Antiviral Research, 17 (1992) 115–131

AVR 00524

A combination of human cytomegalovirus (HCMV)-specific murine monoclonal antibodies exhibits synergistic antiviral activity in vitro

Richard C. Gehrz, Curtis M. Nelson and Bruce E. Kari

Children's Biomedical Research Institute, St. Paul, and Medimorphics, Inc., St. Paul, Minnesota, U.S.A.

(Received 10 April 1991; accepted 17 July 1991)

Summary

A combination of HCMV-specific monoclonal antibodies (MAbs) reactive with glycoproteins in gcI complexes which exhibit synergistic antiviral activity in vitro is described. MAbs directed against different structural and biological properties of HCMV have been selected to increase the antiviral activity against all possible strains, and to reduce the likelihood that resistant strains will emerge with prolonged exposure. Furthermore, in vitro analysis demonstrates that certain of the MAbs in the combination augment the virus-neutralizing activity of other component antibodies, thereby decreasing the amount of total antibody protein required to inhibit HCMV infection. Certain MAbs have been selected to inactivate extracellular virus during the early phase of HCMV infection, whereas others have been selected to prevent its spread once cells have been infected. These data suggest that a MAb cocktail may be useful for prophylaxis and treatment of patients at risk of life-threatening HCMV infections.

Monoclonal antibody; Human cytomegalovirus, HCMV; Monoclonal antibody, HCMV-specific

Correspondence to: Richard C. Gehrz, M.D., Children's Biomedical Research Institute, 345 No. Smith Ave., St. Paul, MN 55102, U.S.A.

Introduction

Human cytomegalovirus (HCMV) is the most common congenital infection leading to birth defects in the United States, and among the most common causes of serious illness in organ and bone marrow transplant patients and patients with acquired immune deficiency syndrome (AIDS) (Gehrz, 1991). A member of the herpesvirus family, HCMV is a species-specific virus which causes productive infection and disease only in man (Stinski, 1990). Following recovery from a primary HCMV infection, which is sub-clinical in most cases, the virus persists in a latent state and can be reactivated to cause disease in immunocompromised patients. HCMV-specific antibodies can be detected in convalescent sera of individuals who have recovered from subclinical or symptomatic HCMV infections. Among the HCMV proteins recognized by human antibodies are envelope glycoproteins (gp52 and gp93 of gcI complexes; group 1 and group 2 gcII glycoproteins; and gp86 of gcIII complexes), internal structural proteins (matrix protein pp28; major lower matrix protein pp65; major capsid protein p150; and a protein of approx. 200 kDa), and the major immediate-early protein (Landini et al., 1985; Mirolo et al., 1986; Rodgers et al., 1987; Liu et al., 1988; Kari and Gehrz, 1990b). However, little is presently known regarding the in vivo antiviral activity of these antibodies during primary infection, or their role in protecting against reinfection or reactivation of latent virus.

Antibodies reactive with HCMV glycoproteins are thought to play an important role in destroying infectious virus in the bloodstream, and are also likely to be important in limiting the spread of HCMV once it has established a productive infection in tissues. Antibody neutralization may involve complement-dependent lysis of extracellular virions, or envelopment with antibody protein or aggregation of the virus to prevent its infectivity. Alternatively, complement-independent neutralizing antibodies may bind specifically to viral glycoproteins expressed on the virion envelope or on the surface of HCMV-infected cells to interfere with attachment, penetration, or cell-to-cell spreading. HCMV-specific antibodies may also limit the progression of HCMV disease by direct cytolytic or lymphocyte-mediated cytotoxic mechanisms (i.e., antibody-mediated cellular cytotoxicity (ADCC)) to eliminate HCMV-infected cells as a repository of infectious virus. In addition, non-specific immunoglobulin may also limit viral infection by mechanisms that are not presently well-defined.

We and others have previously described MAbs reactive with a family of glycoprotein complexes designated as gcI (gB) which neutralize HCMV in the presence or absence of complement (Pereira et al., 1982; Britt, 1984; Kari et al., 1986; Britt et al., 1988; Kari et al., 1990a). These complexes contain 2 mature glycoproteins with M_rs of 93 to 130 kDa (gp93–130) and 52 kDa (gp52) derived by proteolytic cleavage of a precursor glycoprotein with an M_r of 158 kDa (gp158) (Gretch et al., 1988a; Gretch et al., 1988b; Spaete et al., 1988; Britt and Vugler, 1989; Kari et al., 1990a). gp93–130 and gp52 represent the amino- and carboxy-terminal portions of the polypeptide backbone of gp158, encoded by a

single gene exhibiting homology with gB of HSV-1 (Cranage et al., 1986; Kari et al., 1990a). Convalescent sera contain antibodies which react with all of the gcI glycoproteins and complexes (Liu et al., 1988; Kari and Gehrz, 1990b). Furthermore, a significant proportion of HCMV-specific neutralizing activity in human sera can be adsorbed with fibroblasts infected with vaccinia recombinant viruses containing either the entire gB open reading frame or an *N*-terminal truncation of gB encoding the first 513 amino acids of gB (Britt et al., 1990; Liu et al., 1991). In the present report, we describe a combination of three murine MAbs exhibiting specificity for unique epitopes on HCMV envelope glycoproteins comprising the gcI family of complexes and synergistic antiviral activity against all strains of HCMV.

Materials and Methods

Viruses

Laboratory strains of HCMV (Towne, AD169, Davis, Toledo) were plaque-purified and infectious units quantitated in an agarose overlay plaque-forming assay (Wentworth and French, 1970). Wild strains of HCMV isolated from clinical specimens from infants with cytomegalic inclusion disease, and transplant and AIDS patients with opportunistic HCMV infections were passaged in tissue culture, and infectious units determined as above. Towne strain HCMV was used as a prototype virus in all experiments unless otherwise specified. Clinical isolates of HCMV, HSV-1, HSV-2, adenoviruses types 2 and 5, VZV, and influenza A were provided by the Diagnostic Virology Laboratory at St. Paul Children's Hospital.

