Humanization of Murine Neutralizing Antibodies against Human Herpesvirus 6B

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ABSTRACT Exanthem subitum is a common childhood illness caused by primary infection with human herpesvirus 6B (HHV-6B). It is occasionally complicated by febrile seizures and even encephalitis. HHV-6B reactivation also causes encephalitis, especially after allogeneic hematopoietic stem cell transplantation. However, no adequate antiviral treatment for HHV-6B has yet been established. Mouse-derived monoclonal antibodies (MAbs) against the HHV-6B envelope glycoprotein complex gH/gL/gQ1/gQ2 have been shown to neutralize the viral infection. These antibodies have the potential to become antiviral agents against HHV-6B despite their inherent immunogenicity to the human immune system. Humanization of MAbs derived from other species is one of the proven solutions to such a dilemma. In this study, we constructed chimeric forms of two neutralizing MAbs against HHV-6B to make humanized antibodies. Both showed neutralizing activities equivalent to those of their original forms. This is the first report of humanized antibodies against HHV-6B and provides a basis for the further development of HHV-6B-specific antivirals.

IMPORTANCE Human herpesvirus 6B (HHV-6B) establishes lifelong latent infection in most individuals after the primary infection. Encephalitis is the most severe complication caused by both the primary infection and the reactivation of HHV-6B and is the cause of considerable mortality in patients, without any established treatments to date. The humanization of the murine neutralizing antibodies described in this research provided a feasible way to reduce the inherent immunogenicity of the antibodies without changing their neutralizing activities. These newly designed chimeric antibodies against HHV-6B have the potential to be candidates for antivirals for future use.

KEYWORDS human herpesvirus 6B, humanized antibody, neutralizing antibody

Human herpesvirus 6B (HHV-6B) is a ubiquitous virus belonging to the betaherpesvirus subfamily (1–3). Primary infection with HHV-6B usually causes a common childhood febrile illness known as 3-day fever, or exanthem subitum (4). Approximately 21% of febrile seizure cases in children under the age of 2 years may be related to HHV-6B infection, and children with more-severe forms of seizure, such as status epilepticus, have been reported as HHV-6B positive (5). Meningitis and acute encephalitis can develop in some cases, and half of these patients experience severe neurological sequelae (6). Like other members of the herpesvirus family, HHV-6B establishes a lifelong latent infection (1). Reactivation of HHV-6B is associated with drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms (DRESS), which is a life-threatening syndrome (7, 8). Reactivation also occurs in patients with immunocompromised status and has been reported in 40% to 70% of patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT) (6, 9). Severe complications, such as delayed engraftment, graft-versus-host disease, and

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encephalitis, occasionally develop after reactivation, with a poor prognosis (9–12). However, there is currently no established prophylaxis or treatment for HHV-6B.

Recently, monoclonal antibodies (MAbs) have shown great success in treatments ranging from malignancies to autoimmune diseases, and more than 50 MAbs have been approved by the Food and Drug Administration (FDA). Palivizumab (Synagis), a MAb that has been approved for the prevention of respiratory syncytial virus (RSV) infections, is one success story. Indeed, MAbs have the potential to be candidates for both prophylactic and therapeutic use in HHV-6B infections.

Envelope glycoproteins play an important role in the cell entry step of herpesvirus infection. The glycoprotein complex based on the conserved glycoprotein H (gH)-gL is known as the viral ligand. It binds to its cell receptor and then mediates membrane fusion between the viral envelope and cell membranes by cooperating with the fusion protein gB (13). In our recent research, we identified the gH/gL/gQ1/gQ2 complex as the ligand for a cellular receptor of HHV-6B, human CD134, which we had identified previously (14–16). Although the detailed function of gH/gL/gQ1/gQ2 is still unclear, it is suggested that gH/gL/gQ1/gQ2 has at least two functions: recognizing the receptor and activating gB fusion activity. gQ1 is the crucial component for receptor recognition, and gQ2 might assist in its function as a chaperone (16, 17). On the other hand, gH and gL are suggested to be responsible for gB activation. In previous studies, we demonstrated that a mouse-derived MAb against gQ1, named KH-1, has great neutralizing activity for HHV-6B infection (18); an anti-gH mouse MAb, OHV-3, was also shown to neutralize HHV-6B infection (19).

However, the inherent immunogenicity of the mouse MAbs needs to be reduced for clinical use. In this study, we humanized two neutralizing MAbs, KH-1 against gQ1 and OHV-3 against gH (18, 19), using antibody-engineering technologies. Our further analysis demonstrated that the neutralizing activities of the newly constructed mouse-human chimeric MAbs were equivalent to those of the original mouse MAbs. These results strongly indicated the utility of the humanization method for neutralizing MAbs against HHV-6B and the possibility of their future clinical use.

