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Identification of the gene coding for rhesus cytomegalovirus glycoprotein B and immunological analysis of the protein

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The nucleotide sequence of the gene encoding glycoprotein B (gB) of rhesus cytomegalovirus (RhCMV) was determined and the protein characterized. The open reading frame of gB encoded a protein of 854 amino acids with 60% identity and 75% similarity at the amino acid level to human cytomegalovirus (HCMV) gB. Cysteine residues in the extraluminal part of the protein are perfectly conserved. Out of the 16 potential *N*-linked glycosylation sites present in HCMV gB, 15 are conserved in RhCMV gB. Immunoblot analyses with antisera detected three bands of 150 kDa, 90–110 kDa and 55 kDa representing the full-length gB as well as the proteolytic cleavage products. Cross-reactivity and cross-neutralization of a number of HCMV gB-specific monoclonal antibodies with RhCMV gB indicated sharing of immunogenic epitopes between the two molecules. The RhCMV gB regions corresponding to antigenic domains AD-1, 2 and 3 of HCMV gB were immunogenic during natural RhCMV infection with the AD-1 region being the immunodominant domain. The data indicate that RhCMV might represent a useful model to investigate pathogenesis and immune surveillance of cytomegaloviruses.

Introduction

Human cytomegalovirus (HCMV) is an important pathogen in immunocompromised persons: allograft recipients can have severe and sometimes fatal infections in the post-transplant period (Ho, 1991), and among the human immunodeficiency virus (HIV)-infected population, whose lives have been extended by more effective therapies for other opportunistic infections, HCMV-related clinical complications are increasing considerably (Jacobson *et al.*, 1988). A particularly serious complication in AIDS patients is HCMV-caused retinitis, which invariably leads to blindness when left untreated. HCMV has also been proposed as a cofactor that may accelerate HIV infection. *In vitro*, HCMV can transactivate the HIV promoter region and coinfection with HCMV enhances HIV replication (Davis *et al.*, 1987). Similarly, replication of HCMV is enhanced by HIV (Skolnik *et al.*, 1988). HIV and HCMV have been found to coinfect brain cells in patients with AIDS (Nelson *et al.*, 1988). The clinical relevance of these observations is unclear. In addition, HCMV is the most common cause of congenital infections and results in a large and varied range of clinical manifestations (Alford *et al.*, 1990). The mechanisms of pathogenesis in these situations await resolution.

The immunological effector functions which control HCMV infections are difficult to analyse since the virus is strictly species specific and no animal model system is available. Indirect support for the importance of the cellular immune response in controlling HCMV infection comes from transplant patients in whom the presence of HCMV-specific cytotoxic T cells correlates with favourable outcome of the infection (Reusser *et al.*, 1991). In the closely related murine cytomegalovirus system protection from a lethal challenge can be mediated by cytotoxic T cells (Reddehase *et al.*, 1987).

The humoral immune response also plays an important role during HCMV infection. Transfer of maternal HCMV-specific antibodies is important in preventing HCMV disease in the newborn (Fowler *et al.*, 1992). In addition, protection from reinfection is correlated with neutralizing antibodies (Adler *et al.*, 1995). In the murine cytomegalovirus system antibodies have been shown to prevent virus dissemination as well as conferring protection from a lethal challenge (Rapp *et al.*, 1992; Jonjic *et al.*, 1994).

With respect to the antiviral humoral immune response glycoprotein B (gB) is the dominant antigen on the envelope of

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The GenBank accession number of the RhCMV gB nucleotide sequence is U76749.