Monoclonal antibody production

MAbs reactive with gp52 were generated by using purified Towne HCMV virions or envelope glycoproteins and complexes obtained by detergent extraction as the immunizing antigen (Kari et al., 1986); biochemically purified gp93-130 was used to generate MAbs reactive with gp93-130 (Kari et al., 1990a); purified gcII complexes were used to obtain the group 2 gcII MAb 15F9, which has been included as a non-neutralizing antibody control (Kari et al., 1990c). Adult female BALB/c mice were immunized intraperitoneally with antigen emulsified in complete Freund's adjuvant, and immune splenocytes were fused with the murine myeloma cell line SP2/0-Ag14 with polyethylene glycol. Fused cells were cloned by limiting dilution according to standard procedures (Kohler and Milstein, 1975), and the resulting hybridomas were screened for HCMV-specific antibody by ELISA using purified virions, detergent extracts, or biochemically purified glycoproteins as antigens. Antibody-producing clones of interest were sub-cloned at least twice and expanded for production of ascites fluid in BALB/c mice. Alternatively, hybridomas have been adapted to low serum medium and MAbs produced in

gram quantities in continuous perfusion bioreactors. For definitive characterization, immunoglobulin proteins were purified by hydroxyapatite HPLC or immunoaffinity using protein A or protein G. Purified MAbs were characterized by isoelectric focusing and immunoglobulin subclasses were determined by enzyme-linked immunosorbent assay (ELISA) using subclass-specific goat anti-mouse antisera.

Western blot analysis

HCMV virions were pelleted by centrifugation from the supernatants of human skin fibroblast cultures infected for 3 to 7 days with Towne strain HCMV, and boiled for 3 min in SDS-PAGE sample solubilization buffer. Insoluble material was removed by centrifugation, and proteins in the supernatant were separated on 10% polyacrylamide gels following the method of Laemmli (1970). Proteins in these gels were electroblotted onto nitrocellulose paper and the paper was then blocked with 3% gelatin in Tris-buffered saline (TBS, 25 mM Tris, 0.8% NaCl, pH 7.5). Strips of blocked paper were incubated for 2 h with MAbs 9B7, 41C2, and 3B10 at a protein concentration of 2 μg/ml in TBS containing 0.05% Tween 20 (TBS-T). Strips were washed with TBS-T and then with TBS before incubation with phosphatase-labeled goat anti-mouse IgG (Kirkegaard and Perry, Gaithersburg, MD) diluted 1 to 1000 with TBS-T. After a 1.5-h incubation, the paper was washed as above, and the substrate 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M Tris buffer (Kirkegaard and Perry) was added. The reaction was stopped by washing the strips in water. Immunoreactive bands in the lanes containing HCMV glycoproteins were compared to co-migrating molecular weight standards.

Immunofluorescence studies

Reactivity of gcI-specific MAbs with wild type and laboratory-adapted strains of HCMV and several unrelated viruses was determined in an indirect immunofluorescence assay. Virus-infected and uninfected fibroblast cultures on glass slides were fixed in cold acetone:methanol (v/v, 1:1). Fixed cultures were incubated with 10 μg/ml MAb in TBS for 30 min, washed with TBS, and then incubated for 1 h with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG $F(ab')_2$ fragments (Cappel, Malvern, PA) diluted 1 to 200 in TBS. Following a final wash, slides were examined with a Zeiss phase fluorescence microscope for characteristic cytoplasmic fluorescence associated with HCMV-infected cells.

Reactivity of MAbs with normal cellular proteins was determined by fixing various cell lines of fibroblast, epithelial, and lymphoid origin with acetone:methanol and staining with FITC-goat anti-mouse IgG $F(ab')_2$ fragments as above. Paraffin sections of autopsy tissues from several patients dying of causes unrelated to HCMV infection were tested in the same way after dewaxing in AmeriClear (Baxter, McGaw Park, IL) and rehydrating by passing

through graded ethanol (absolute-50%). Sections were examined for HCMV-specific immunofluorescence relative to background fluorescence in sections treated with FITC-anti-IgG in the absence of HCMV-specific MAb.

Microneutralization assay

A microneutralization assay was adapted from the method of Gonczol et al. (1986) to examine the inhibitory activity of individual HCMV-specific MAbs and various combinations of MAbs against several laboratory-adapted and wild strains of HCMV in the presence or absence of complement. Serial dilutions of a primary MAb alone or in combination with several concentrations of a second MAb were made in Dulbecco's minimum essential medium (DMEM) supplemented with L-glutamine (2 mM), glucose (1 g/l), $NaHCO_3$ (0.81 g/l, pH 7.0), 5% heat-inactivated fetal bovine serum and 5% heat-inactivated newborn calf serum (HyClone, Logan, UT), and aliquoted into individual wells of 96-well microtiter plates. 40–50 plaque-forming units (PFU) of Towne HCMV (or other HCMV strains) alone or in the presence of 8.3% guinea pig complement were added to each well and incubated for 1 h at 37°C in 5% CO_2. Skin fibroblast cells were harvested using trypsin-EDTA and suspended in medium at 1×10^5 cells/ml. 150 μl of cell suspension (1.5×10^4 cells) was added to each well and the plates were incubated at 37°C in 5% CO_2 for 4 days. The medium was then removed from each well by aspiration, the monolayer fixed with 10% formalin in 70% ethanol, and 0.1% methylene blue was added to each well for approximately 5 min. The methylene blue was removed by aspiration, the wells washed with water, and the plates dried and examined for the number of plaques in each well using an inverted microscope. Inhibition of infectious virus was determined according to the following formula:

$$\% \text{ plaque inhibition} = 1 - \frac{\text{PFU in treated well}}{\text{PFU in untreated well}} \times 100$$

Results

Characterization of HCMV-specific MAbs

The IgG subclasses were determined by ELISA using subclass-specific goat-anti-mouse antibodies. MAb 9B7 was a IgG_{2b} antibody; MAbs 41C2 and 3B10 were IgG_1 antibodies. The specificity of these MAbs for gcI glycoproteins was determined by Western blot analysis. 9B7 (Fig. 1, lane A) and 41C2 (Fig. 1, lane B) reacted with gp52 and the precursor glycoprotein, gp158 but not with gp93-130; 3B10 reacted with gp93-130 and gp158 but not gp52.