RESULTS

Construction of expression plasmids for the mouse-human chimeric MAbs. To humanize the mouse-derived neutralizing MAbs, we designed a mouse-human chimeric form consisting of a mouse variable domain and human constant domains for each MAb (Fig. 1A). The sequences of the variable domains of the heavy chain (V_H) and light chain (V_L) were fused to the sequences of the constant domains of the human IgG1 heavy chain and human IgG(κ) light chain encoded in the pFUSE-CHlg-hG1 and pFUSE2-CLlg-hK vectors, respectively. The DNA fragments of the variable domains were amplified from cDNAs derived from the hybridoma clones, and the sequences were determined. The complementarity-determining regions (CDRs) of each V_H and V_L are shown in Fig. 1B. All the sequences of the V_H and V_L from the two murine MAbs exhibited four frame regions (FRs) and three CDRs, which were highly conserved with the sequences of open reading frames from the V gene and J gene (Fig. 1B). According to these sequences, we designed new primers for each MAb and constructed the expression plasmids (Table 1 and Fig. 1C).

Preparation of purified chimeric MAbs. Each chimeric MAb was expressed in HEK293T cells by cotransfection of the pair of constructed plasmids for heavy and light chains and was purified by protein A resin. Purified chimeric MAbs were analyzed by SDS-PAGE and Coomassie blue staining (Fig. 2). Bands of heavy chains (about 50 kDa) and light chains (about 25 kDa) of chimeric MAbs KH-1 and OHV-3 were detected, indicating the successful production of the chimeric MAbs.

To confirm that the chimeric MAbs recognize the same antigens as the original MAbs, indirect immunofluorescence assays (IFAs) were performed. HEK293T cells were transfected with either the gQ1 or the gH expression plasmid. Two days later, expression was examined with the chimeric and mouse MAbs. As shown in Fig. 3A, gQ1 or gH could be detected by the chimeric KH-1 or OHV-1 MAb as well as by the mouse KH-1

FIG 1 Construction of the chimeric forms of KH-1 and OHV-3. (A) Model of the chimeric form of the antibody. Constant domains of the heavy chain and light chain are shown as shaded rectangles, while variable domains are shown as filled rectangles. (B) Amino acid sequences of the variable domains of MABs KH-1 and OHV-3. Sequences of complementarity-determining regions (CDRs) of the variable domains of the heavy chain (V_H) and light chain (V_L) are shaded. (C) Construction of the expression plasmids. Sequences of V domains of both the heavy chain and the light chain were inserted into vectors containing the respective constant domains.

or OHV-1 MAB. HHV-6B-infected MT4 cells were also detected by both the chimeric MABs and the mouse MABs. These results indicated that the chimeric MABs retained antigen recognition ability.

Neutralizing activities of chimeric MABs. In order to identify the neutralizing activities of the chimeric MABs, virus-neutralizing assays were performed. We first prepared serially diluted solutions that contained the purified chimeric MABs or original mouse MABs at concentrations of 4.0, 2.0, 1.0, 0.5, 0.25, 0.1, and 0.05 μ g/ml after mixing with HHV-6B. After incubation, each mixed solution was used for the infection of MT4 cells. IFAs were performed at 3 days postinfection (dpi) using a rabbit antiserum specific to HHV-6B immediate early protein 1 (IE1). From the results, we found that chimeric MAB KH-1 prevented HHV-6B infection in a concentration-dependent manner. When this MAB was used at 4.0 μ g/ml, no IE1-positive cells could be detected, indicating the complete inhibition of infection (Fig. 4A and B). This neutralizing activity was the same as that observed for the original mouse MAB (Fig. 4B). On the other hand, no reduction

TABLE 1 List of primers

No.	Name	Sequence ^a
1	M13 Forward	5'-GTAAAACGACGGCCAG-3'
2	M13 Reverse	5'-CAGGAAACAGCTATGAC-3'
3	KH1_VH_for_EcoRI	5'-AGATC GAATTC GGAGGTCCAGCTGCAGCAG-3'
4	KH1_VH_rev_XhoI	5'-GTCA CTCGAG ACGGTGACCGCGGTC-3'
5	KH1_VL_for_EcoRI	5'-TGATC GAATTC GCAAATTGTCTCACCCAGTCTC-3'
6	KH1_VL_rev_BsiWI	5'-AT CGTACG TTTTATTCCAGCCTGGTCC-3'
7	OHV3_VH_for_EcoRI	5'-AGATC GAATTC GCAGGTAAGCTGGAGGAGTC-3'
8	OHV3_rev_XhoI	5'-GTCA CTCGAG ACGGTGACTGAGGTTC-3'
9	OHV3_VL_for_EcoRI	5'-TGATC GAATTC GACATTGTGCTGACACAGTCTC-3'
10	OHV3_VL_rev_BsiWI	5'-AT CGTACG TTTGATTCCAGCTTGGTG-3'

^aBoldface letters represent the sites of restriction enzymes.