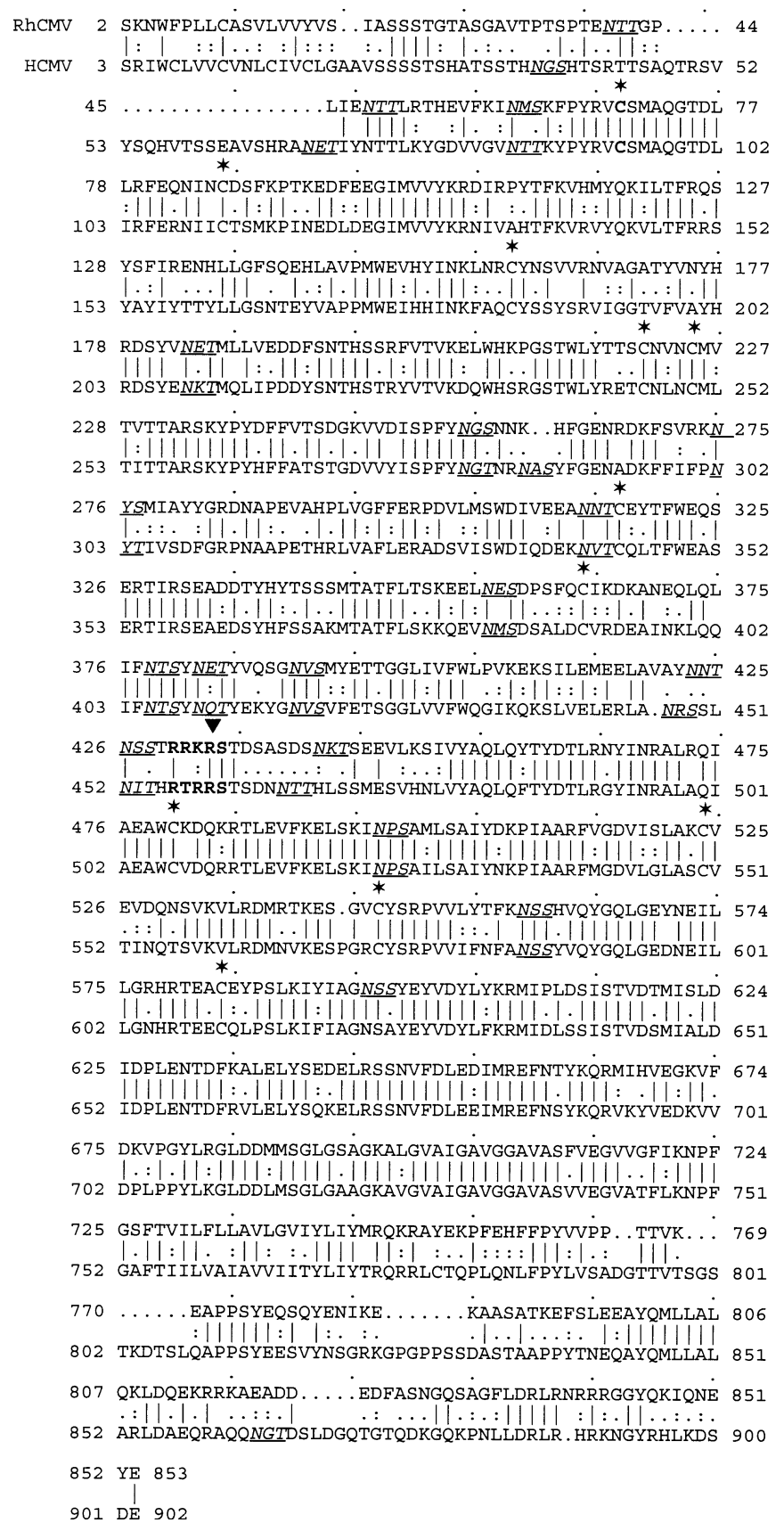

Fig. 1. For legend see facing page.

HCMV and nearly 100% of HCMV-infected individuals develop antibodies against this protein (Kniess *et al.*, 1991). Antibody preadsorption experiments with recombinant-derived gB have also shown that in some human sera a considerable fraction of the neutralizing response is directed against gB (Britt *et al.*, 1990; Marshall *et al.*, 1994). Consequently, gB has been proposed as a candidate subunit vaccine and clinical trials have been initiated.

Nevertheless, due to the exquisite species specificity of cytomegaloviruses progress in immunoprophylaxis or treatment of infections has been slow. However, questions concerning pathogenesis and immune surveillance might be appropriately addressed in non-human primate systems because of the intimate coevolutionary relationship. For example, macaques infected with simian immunodeficiency virus (SIV) frequently present with simian cytomegalovirus-induced complications which closely parallel those found in humans. Sites of rhesus CMV (RhCMV) infection in these animals include brain, gastrointestinal tract, spleen, lungs, testicles, nerves and lymph nodes (Baskin, 1987).

As a first step towards the characterization of the humoral immune response against primate CMV we have investigated the nucleotide sequence of the RhCMV gB gene and the immune response against the protein. Our data show that the protein is highly homologous to HCMV gB and that it shares important immunogenic properties.

Methods

■ **Virus and cell culture.** RhCMV strain 68-1 was obtained from the American Type Culture Collection. Virus was propagated in human foreskin fibroblasts (HFF) by standard methods. Extracellular virus was purified from tissue culture supernatant through a glycerol–tartrate gradient (Almeida *et al.*, 1978).

■ **Antibodies.** The HCMV-specific human and murine monoclonal antibodies have been described before: 89-104 (Wagner *et al.*, 1992), MSL-109 (Ehrlich *et al.*, 1988), the ITC series (Ohlin *et al.*, 1993), 27-156, 27-287 (Schoppel *et al.*, 1996). The antisera aSgB-1 and aSgBNH were raised by standard methods in rabbits by immunizing the animals with purified recombinant proteins (Harlow *et al.*, 1988).

■ **Construction of recombinant cosmids and plasmids.** To construct a cosmid library, DNA from RhCMV virions was prepared and fragmented by a partial *Bam*HI digest and ligated into the cosmid vector pWE15 (Stratagene). The cosmid library was screened by hybridization using the 3·2 kb coding sequence of HCMV gB as probe. Working procedures were according to Sambrook *et al.* (1989). To construct expression plasmids pSgBNH, pSgB, pSAD-1 and pSgBCO the respective DNA fragments were amplified by PCR, ligated into the vector pQE9 (Qiagen) and transfected into *E. coli* M15(pREP4). Correct insertion of the respective DNA fragments was monitored by nucleotide sequence analysis. All cloning procedures were performed using standard methods. Plasmid XP1, expressing amino acids (aa) 555–705 of HCMV pp150 (pUL32), has been described previously (Schoppel *et al.*, 1996).