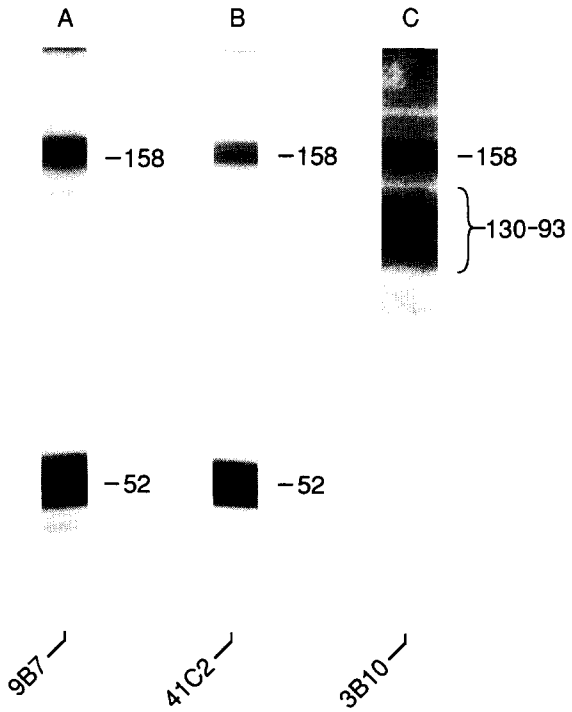

Fig. 1. Western blot analysis of HCMV-gcI specific monoclonal antibodies. Lane A: MAb 9B7 reactive with gp52 of gcI complexes; lane B: MAb 41C2 reactive with gp52 of gcI complexes; lane C: MAb 3B10 reactive with gp93–130 of gcI complexes. Molecular weights $\times 10^{-3}$ are indicated to the right of each lane.

Reactivity with various strains of HCMV and unrelated viruses

HCMV contains glycoproteins exhibiting structural and functional homology with those of other herpesviruses (Stinski, 1990), and immunologic cross-reactivity has been described among proteins from a number of viruses including HCMV (Balachandran et al., 1987; Tsai et al., 1990). The MAbs were therefore evaluated for reactivity with human fibroblasts infected with 4 laboratory-adapted strains of HCMV; 10 wild strains isolated from patients with congenital or opportunistic HCMV infection; and 6 unrelated viruses in an indirect immunofluorescence assay. All 3 MAbs reacted with all strains of HCMV tested, but not with other viruses (Table 1).

Reactivity with normal cellular proteins

DNA and amino acid homologies have been described between HCMV proteins and products of HLA class I α chain (Beck and Barrell, 1988) and DRβ chain (Fujinami et al., 1988) genes, and a MAb reactive with a 94-kDa HCMV envelope glycoprotein was recently reported by Michelson et al. (1989) which cross-reacts with a cellular protein expressed on uninfected fibroblasts.

TABLE 1

Reactivity of HCMV-specific MAbs with various strains of HCMV and unrelated viruses

Virus-infected fibroblasts[a]	MAb reactivity (Indirect immunofluorescence)[b]		
	9B7	41C2	3B10
Lab-adapted HCMV strains			
Towne	+	+	+
AD169	+	+	+
Davis	+	+	+
Wild strains from clinical HCMV isolates			
MC	+	+	+
LJ	+	+	+
CD	+	+	+
BT5036	+	+	+
KS	+	+	+
SG	+	+	+
BT5048	+	+	+
RT	+	+	+
TL	+	+	+
BGYCA	+	+	+
Unrelated viruses			
HSV-1	−	−	−
HSV-2	−	−	−
Adeno-2	−	−	−
Adeno-5	−	−	−
VZV	−	−	−
Influenza-A	−	−	−

[a]Human skin fibroblast monolayers in 8-chamber glass slides were infected with viral isolates and observed for cytopathic effects. The infected monolayer was then fixed with acetone:methanol (v/v, 1:1).

[b]Slides were incubated with 10 μg/ml of HCMV-specific MAbs, washed, and then incubated with FITC-labeled goat anti-mouse IgG $F(ab')_2$ fragments and examined using a fluorescence microscope.

The MAbs were therefore tested by indirect immunofluorescence for reactivity with normal cellular proteins on cell lines of fibroblast, epithelial, and lymphoid origin; and on post-mortem tissue sections from autopsy tissues obtained from patients dying of causes unrelated to HCMV (Table 2). No cross-reactivity was observed with any cells or tissues tested.

Virus neutralizing activity of HCMV-specific MAbs

The gp52-specific MAb 9B7 exhibited 50% plaque reduction at an antibody protein concentration of 1.1 μg/ml in the presence of complement (Fig. 2), but did not inhibit HCMV replication significantly in the absence of complement (data not shown). In contrast, gp52-specific MAb 41C2 did not inhibit HCMV in the presence or absence of complement at concentrations as high as 50 μg/ml (Fig. 2). MAb 3B10, which reacts with gp93–130, exhibited 50% plaque reduction at 1.8 μg/ml in the absence of complement, and was unaffected by the

TABLE 2

Reactivity of HCMV-specific MAbs with normal cellular proteins

	MAb reactivity[a] (Indirect immunofluorescence)		
	9B7	41C2	3B10
Cell lines[b]			
Fibroblasts	–	–	–
CMK	–	–	–
Hep-2	–	–	–
LCL	–	–	–
T_h clone	–	–	–
Mph line	–	–	–
Post-mortem tissue sections[c]			
Lung	–	–	–
Kidney	–	–	–
Heart	–	–	–
Muscle	–	–	–
Pancreas	–	–	–
Liver	–	–	–
Spleen	–	–	–
Parotid	–	–	–
Cerebral cortex	–	–	–
grey matter	–	–	–
white matter	–	–	–
Cerebellum	–	–	–
Bladder	–	–	–
Testis	–	–	–
Adrenal gland	–	–	–
Endometrium	–	–	–
Ovary	–	–	–
Myocardium	–	–	–

[a]Cell lines and tissue sections were fixed to glass slides, incubated with 10 μg/ml HCMV-specific MAbs, washed, and then incubated with FITC-labeled goat anti-mouse IgG $F(ab')_2$ fragments and examined using a fluorescence microscope.

[b]Fibroblasts were obtained from human foreskin or skin biopsies; CMK and Hep-2 are epithelial cell lines of monkey and human origin, respectively; LCL, T_h clones, and Mph lines are human lymphoid cell lines.

[c]Autopsy tissues were obtained from several patients dying of causes unrelated to HCMV.

addition of complement (Fig. 2). A non-neutralizing MAb 15F9 reactive with HCMV gcII complexes was included as a control.

