprising providing an IgG, scFv, Fab or bispecific antibody molecule or multi-specific antibody comprising the product V_H domain and a V_L domain.

76. A method according to any one of claims 67 to 75, further comprising testing the antibody antigen-binding domain or binding member that binds hCMV for ability to neutralise hCMV.

77. A method according to claim 76, wherein a binding member that comprises an antibody molecule that binds and neutralises hCMV is obtained.

78. A method of neutralising hCMV in a subject or sample, comprising administering to said subject or sample a binding member according to any of claims 1 to 25 and 28 to 41, or an antibody molecule according to any of claims 26, 27 and 42 to 51, in an amount sufficient to reduce hCMV infectivity by at least 50% at a concentration of from about 0.1 to about 5.0 µg/ml.