■ **Expression of recombinant proteins, SDS–PAGE and immunoblotting.** Recombinant proteins were purified on Ni-chelate columns according to the manufacturer's instructions (Qiagen). When analysed on Coomassie Brilliant Blue-stained polyacrylamide gels, the proteins appeared as a single band indicating a purity of $> 90\%$. Immunoblot analysis following electrophoretic separation of proteins through 15% SDS–PAGE was done by standard procedures. The blots were blocked in PBS, 0·1% Tween 20 and incubated overnight at 4 °C with monoclonal antibodies or rhesus sera in PBS containing 0·1% Tween 20. Serum dilution was 1:150. Antibody binding was detected after incubation with alkaline phosphatase-coupled anti-immunoglobulins by staining with BCIP and NBT.

■ **RhCMV neutralization assay.** Neutralizing capacity of sera and monoclonal antibodies was carried out using a conventional plaque reduction assay. RhCMV (100–200 p.f.u.) was incubated with sera in appropriate dilutions for 1 h at 37 °C in a volume of 100 µl. The mixture was added to 30000 HFF, which had been seeded in 96-well microtitre plates. After a 4 h incubation period the medium was removed and 300 µl of fresh medium was added. Plates were analysed for cytopathic effect (CPE) 4–5 days after infection. 100% neutralization was assigned to wells showing no CPE.

■ **ELISA.** Polystyrene 96-well microtitre plates were coated with 50 µl per well purified fusion protein at a concentration of 3 µg/ml in a buffer containing 6 M urea, pH 9·5. Microtitre plates were incubated for 16 h at 4 °C in a humid chamber. All subsequent steps were carried out at 37 °C. Reaction wells were rinsed three times with buffer A (PBS, 0·05% Tween 20) and blocked for 2 h with PBS containing 2% foetal calf serum (FCS). Plates were rinsed again with buffer A and incubated with sera (dilution 1:50) or monoclonal antibodies for 2 h (50 µl per well). After four additional washes with buffer A, 50 µl peroxidase-conjugated second antibody was added in appropriate dilutions for 45 min. The reaction was stopped by addition of 100 µl 1 M H_2SO_4 and the absorbance at 492 nm was determined. Dilution of all antibodies was done in the dilution buffer provided with the HIV 1/2 ELISA kit (Biotest).

■ **Nucleotide sequence analysis and accession number.** Nucleotide sequence analysis was performed on an ABI 377 using the dye terminator cycle sequencing chemistry according to the manufacturer's instructions (Perkin Elmer). Accession number U76749 was assigned to the sequence. DNA sequence editing, translation, comparison and alignment was performed by use of the UWGCG (University of Wisconsin Genetics Computer Group) software.

Results

Primary amino acid sequence of RhCMV gB

To identify the gene coding for RhCMV gB a cosmid library made from viral DNA was established by standard procedures (see Methods). The library was screened using

Fig. 1. Alignment of the gB polypeptides of RhCMV strain 68-1 and HCMV strain AD169. The sequences are displayed in the one-letter amino acid code. Gaps have been introduced into the sequences to generate maximum alignment. Identical amino acids are marked (±) and similar amino acids (:). Cysteines are marked (*) and potential *N*-linked glycosylation sites are underlined. The proteolytic cleavage site is indicated by the black triangle.

DNA from HCMV gB as hybridization probe. Eight positive signals were obtained and one clone (designated pC36) was purified and characterized in detail. The nucleotide sequence of a 3·2 kb segment was determined. This stretch of DNA contained an open reading frame between nucleotides 298–2859 which showed 63·9% identity to the HCMV gB (strain AD169) nucleotide sequence (data not shown).

The gB protein predicted from the nucleotide sequence is 854 aa long with a molecular mass of 97·8 kDa (Fig. 1). The putative protein has the characteristics of a membrane protein. It contains 16 potential *N*-linked glycosylation sites, a hydrophobic N-terminal domain between residues 1–20 which is likely to be a cleavable signal sequence and stretches of hydrophobic sequences close to the carboxy terminus (aa 688–745) which may function as anchor sequences. The long stretch of hydrophobic residues is also found in HCMV gB. It was suggested that the most hydrophobic domain located between aa 751–771 of HCMV gB is responsible for membrane insertion of the molecule (Reschke *et al.*, 1995). Using the program Bestfit we compared the predicted translation product of this gene with gB from HCMV and Fig. 1 shows an alignment of the two sequences. RhCMV gB has 75% amino acid similarity and 60% identity to HCMV gB. Overall, RhCMV gB is 52 aa shorter than its human counterpart. The differences are mainly accounted for by deletions at the amino- and carboxy-terminal ends of the protein.