Synergistic activity of HCMV-specific MAbs

Previous studies using a standard agarose plaque reduction assay demonstrated that the neutralizing activity of 9B7 could be augmented by 20-fold in the presence of non-neutralizing MAb 41C2 (Lussenhop et al., 1988). In the present study, we have employed the microneutralization assay to show that the antiviral activity of 9B7 is increased approximately 10-fold by the presence of 0.05 μg/ml 41C2, and an additional 5-fold in the presence of 0.5 μg/

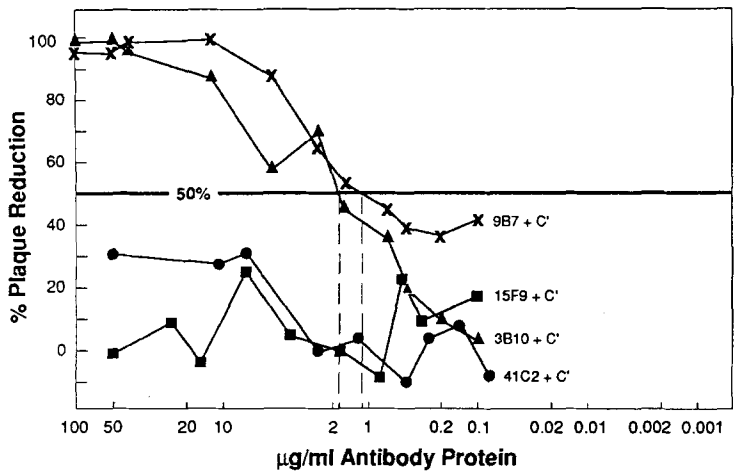

Fig. 2. Virus neutralizing activity of HCMV-specific monoclonal antibodies. The anti-HCMV activity of serial concentrations of gp52-specific and gp93-specific MAbs in the presence of guinea pig complement was determined in triplicate wells using the microneutralization assay described in Materials and Methods. A non-neutralizing gcII-specific MAb 15F9 was included as a control. Results are expressed as the mean percent plaque inhibition in treated wells relative to untreated wells, and are representative of results obtained in 3 separate experiments.

ml 41C2 (Fig. 3A). Thus, 50% plaque reduction can be achieved at a total IgG concentration of as little as 0.1 μg/ml. In contrast, no significant augmentation of 9B7 was observed at 2 μg/ml of 3B10 (Fig. 3B), which is consistent with the fact that these MAbs react with entirely different glycoproteins.

Virus neutralizing activity of an equimolar mixture of HCMV-specific MAbs

When the 3 gcI-specific MAbs were combined at a ratio of 1:1:1, 50% plaque reduction of Towne HCMV was observed at a total IgG concentration of 0.175 μg/ml, a level approximately 5–10 fold less than that for any component MAb (Fig. 4A). Similar experiments were then performed with 4 laboratory-adapted (Fig. 4A) and 5 wild strains (Fig. 4B) of HCMV. In all cases, 50% plaque reduction was observed at concentrations of total antibody protein of 0.5 μg/ml, and 0.2 μg/ml for most strains. Although 95% plaque inhibition was observed at 2 μg/ml antibody protein for all strains tested, we wanted to determine if the low levels of residual virus could still be neutralized by the MAbs. To address this question, residual Towne HCMV was recovered from the culture supernatant following 7 days of exposure to 10 μg/ml of an equimolar mixture of the MAbs followed by 7 days of culture in the absence of the MAbs, and re-incubated with serial concentrations of the MAb cocktail. The MAbs exhibited 50% plaque reduction at 1 μg/ml, suggesting that virus which had reactivated following removal of antibody was still susceptible to neutralization by the MAb cocktail.

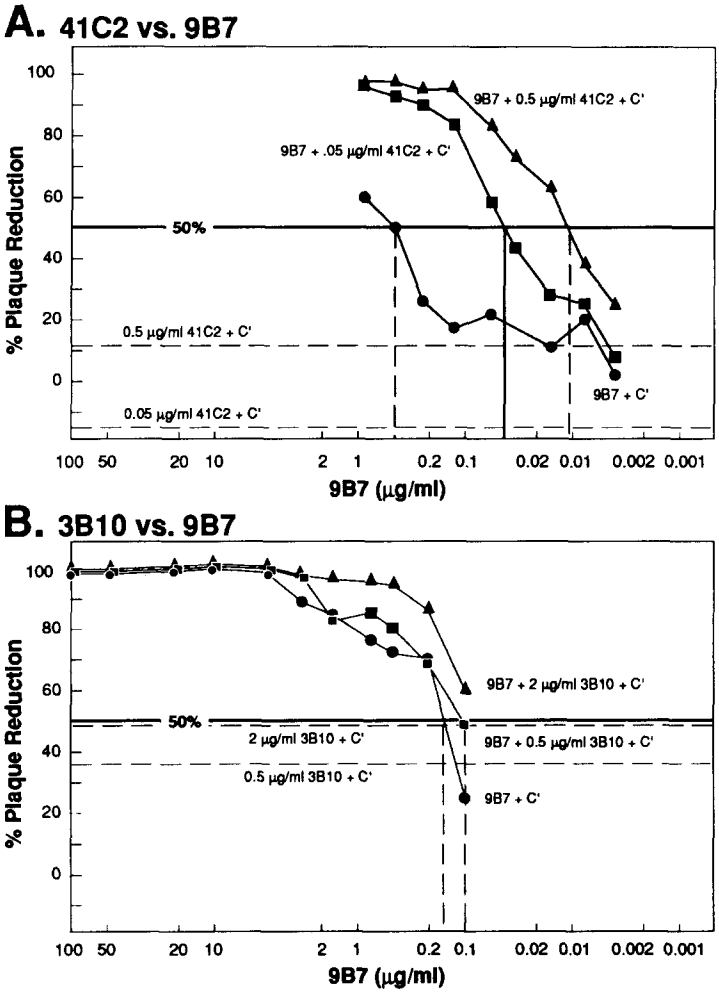

Fig. 3. Synergistic virus neutralizing activity of HCMV-specific monoclonal antibodies. Serial concentrations of a primary MAb added to suboptimal concentrations of a second MAb were incubated with Towne strain HCMV in the presence of guinea pig complement in triplicate wells to determine the augmenting effect of the secondary antibody on the antiviral activity of the primary antibody using the microneutralization assay described in Materials and Methods. (A) Neutralizing activity of MAb 9B7 in the presence of 0.05 μg/ml or 0.5 μg/ml MAb 41C2; (B) Neutralizing activity of MAb 9B7 in the presence of 0.5 μg/ml or 2 μg/ml MAb 3B10. Results are representative of those obtained in 3 separate experiments.