FIG 2 Purified chimeric MABs. Purified chimeric KH-1 and OHV-3 were analyzed by SDS-PAGE and Coomassie blue staining. Both chimeric MABs, KH-1 and OHV-3, were prepared by cotransfecting the expression plasmids of both the heavy chain and the light chain into HEK293T cells. The supernatants were collected 4 days later, after the medium was changed to serum-free CD 293. Chimeric antibodies were purified using rProtein A Sepharose.

in the IE1 expression level was observed at $4.0 \mu\text{g/ml}$ with either chimeric OHV-3 or its original MAB, suggesting that the neutralizing activity of OHV-3 was very weak or nonexistent at this concentration (Fig. 4C). Because the neutralizing activity of the mouse OHV-3 MAB was checked directly using dilutions of mouse ascites in a previous study (19), the effective concentration of purified OHV-3 needed to be investigated. Therefore, for OHV-3, we prepared much higher concentrations of 100, 50, 25, 12, and $6 \mu\text{g/ml}$, as well as 3 and $1.5 \mu\text{g/ml}$. As shown in Fig. 4D, both the chimeric and the mouse OHV-3 MAB neutralized the infection in a concentration-dependent manner and could totally inhibit HHV-6B infection at $100 \mu\text{g/ml}$. To further confirm these neutral-

FIG 3 Antigen recognition by the chimeric MABs. (A) HEK293T cells were transfected with an empty plasmid or a plasmid expressing either gQ1 or gH and were stained with either the mouse MAB or the chimeric MAB (green) along with Hoechst 33258 (blue) 2 days later. (B) MT4 cells either infected with HHV-6B (strain HST) or mock infected were stained with either the mouse or the chimeric MAB (green) and Hoechst 33258 (blue) at 3 days postinfection. Representative micrographs are shown. Bars, $50 \mu\text{m}$.

FIG 4 Neutralizing activities of chimeric antibodies analyzed by immunofluorescence staining. MT4 cells were either left untreated or preincubated with a serial dilution of the indicated antibodies using the centrifuge method and were then infected with HHV-6B (strain HST) stock (3.2×10^4 TCID₅₀/ml). After the remaining antibodies were washed away, cells were cultured for 3 days. HHV-6B infection was detected by a rabbit antiserum against immediate early protein 1 (IE1) (green) along with Hoechst 33258 for staining nuclei (blue). (A) Results with HHV-6B-infected or mock-infected cells and no neutralizing MAbs. (B) Representative micrographs with KH-1. (C and D) Results using low (4.0 μg/ml) and high (up to 100 μg/ml) concentrations of OHV-3 for neutralization, respectively. Bars, 50 μm.

izing effects, viral proteins in infected MT4 cells were detected by immunoblotting with a rabbit anti-IE1 serum and a MAb against the HHV-6B tegument protein U14, as described previously (20). In agreement with the IFA results, neither IE1 nor U14 could be detected at a concentration of 4.0 μg/ml of chimeric KH-1 or 100 μg/ml of chimeric OHV-3 (Fig. 5). These results suggest that the newly devised chimeric MAbs effectively neutralized HHV-6B infection.

Quantitative evaluation of neutralizing activity for the chimeric MAbs. Collectively, the results described above show that there was no significant loss of the neutralizing activities of these MAbs after humanization. To further evaluate their neutralizing activities, we quantified the IFA results. We counted the IE1-positive cells, which represent the infection, and the nuclei in each sample and calculated the infection rates. The inhibition rates of the MAbs were calculated by normalization with a control sample and are shown in Fig. 6. All MAbs showed dose-dependent neutralizing activities. The half-maximal inhibitory concentrations (IC₅₀) were calculated by conducting a four-parameter logistic regression depending on the neutralizing titers and concentrations of MAbs. The results are summarized in Table 2. The IC₅₀ of the chimeric KH-1 MAb (0.18 μg/ml) was similar to the IC₅₀ for the mouse MAb (0.17 μg/ml). For mouse and chimeric OHV-3, the IC₅₀ were also at the same level (7.7 and 6.9 μg/ml, respectively), although both were relatively higher than the IC₅₀ for KH-1.

FIG 5 Neutralizing activities of chimeric antibodies analyzed by immunoblotting. MT4 cells were either left untreated or preincubated either with mouse or chimeric OHV-3 at a concentration of 100 or 3 μ g/ml or with mouse or chimeric KH-1 at a concentration of 4.0 or 0.1 μ g/ml. Then the cells were infected with HHV-6B (strain HST) stock (3.2×10^4 TCID₅₀/ml). After washing, cells were cultured for 3 days and then lysed for immunoblotting. HHV-6B immediate early protein 1 (IE1) and tegument protein U14 were detected by a rabbit antiserum and a specific MAb, respectively. α -Tubulin was detected as a loading control. These data show the results of one of three independent experiments.