Similarities between the two proteins include:

(i) The positions of 11 cysteine residues which are present between the putative signal and anchor sequence are perfectly aligned suggesting that the extracellular portion of the protein may possess a similar overall structure. The cysteine residue at position 778 of HCMV gB which is located at the luminal part of the protein is missing in RhCMV gB.

(ii) In the extraluminal part of the molecule RhCMV gB contains 16 potential *N*-linked glycosylation sites of which 15 are nearly perfectly conserved in HCMV gB. The motif NSS located between aa 595–597 of RhCMV is missing in HCMV gB.

(iii) A putative recognition site (RRKR) for proteolytic cleavage of gB by furinlike proteases is present between aa 430–433 of RhCMV gB (Vey *et al.*, 1995).

The extent of homology between the two proteins provides convincing evidence that the putative RhCMV glycoprotein shown in Fig. 1 is gB.

Characterization of the RhCMV gB polypeptide

To characterize the RhCMV gB, extracellular virions were purified on glycerol–tartrate gradients, disrupted with SDS and analysed in immunoblots using as reagents:

(i) Rabbit antisera raised against aa 24–95 and aa 458–635 of RhCMV gB, respectively.

(ii) Murine monoclonal antibody 27-287 recognizing AD-1 (aa 552–635) of HCMV gB (Schoppel *et al.*, 1996).

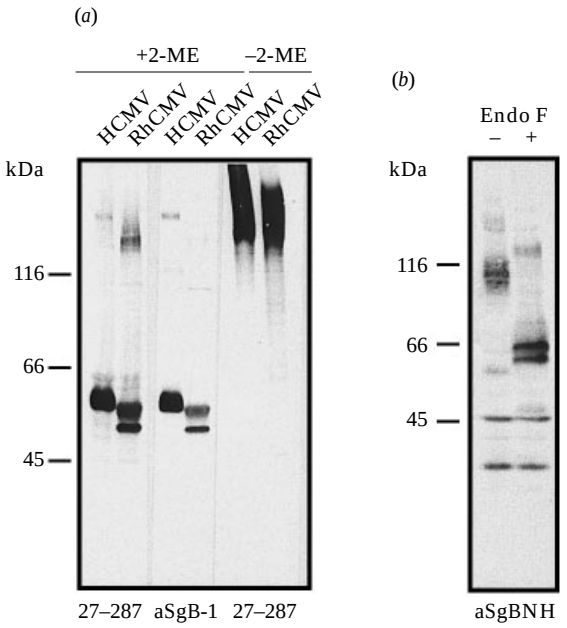

Fig. 2. Analysis of gB in lysates of HCMV and RhCMV virions. Extracellular virus particles were purified by centrifugation through a glycerol–tartrate gradient. Lysates were prepared, separated on PAGE and analysed in immunoblots. Antibody binding was detected with alkaline phosphatase-conjugated secondary antibodies and BCIP/NBT staining. (*a*) Lysates of HCMV and RhCMV virions were analysed in the presence (+2-ME) or absence (−2-ME) of 2-mercaptoethanol. (*b*) RhCMV virions were treated with endoglycosidase F prior to PAGE. Blots were developed with either monoclonal antibody 27-287, specific for the carboxy-terminal cleavage product of HCMV gB, a rabbit serum raised against aa 458–635 of RhCMV gB (aSgB-1) or a rabbit serum raised against aa 24–95 of RhCMV gB (aSgBNH).

For comparison, gradient-purified extracellular HCMV virions were also analysed. In accordance with previously published results proteins of 160 kDa and 58 kDa were detected in lysates from HCMV particles by antibody 27-287, with gp160 being the weaker signal (Fig. 2*a*) (Britt *et al.*, 1992). Gp160 and gp58 of HCMV represent the uncleaved full length gB protein and the carboxy-terminal cleavage product gp58, respectively. The rabbit antiserum raised against aa 458–635 of RhCMV gB also recognized gp160 and gp58 in lysates from HCMV virions. In lysates of RhCMV particles proteins of 150 kDa, 55 kDa and 52 kDa were detected by both reagents. These data indicate cross-reactivity between RhCMV and HCMV gB-specific immunoglobulins as well as similar proteolytic processing of the protein. Glycosylation of the protein, however, may be different, since the HCMV gp58 polypeptide is found as a homogeneous band whereas the corresponding part of RhCMV gB was detected as a doublet in different virus preparations. Both the 55 kDa and the 52 kDa polypeptides represent glycosylated proteins since, after treatment with endoglycosidase F, a single protein of 50 kDa was detected in immunoblots (data not shown).