Neutralizing activity of HCMV-specific MAbs in the presence of human sera

We have previously described gp52-specific MAbs which inhibit the binding of 9B7 and 41C2 in an ELISA assay, and which also inhibit their neutralizing activity in a standard agarose plaque reduction assay (Lussenhop et al., 1988). Since we have also shown that human sera from HCMV-seropositive donors

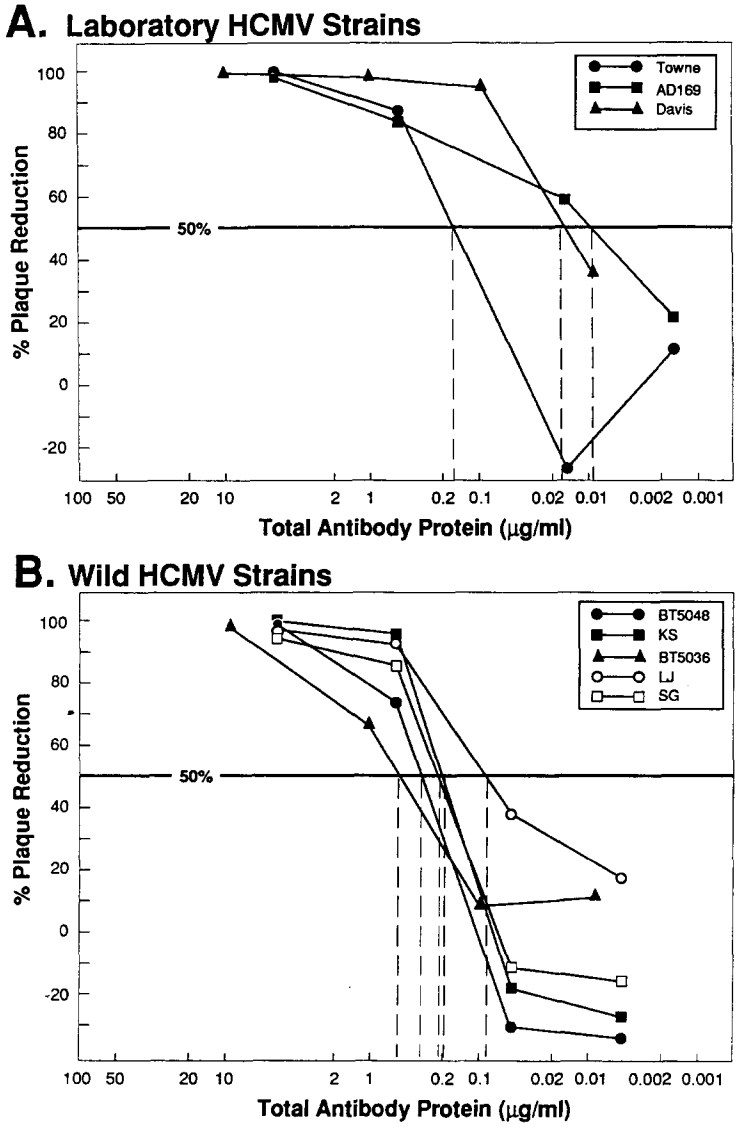

Fig. 4. Virus neutralizing activity of an HCMV-specific monoclonal antibody cocktail against various strains of HCMV. The antiviral activity of serial concentrations of a cocktail containing equimolar concentrations of MAbs 9B7, 41C2, and 3B10 were tested in triplicate wells against different strains of HCMV in the microneutralization assay described in Materials and Methods. (A) Neutralization of laboratory-adapted strains of HCMV; (B) Neutralization of wild strains of HCMV isolated from patients with congenital or acquired HCMV infections. Results are representative of those obtained in 3 separate experiments.

contain antibodies which recognize gp52 (Kari and Gehrz, 1990b), we postulated that HCMV might induce inhibitory antibodies to evade the

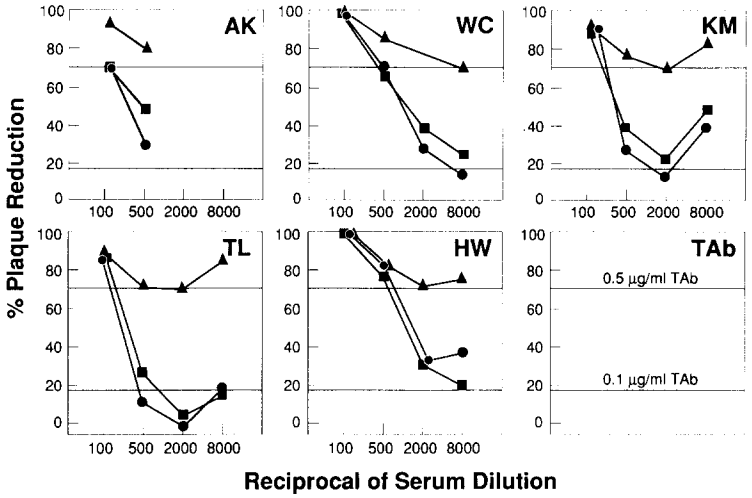

Fig. 5. Virus neutralizing activity of an HCMV-specific monoclonal antibody cocktail in the presence of human sera. The antiviral activity of 0.1 μg/ml or 0.5 μg/ml of a cocktail containing equimolar concentrations of MAbs 9B7, 41C2, and 3B10 (TAb = triclonal cocktail) against Towne strain HCMV was determined in triplicate wells in the presence of serial dilutions of human sera from an HCMV-antibody negative adult donor (AK), two HCMV-antibody positive adult donors (WC, KM), and two HCMV-antibody positive infants with congenital HCMV infection (TL, HW) using the microneutralization assay described in Materials and Methods. ●—● serum alone; ■—■ serum + 0.1 μg/ml TAb; ▲—▲ serum + 0.5 μg/ml TAb. The TAb lines in the lower right hand figure represent the % plaque reduction of Towne strain HCMV by 0.1 μg/ml or 0.5 μg/ml of the TAb in the absence of human serum. Results are representative of those obtained in 3 separate experiments.

human immune response. To test this hypothesis, the neutralizing activity of 0.5 μg/ml and 0.1 μg/ml of an equimolar mixture of 9B7, 41C2, and 3B10 in the presence of 4 dilutions of human sera was compared to that of equivalent amounts of the MAb cocktail in the absence of human serum from 3 adults and 2 congenitally infected infants (Fig. 5). Human sera alone at high concentrations inhibited HCMV plaque formation irrespective of the HCMV-immune status of the donor, suggesting that humoral mechanisms other than HCMV-specific antibodies may contribute to host defense against this virus. In every case, the neutralizing activity of the MAb cocktail combined with human serum was equal to or greater than that of the MAbs alone, suggesting that human sera do not contain obvious inhibitory antibodies.