When the rabbit antiserum raised against aa 24–95 of RhCMV gB was used in immunoblots, a diffusely migrating

band between 90–110 kDa was detected in addition to the 150 kDa full-length gB (Fig. 2 *b*). Upon treatment with endoglycosidase F the proteins were reduced in size to 120 kDa and 60–65 kDa, respectively. This indicated that as with HCMV gB the amino-terminal part of the protein is extensively modified by *N*-linked glycosylation. When lysates from RhCMV or HCMV virions were analysed in the absence of the reducing agent 2-mercaptoethanol (2-ME), a smear of larger proteins ranging in size from 150–300 kDa in HCMV lysates and from 150–250 kDa in RhCMV lysates was observed (Fig. 2 *a*). In addition, the low molecular mass proteins were no longer detectable in the absence of 2-ME, indicating that RhCMV gB exhibits disulfide bonding similar to HCMV gB. We therefore conclude that RhCMV gB is processed and inserted into the membrane of virions in a way that is identical to HCMV (Britt *et al.*, 1992).

Immune response to RhCMV gB

HCMV gB is the immunodominant envelope glycoprotein and represents the only glycoprotein that is regularly detected in virus lysates using immunoblotting with sera from HCMV-seropositive persons. To analyse whether RhCMV gB is similarly dominant we performed immunoblots using purified RhCMV virions and sera from RhCMV-seropositive rhesus monkeys. Characteristic bands of 180–200 kDa and a doublet at 110 kDa were detected by all sera (Fig. 3). In 18 out of 20 sera a prominent protein at 55 kDa corresponding in size to the carboxy-terminal part of gB was also seen. Potential antibody reactivity against the amino-terminal part of gB could not be determined due to a number of reactive proteins migrating between 90–120 kDa.

During natural HCMV infection antibodies against three major continuous regions of HCMV gB are produced. To investigate whether a similar situation is found in naturally infected rhesus monkeys we analysed the humoral immune response against the corresponding regions of RhCMV gB (the regions are shown schematically in Fig. 4 *b*):

(i) aa 24–95 (SgBNH). This area corresponds to antigenic domain 2 (AD-2) of HCMV gB, which induces neutralizing antibodies during natural infection (Meyer *et al.*, 1992).

(ii) aa 458–635 (SgB) and 525–618 (SgBAD-1), respectively. The corresponding determinant on HCMV represents the immunodominant structure on gB which is recognized by nearly 100% of HCMV convalescent human sera. SgBAD-1 corresponds to the minimal HCMV AD-1 sequence which has been shown to be necessary for binding of human antibodies (Wagner *et al.*, 1992). Antibodies binding to AD-1 can be of the neutralizing or non-neutralizing type (Schoppel *et al.*, 1996).

(iii) aa 756–854 (SgBCO), corresponding to AD-3 of HCMV gB. Antibodies reacting with this region lack neutralizing activity most probably because this part is not exposed on the surface of infected cells or virions (Kniess *et al.*, 1991).

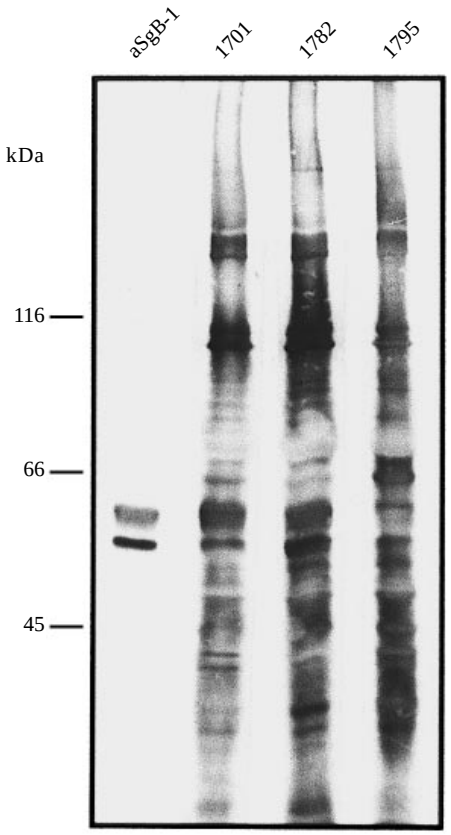

Fig. 3. Immunoblot analysis of the RhCMV-specific antibody response. Lysates from purified extracellular virions were subjected to PAGE, transferred to nitrocellulose and developed with sera from rhesus macaques (1701, 1782, 1795) as well as a rabbit serum specific for aa 458–635 of RhCMV gB (aSgB-1).

Respective RhCMV gB fragments were generated by PCR amplification using pC36 DNA as template. Fragments were inserted into the vector pQE9, which directs the synthesis in bacteria of recombinant polypeptides containing a histidine tag at the amino terminus (see Methods). Proteins were purified from lysates of *E. coli* and used in immunoblot analyses with sera from eight individual rhesus monkeys. A representative result is shown in Fig. 4 (*a*). All sera were positive for the SgB (aa 458–635) and SgBAD-1 (aa 525–618) domains, whereas the regions SgBNH (aa 24–95) and SgBCO (aa 756–854) each reacted with a single serum only. All sera cross-reacted with the HCMV AD-1 region. However, none of the sera was positive for an identically constructed recombinant protein containing aa 550–705 of HCMV pp150, indicating that the non-viral part of the polypeptides was not recognized by the monkey sera. In this assay, a number of HCMV gB-specific monoclonal antibodies were also tested and results from a murine antibody (27-156) and a human antibody (89-104) are shown. Both immunoglobulins cross-reacted with recombinant proteins containing the region corresponding to AD-1 of HCMV gB (Fig. 4 *b*). It should be noted, however,