Discussion

In the present study, we describe a combination of three HCMV-specific MAbs exhibiting synergistic antiviral activity in vitro. MAbs reactive with unique epitopes on 2 different HCMV envelope glycoproteins were selected because of novel antiviral characteristics determined by the binding specificities and biological properties of individual immunoglobulin molecules, as well as

unique molecular interactions among component MAbs. MAb 9B7 is a complement-dependent neutralizing antibody which reacts with gp52 of gcI complexes. MAb 41C2 also reacts with gp52 and is non-neutralizing, but augments the neutralizing activity of 9B7 by approximately 10-fold. The epitopes recognized by these MAbs have previously been assigned to independent domains expressed in close physical proximity on the topographical structure of native gcI complexes, as determined by their mutual augmentation of binding in a simultaneous competitive binding immunoassay (Lussenhop et al., 1988; Kari et al., 1990a). Therefore, we postulate that binding of 41C2 to its epitope alters the conformation of gcI to increase accessibility of the proximate epitope for 9B7, thereby potentiating its neutralizing activity. MAb 3B10 is a complement-independent neutralizing antibody which reacts with gp93–130 of gcI complexes. We have confirmed that 3B10 recognizes an epitope on the N-terminal portion of the gB polypeptide represented in gp93 by reactivity with a truncated protein expressed in vaccinia virus (Kari et al., 1990a). The gcI glycoproteins are presumably related to a primordial family of herpesvirus glycoproteins including gB of HSV-1, which has been shown to be involved in fusion of the virus with the host cell membrane (Highlander et al., 1988; Stinski, 1990). Therefore, 3B10 may inhibit this critical stage in the replicative cycle of HCMV.

These data suggest that a combination of synergistic HCMV-specific MAbs may prove useful for the prevention and treatment of life-threatening HCMV infections in immunocompromised patients. Prophylactic administration of HCMV hyperimmune globulin has variably reduced the incidence and severity of opportunistic HCMV infections in transplant patients, presumably by limiting hematogenous dissemination of exogenous or reactivated virus (Condie and O'Reilly, 1984; Bowden et al., 1986; Ringdis et al., 1987; Winston et al., 1987; Snydman et al., 1987; Einsele et al., 1988). However, it is unclear whether the protective effects of hyperimmune globulin are related to HCMV-specific antibodies or non-specific immunoglobulin. Since HCMV induces Fc receptor (FcR) expression on the surface of infected cells, non-specific attachment of IgG to FcR might facilitate ADCC or other undefined antiviral mechanisms. In contrast, passive immunization of patients with symptomatic HCMV disease reduces viral shedding but has little effect on outcome in most cases (Pettersson et al., 1986; Lautenschlager et al., 1989). The effectiveness of hyperimmune globulin preparations is likely to be limited by their low levels of neutralizing HCMV-specific antibodies relative to total immunoglobulin. Furthermore, human antibodies in convalescent donor sera may not exhibit the same specificities and antiviral activity as those which apparently restrict viral dissemination and disease in the early stages of HCMV infection. HCMV-specific MAbs might prove more effective than HCMV-hyperimmune globulin, since they exhibit greater specificity and antiviral activity in vitro. However, the efficacy of HCMV-specific MAbs is likely to be limited by several factors. There is little direct evidence that in vitro

neutralizing activity correlates with antibody-mediated host defense mechanisms in vivo. Neutralizing antibodies may inactivate extracellular virus or prevent its spread to uninfected cells, but will have little effect on HCMV replication in infected cells. Furthermore, the pharmacokinetics and biodistribution of the MAbs may limit the feasibility of achieving therapeutic levels in HCMV-infected tissues (Press et al., 1987). Natural HCMV infection appears to confer cross-reactive humoral immunity against most, if not all, strains of HCMV, since convalescent sera from seropositive individuals appear to be reactive with a variety of laboratory-adapted and wild strains. However, immunosuppressed patients are frequently infected with multiple strains of HCMV, suggesting that antibodies elicited by primary infection with one strain are not necessarily protective against reinfection with other strains of the virus (Spector et al., 1984; Drew et al., 1984). Although most HCMV-specific MAbs recognize conserved determinants, epitopes have been identified which are either uniquely expressed by particular strains or which have been deleted from certain strains of HCMV (Kari et al., 1986). Furthermore, prolonged exposure of viruses in vitro to a single MAb has been shown to select for MAb-resistant (mar) mutant viruses which have deleted the epitope recognized by that antibody (Holland et al., 1983; Highlander et al., 1989). A cocktail containing a combination of MAbs directed against different epitopes on HCMV glycoproteins markedly increases the likelihood of antiviral activity against all possible strains of HCMV, and virtually eliminates the possibility that resistant strains will emerge.

An additional problem with murine HCMV-specific MAbs is that they frequently elicit human anti-mouse antibody (HAMA) responses to isotypic or idiotypic determinants on the antibody molecule which may affect the safety or efficacy of therapeutic MAbs in vivo (Jaffer et al., 1983; Harkonen et al., 1987). Anti-isotypic antibody responses can be prevented by using 'humanized' MAbs exhibiting equivalent specificity and antiviral activity (Morrison et al., 1984; Strelkauskas, 1987). A number of HCMV-specific human MAbs have been described, and several are currently being evaluated in clinical trials (Emanuel et al., 1984; Masuho et al., 1987; Foung et al., 1989). However, anti-idiotypic antibody responses may occur with repeated or prolonged exposure even when human MAbs are used. The increased antiviral potency of a combination of synergistic MAbs should reduce the frequency and amount of individual antibodies required, thereby limiting exposure to immunogenic determinants. In any event, these problems should not pose an insurmountable obstacle to passive immunization of transplant and AIDS patients with life-threatening HCMV infections, since their primary antibody responses are impaired.