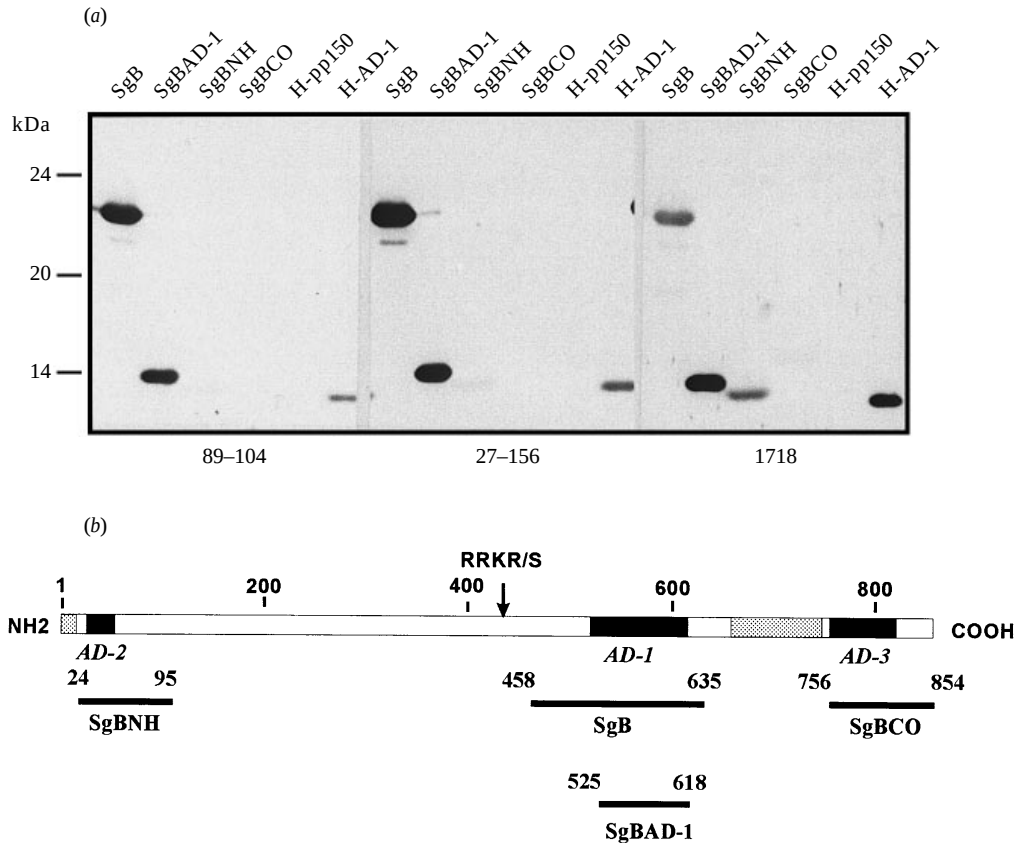

Fig. 4. (*a*) Reactivity of monoclonal antibodies and monkey sera for bacterial fusion proteins containing various fragments of RhCMV or HCMV gB. Fusion proteins were purified from bacterial lysates as described in Methods, separated on PAGE and transferred to nitrocellulose membranes. After blocking, individual blots were incubated with either the HCMV gB-specific human monoclonal antibody 89-104, the HCMV gB-specific murine monoclonal antibody 27-156 or a monkey serum (1718). Bound antibody was detected as described in the legend to Fig. 2. RhCMV gB-specific antigens included: SgB (aa 458–635); SgBAD-1 (aa 525–618); SgBNH (aa 24–95); SgBCO (aa 756–854). HCMV-specific antigens included H-AD-1 (aa 552–635 of gB) and H-pp150 [aa 555–705 of pp150 (ppUL32)]. (*b*) Schematic diagram of functional and immunogenic regions of RhCMV gB. Potential signal and anchor sequences (shaded boxes) as well as the proteolytic cleavage site (arrow) are indicated. Regions that were used for construction of bacterial fusion proteins are shown. Immunogenic regions corresponding to AD1–3 of HCMV gB are marked by black boxes.

that cross-reactivity between HCMV gB-specific monoclonal antibodies and RhCMV gB was not a general phenomenon since we observed a number of HCMV-specific antibodies which did not react with the RhCMV-derived recombinant proteins (data not shown).

In order to assess gB reactivity in rhesus sera more specifically an ELISA was carried out using purified SgB protein as antigen. As mentioned above a recombinant protein representing aa 555–705 of HCMV pp150 was used as control antigen. Since adult rhesus macaques have a 100% seropositivity rate for RhCMV it was necessary to use an unrelated polypeptide as control rather than determining a cut-off value using samples from RhCMV-seronegative monkeys. A total of 98 sera from 47 monkeys was tested and all contained antibodies against gB. However, antibody titres between individual animals varied considerably with some animals having very low antibody levels as exemplified by sera shown in Fig. 5 (*a*). Moreover, antibody titres also changed over time in a single animal, as shown in Fig. 5 (*b*).

Lastly, since some HCMV gB-specific murine and human monoclonal antibodies cross-reacted with RhCMV-gB, we were interested to see whether these antibodies could also neutralize RhCMV. To analyse this, a standard plaque reduction assay was performed and antibody dilutions which resulted in 100% virus neutralization were determined. We found the determination of the 100% neutralization titre superior to the conventional 50% neutralization titre since the 100% titre could be unequivocally determined, whereas the 50% titre was difficult to determine for some animal sera due to toxic effects on the cells. Pooled as well as individual sera from RhCMV-infected monkeys were also included in these analyses and were found to neutralize virus at dilutions ranging from 1:50 to 1:500 (Table 1). At concentrations of 5 μg/ml the human monoclonal antibody 89-104 was also

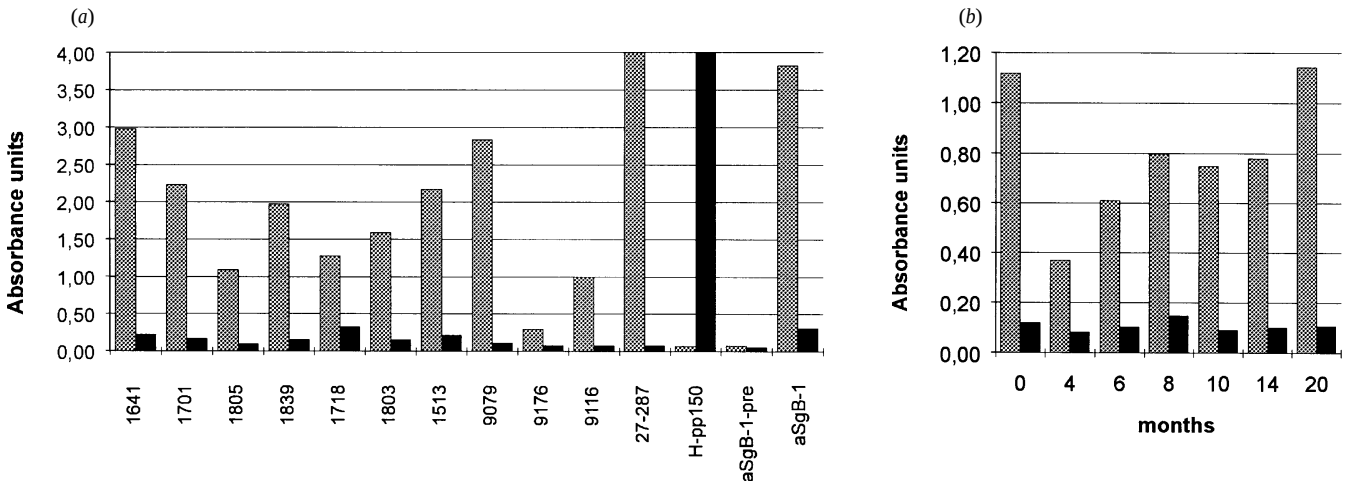

Fig. 5. ELISA scores for representative sera from naturally RhCMV-infected rhesus macaques. Antigens included sgB (aa 458–635) of RhCMV gB (grey bars) and XP-1 of HCMV pp150 (aa 555–705) (black bars). (*a*) Individual sera from different animals are indicated by numbers. (*b*) Sequential sera from a single animal were tested. Antibodies specific for gB (27-287, aSgB-1), HCMV pp150 (H-pp150) as well as a rabbit preimmune serum (aSgB-1-pre) were used as controls.

Table 1. Neutralization of RhCMV

Neutralization*	Antibody†	Dilution‡	Specificity§
100%	RhCMV (pool)	1/500	
	RhCMV (7207)	1/50	
	RhCMV (5570)	1/500	
	RhCMV (5569)	1/200	
	89-104	5 µg/ml	HCMV gB(AD-1)
0%	HCMV (pool)	1/50	
	HCMV (BK1-8)	1/50	
	C23	20 µg/ml	HCMV gB(AD-2)
	27-156, 7-17	20 µg/ml	HCMV gB(AD-1)
	ITC63, ITC39, ITC52	20 µg/ml	HCMV gB(AD-1)
	p63-27	TC	HCMV IE-1
	MSL-109	20 µg/ml	HCMV gH

* Neutralization assays were performed as described in Methods. Cytopathic effect of infection was assayed on HFF 4 days after infection and expressed as 100% and 0% neutralization, respectively.
† Antibody preparation represented: pooled sera from rhesus macaques or healthy HCMV-seropositive donors ($n = 8$), individual sera or monoclonal antibodies.
‡ For the 100% neutralization panel numbers indicate the lowest concentration needed for 100% virus neutralization. For the 0% panel the highest concentrations that were tested are given. TC, tissue culture supernatant.
§ For specificity of antibodies see Methods.

capable of completely neutralizing infectivity of RhCMV. Other HCMV-specific antibodies did not reduce viral titres. This included pooled or individual human sera from HCMV-seropositive donors ($n = 8$) as well as monoclonal antibodies specific for AD-1, AD-2, glycoprotein H (gpUL75) and IE-1 (pUL123) (Table 1).