Acknowledgements

The authors wish to thank Mrs. Victoria Halverson and Mrs. Sheryl Schroeder for their excellent preparation of this manuscript. Support for this

work was provided by grants from Medimorphics, Inc., Children's Biomedical Research Institute, and PO1-HD19937 from the National Institute of Child Health and Human Development.

References

Balachandran, N., Oba, D.E. and Hutt-Fletcher, L.M. (1987) Antigenic cross-reactions among herpes simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus. J. Virol. 61, 1125–1135.

Beck, S. and Barrell, B.G. (1988) Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. Nature (London) 331, 269–272.

Bowden, R.A., Sayers, M., Flournoy, N., Newton, B., Banaji, M., Thomas, E.D. and Meyers, J.D. (1986) Cytomegalovirus immunoglobulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. N. Engl. J. Med. 314, 1006–1010.

Britt, W.J. (1984) Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. Virology 135, 369–378.

Britt, W.J., Vugler, L. and Stephens, E.B. (1988) Induction of complement-dependent and -independent neutralizing antibodies by recombinant-derived human cytomegalovirus gp55–116 (gB). J. Virol. 62, 3309–3318.

Britt, W.J. and Vugler, L.G. (1989) Processing of the gp55–116 envelope glycoprotein complex (gB) of human cytomegalovirus. J. Virol. 63, 403–410.

Britt, W.J., Vugler, L., Butfiloski, E.J. and Stephens, E.B. (1990) Cell surface expression of human cytomegalovirus (HCMV) gp 55–116 (gB): use of HCMV-infected recombinant vaccinia virus-infected cells in analysis of human neutralizing antibody response. J. Virol. 64, 1079–1085.

Condie, R.M. and O'Reilly, R.J. (1984) Prevention of cytomegalovirus infection by prophylaxis with an intravenous, hyperimmune, native, unmodified cytomegalovirus globulin. Randomized trial in bone marrow transplant recipients. Amer. J. Med. 76 (3A), 134–141.

Cranage, M.P., Kouzarides, T., Bankier, A.T., Satchwell, S., Weston, K., Tomlinson, P., Barrell, B., Hart, H., Bell, S.E., Minson, A.C. and Smith, G.L. (1986) Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in vaccinia virus. EMBO J. 5, 3057–3063.

Drew, W.L., Sweet, E.S., Miner, R.C. and Mocarski, E.S. (1984) Multiple infections by cytomegalovirus in patients with acquired immunodeficiency syndrome: documentation by Southern blot hybridization. J. Infect. Dis. 150, 952–953.

Einsele, H., Vallbracht, A., Schmidt, H., Friese, M., Shuch, K., Haen, M., Dopfer, R., Niethammer, D., Waller, H.D. and Ehninger, G. (1988) Prevention of CMV infection after BMT in high risk patients using CMV hyperimmune globulin. Cancer Detect. Prevent. 12, 637–641.

Emanuel, D., Gold, J., Colacino, J., Lopez, C. and Hammerling, U. (1984) A human monoclonal antibody to cytomegalovirus (CMV). J. Immunol. 133, 2202–2205.

Foung, S.K.H., Perkins, S., Bradshaw, P., Rowe, J., Rabin, L.B., Reyes, G.R. and Lennette, E.T. (1989) Human monoclonal antibodies to human cytomegalovirus. J. Infect. Dis. 159, 436–443.

Fujinami, R.S., Nelson, J.A., Walker, L. and Oldstone, M.B.A. (1988) Sequence homology and immunologic cross-reactivity in human cytomegalovirus with HLA-DR β chain: a means for graft rejection and immunosuppression. J. Virol. 62, 100–105.

Gehrz, R.C. (1991) Human cytomegalovirus: biology and clinical perspectives. Adv. Pediatr. 38, 190–219.

Gonczol, E., Furlini, G., Ianacone, J. and Plotkin, S.A. (1986) A rapid microneutralization assay for cytomegalovirus. J. Virol. Methods 14, 37–41.

Gretch, D.R., Suter, M. and Stinski, M.F. (1987) The use of biotinylated monoclonal antibodies and streptavidin affinity chromatography to isolate herpes virus hydrophobic proteins or glycoproteins. Anal. Biochem. 163, 270–277.

Gretch, D.R., Kari, B., Rasmussen, L., Gehrz, R.C. and Stinski, M.F. (1988a) Identification and characterization of three distinct families of glycoprotein complexes present in the envelopes of human cytomegalovirus. J. Virol. 62, 875–881.

Gretch, D.R., Gehrz, R.C. and Stinski, M.F. (1988b) Characterization of a human cytomegalovirus glycoprotein complex (gcI). J. Gen. Virol. 69, 1205–1215.

Harkonen, S., Stoudemire, J., Mischak, R., Spitler, L.E., Lopez, H. and Scannon, P. (1987) Toxicity and immunogenicity of monoclonal antimelanoma antibody-ricin A chain immunotoxin in rats. Cancer Res. 47, 1377–1382.

Highlander, S.L., Cai, W., Person, S., Levine, M. and Glorioso, J.C. (1988) Monoclonal antibodies define a domain on herpes simplex virus glycoprotein B involved in virus penetration. J. Virol. 62, 1881–1888.

Highlander, S.L., Dorney, D.J., Gage, P.J., Holland, T.C., Cai, W., Person, S., Levine, M. and Glorioso, J.C. (1989) Identification of 'mar' mutants in herpes simplex virus type 1 glycoprotein B which alter antigenic structure and function in virus penetration. J. Virol. 63, 730–738.

Holland, T.C., Marlin, S.D., Levine, M. and Glorioso, J. (1983) Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. 45, 672–682.

Jaffer, G.J., Colvin, R.B., Cosimi, J.V., Giorgi, G., Goldstein, C., Fuller, J.T., Kurnick, C., Lillehi, C. and Russell, P.S. (1983) The human immune response to murine OKT3 monoclonal antibody. Transplant. Proc. 15, 646–648.

Kari, B.E., Lussenhop, N., Goertz, R., Wabuke-Bunoti, M. and Gehrz, R.C. (1986) Characterization of monoclonal antibodies reactive to three biochemically distinct human cytomegalovirus glycoprotein complexes. J. Virol. 60, 345–352.

Kari, B., Liu, Y-N.C., Goertz, R., Lussenhop, N., Stinski, M.F. and Gehrz, R.C. (1990a) Structure and composition of a family of human cytomegalovirus glycoprotein complexes designated gC-I (gB). J. Gen Virol. 71, 2673–2680.

Kari, B. and Gehrz, R. (1990b) Analysis of human antibody responses to human cytomegalovirus envelope glycoproteins found in two families of disulfide-linked glycoprotein complexes designated gC-I and gC-II. Arch. Virol. 114, 213–228.

Kari, B., Goertz, R. and Gehrz, R. (1990c) Characterization of cytomegalovirus glycoproteins in a family of complexes designated gC-II with murine monoclonal antibodies. Arch. Virol. 112, 55–65.

Kohler, G. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) 256, 495–497.

Laemmli, U.K. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) 227, 680–684.

Landini, M.P., Re, M.C., Mirolo, G., Baldassari, B. and LaPlaca, M. (1985) Human immune response to cytomegalovirus structural polypeptides studied by immunoblotting. J. Med. Virol. 17, 303–311.

Lautenschlager, S., Ahonen, J., Eklund, B., Hockerstedt, K., Salmela, K., Isoniemi, H., Korsback, C., Suni, J. and Hayry, P. (1989) Hyperimmune globulin therapy for clinical cytomegalovirus infection in renal allograft recipients. Scand. J. Infect. Dis. 21, 139–143.

Liu, Y-N.C., Kari, B. and Gehrz, R.C. (1988) Immune responses to major human cytomegalovirus glycoprotein complexes. J. Virol. 98, 171–188.

Liu, Y-N.C., Klaus, A., Kari, B., Stinski, M.F., Eckhardt, J. and Gehrz, R.C. (1991) The N-terminal 513 amino acids of the envelope glycoprotein gB of human cytomegalovirus stimulates both B and T cell immune responses in humans. J. Virol. 65, 1644–1648.

Lussenhop, N., Goertz, R., Wabuke-Bunoti, M., Gehrz, R.C. and Kari, B. (1988) Epitope analysis of human cytomegalovirus glycoprotein complexes using murine monoclonal antibodies. Virology 164, 362–372.

Masuho, Y., Matsumoto, Y-I., Sugano, T., Fuzinaga, S. and Minamishima, Y. (1987) Human monoclonal antibodies neutralizing human cytomegalovirus. J. Gen. Virol. 68, 1457–1461.

Michelson, S., Tardy-Panit, M., Colimon, R. and Landini, M.P. (1989) A human cytomegalovirus-neutralizing monoclonal antibody recognizes a normal cell protein. J. Gen. Virol. 70, 673–684.

Mirolo, G., Baldassarri, B., Ripalti, A., Re, M.C., Clementi, M., Manzin, A. and Landini, M.P.

(1986) Antibody responses to individual cytomegalovirus structural proteins in different groups of subjects. Eur. J. Clin. Microbiol. 6, 207–210.

Morrison, S.L., Johnson, M.J., Herzenberg, L.A. and Oi, V.T. (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. Proc. Natl. Acad. Sci. USA 81, 6851–6855.

Pereira, L., Hoffman, M., Gallo, D. and Cremer, N. (1982) Monoclonal antibodies to human cytomegalovirus: three surface membrane proteins with unique immunological and electrophoretic properties specify cross-reactive determinants. Infect. Immunity 36, 924–932.

Pettersson, E., Edlund, B., Hockerstedt, K., Salmela, K., Von Willebrand, E., Hayry, P. and Ahonen, J. (1986) Passive immune therapy of clinical CMV disease in renal allograft recipients. Transplant. Proc. 18, 1384–1386.

Press, O.W., Appelbaum, F., Ledbetter, J.A., Martin, P.J., Zarling, J., Kidd, P. and Thomas, E.D. (1987) Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. Blood 69, 584–591.

Ringdis, O., Pihlstedt, P., Volin, L., Nikoskelainen, J., Lonnqvist, B., Ruutu, P., Ruutu, T., Toivanen, A. and Wahren, B. (1987) Failure to prevent cytomegalovirus infection by cytomegalovirus hyperimmune plasma: a randomized trial by the Nordic bone marrow transplantation group. Bone Marrow Transplant. 106, 12–18.

Rodgers, B., Borysiewicz, L., Mundin, J., Graham, S. and Sissons, P. (1987) Immunoaffinity purification of a 72 K early antigen of human cytomegalovirus: analysis of humoral and cell-mediated immunity to the purified polypeptide. J. Gen. Virol. 68, 2371–2378.

Snydman, D.R., Warner, B.F., Heinze-Lacey et al. (24 authors) (1987) Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. N. Engl. J. Med. 317, 1049–1054.

Spaete, R.R., Thayer, R.M., Probert, W.S., Masiarz, F.R., Chamberlin, S.H., Rasmussen, L., Merigan, T.C. and Pachl, C. (1988) Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. Virology 167, 207–225.

Spector, S.A., Hirata, K.K. and Newman, T.R. (1984) Identification of multiple cytomegalovirus strains in homosexual men with acquired immunodeficiency syndrome. J. Infect. Dis. 150, 953–956.

Stinski, M.F. (1990) Cytomegalovirus and its replication. In: B.N. Fields and D.M. Knipe (Eds), Virology, 2nd edit., pp. 1959–1980. Raven Press, New York.

Strelkauskas, A.J. (Ed.) (1987) Human hybridomas. Diagnostic and therapeutic applications. Marcel Dekker, Inc., New York and Basel.

Tsai, C-H.A., Williams, M.V. and Glaser, R. (1990) A monoclonal antibody that neutralizes Epstein-Barr virus, human cytomegalovirus, human herpes virus 6, and bacteriophage T4 DNA polymerases. Proc. Natl. Acad. Sci USA 87, 7963–7967.

Wentworth, B.B. and French, L. (1970) Plaque assay of cytomegalovirus strains of human origin. Proc. Soc. Exp. Med. Biol. 135, 253–258.

Winston, O.J., Ho, W.G., Lin, C-H., Bartoni, K., Budinger, M.D., Gale, R.P. and Champlin, R.E. (1987) Intravenous immunoglobulin for prevention of cytomegalovirus infection and interstitial pneumonia after bone marrow transplantation. Ann. Intern. Med. 106, 12–18.