Discussion

Nucleotide sequencing of an RhCMV DNA-derived cosmid clone hybridizing to gB of HCMV identified an open reading frame with characteristics of a typical glycoprotein. The deduced amino acid sequence of this gene has extensive homology to gB of HCMV (75% similarity, 60% identity). The gB molecules of RhCMV and HCMV are of similar overall size (854 aa vs 907 aa) and positional conservation of the cysteine residues as well as consensus motifs for *N*-linked glycosylation suggests that external domains exist in the proteins with similar structure and function. In addition, proteolytic processing of the full-length gB seems to follow identical pathways between RhCMV-gB and HCMV-gB.

To potentially exploit RhCMV as an animal model for HCMV analysis of the humoral immune response against the virus and a comparison of the two systems are of interest. In general, the antigenicity of RhCMV gB in macaques seems to be comparable to HCMV. Although only parts of RhCMV were used as bacterially derived antigen to detect gB-specific antibodies in sera from infected monkeys the seropositivity rate was found to be close to 100%. Whether those sera which were found to be non-reactive with the gB-specific fusion proteins did not also contain antibodies for the native gB molecule cannot be decided at this point. However, it is clear from our study that gB-specific antibody titres in naturally infected monkeys vary considerably between animals as well as over time in individual animals and this may have led to a negative result in some sera. Glycoprotein B is the dominant target for the neutralizing antibody response after natural infection with HCMV (Marshall *et al.*, 1992; Britt *et al.*, 1990). Whether RhCMV gB also induces neutralizing antibodies during natural infection remains to be determined. However, recognition as well as neutralization of RhCMV gB by HCMV

gB-specific immunoglobulins indirectly supports a role of RhCMV gB in induction of a virus-neutralizing response.

Antigenic properties of HCMV gB have been studied extensively and a number of antibody-binding domains have been described. Three antibody binding sites on HCMV gB have been characterized in detail:

(i) The amino-terminal domain AD-2. This region comprises two epitopes located between aa 68–77 (site I) and aa 50–54 (site II), respectively. Antibodies binding to site I are capable of neutralizing HCMV (Meyer *et al.*, 1990, 1992; Basgoz *et al.*, 1992). The amino acid sequence corresponding to site II is completely missing in RhCMV gB while the site I homologous sequence lacks the three amino-terminal residues necessary for antibody binding. Nevertheless, the amino-terminal part of RhCMV between aa 24–95 contains antigenic sites since antibody reactivity was detected in immunoblots using sera from RhCMV-infected monkeys.

(ii) The carboxy-terminal domain located between aa 783–906 of HCMV gB. Although this part of the protein does not induce neutralizing antibodies it represents a highly immunogenic structure of HCMV gB since it is recognized by the majority of HCMV-seropositive sera. The corresponding region of RhCMV gB seems to be considerably less immunogenic since we have observed antibody reactivity only in one out of eight monkey sera. Again, the reduced immunogenicity of this part of the molecule may be attributed to the deletions in the RhCMV gB protein.

(iii) The antigenic domain 1 (AD-1) located between aa 552–635 of HCMV gB. AD-1 represents a complex immunodominant structure on HCMV gB which is recognized as an entity by polyclonal sera as well as monoclonal antibodies (Schoppel *et al.*, 1996; Wagner *et al.*, 1992). The homologous region of RhCMV gB is located between aa 526–618. It is interesting to note that the amino acid similarity between the AD-1 regions is higher (84% similarity, 75% identity) than between the entire proteins. Recognition by almost 100% of sera that were tested indicated that immunogenicity of RhCMV AD-1 is similar to the human counterpart. Given the complex interaction between antibodies and HCMV AD-1 it was surprising to find a number of monoclonal antibodies reacting with RhCMV AD-1. It suggests that the AD-1 from both viruses forms a similar overall structure. However, the lack of reaction of some HCMV gB-specific antibodies with RhCMV AD-1 might also provide a tool to investigate the structural requirements for antibody binding via site directed mutagenesis.

RhCMV infection in rhesus macaques closely mimics the situation found in humans including inapparent primary infection, reactivation and generalized disease in immunosuppressed animals. Our study has demonstrated that the humoral immune response to a dominant viral antigen in rhesus monkeys seems to mimic that found in humans. The cytotoxic cellular immune response has also recently been reported to parallel that in humans (Kaur *et al.*, 1996). RhCMV might therefore represent a relevant animal model to study viral pathogenesis, antiviral immunotherapy and vaccine development. Our data show that RhCMV gB represents a molecule with close homology to HCMV gB and the cross-reactivity of virus-neutralizing antibodies opens the possibility of testing HCMV-specific reagents under defined conditions.

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