DISCUSSION

Reactivation of the betaherpesviruses HHV-6B and human cytomegalovirus (HCMV), along with their complications, has been reported as a major problem in allo-HSCT (12, 21). Prophylactic treatments for HCMV have gradually decreased the frequency of HCMV-associated diseases. However, no treatment has been developed for HHV-6B. Although many issues remain to be solved, a neutralizing MAb is one of the leading candidates for such therapy.

The inherent immunogenicity of mouse-derived MABs can be reduced by constructing a mouse-human chimeric MAb, which is a classic technique (22). Already nine chimeric antibodies have been approved by the FDA and are in use in different clinical fields. In this study, we constructed chimeric forms of two neutralizing MABs against HHV-6B. These chimeric MABs were found to have the same levels of neutralizing activities as the respective original mouse MABs (Fig. 4, 5, and 6). KH-1 showed relatively strong neutralizing activities in *in vitro* assays (Fig. 6 and Table 2). Since our

FIG 6 Determination of neutralizing titers. The inhibition rates of MABs KH-1 and OHV-3 for this study were defined as 100 – relative infection rate. The relative infection rates were determined from the numbers of infected cells and total cells used in the neutralizing assays. The inhibition rates and standard deviations of KH-1 (A) and OHV-3 (B) are plotted against the concentrations of MABs used in the neutralizing assays. These data show representative results from one of three independent experiments. Each point represents the average result for triplicate samples $\pm$ the standard deviation. Student *t* tests were performed to determine significance, and *P* values are shown above each data set.

TABLE 2 Half-maximal inhibitory concentrations of MAbs

MAb	IC ₅₀ (μg/ml) ^a	
	Mouse	Chimeric
KH-1	0.17	0.18
OHV-3	7.70	6.87

^aIC₅₀, half-maximal inhibitory concentration.

neutralizing antibodies are candidates for therapeutic antibodies, further evaluation would be required *in vivo*. However, no experimental *in vivo* model of HHV-6B infection is currently available (23). Thus, establishment of an animal model of HHV-6B infection will be in significant demand for the development of therapeutic antibodies for HHV-6B. These first chimeric MAbs generated against HHV-6B are considered to be less immunogenic than the corresponding mouse MAbs and to exhibit increased half-lives and efficacy. However, the chimeric MAbs are not totally free of immunogenic problems in human recipients (24). To further reduce their immunogenicity against mice, it might be necessary to graft the CDRs of the original mouse MAbs onto human immunoglobulin FRs.

The envelope glycoprotein complex plays a key role in herpesvirus infection. The entry of herpesvirus into host cells is usually described as a cascade (13). For HHV-6B, according to our results, the binding of the gH/gL/gQ1/gQ2 complex to its cell receptor, CD134, may activate glycoprotein B (gB) function (15, 17). The conserved fusogens of herpesvirus, gB and gH, collaborate in mediating the fusion between the viral envelope and the cell membrane in the entry process (17). The different binding domains of each MAb may exert different effects at each step of viral entry. Binding affinity is also an important factor in describing the neutralizing activities of MAbs. In this study, we showed that the neutralizing activity of OHV-3 is weaker than that of KH-1. The reason would be that KH-1 recognizes gQ1, and gQ1 is directly responsible for CD134 binding (25).The direct binding of KH-1 to gQ1 of the gH/gL/gQ1/gQ2 complex could disturb the interaction of gQ1 with CD134, because the tetrameric complex with a gQ1 mutant that was not recognized by KH-1 could not bind to CD134 (18, 25) (Fig. 7A). OHV-3, which recognizes gH, may have a different effect on the entry step, such as inhibition of the fusion activity of gH or of the association with gB for collaboration (Fig. 7B). Since both MAbs are not available for Western blotting, each MAb is thought to recognize the conformation of each protein. Steric hindrance of

FIG 7 Model of the possible mechanisms of neutralization by MAbs KH-1 and OHV-3. For HHV-6B entry, the gH/gL/gQ1/gQ2 glycoprotein complex, especially gQ1, binds directly to the cell receptor CD134. (A) KH-1, which interacts with gQ1, could directly inhibit receptor binding. (B) The interaction of gH with OHV-3 could affect gH function (fusion or association with gB) and thus inhibit viral entry.

proteins by MAb may affect their functions for entry. Further studies are required to answer these questions.

MATERIALS AND METHODS

Cells and viruses. Human embryonic kidney (HEK) 293T cells were cultured at 37°C under 5% CO₂ in Dulbecco's modified Eagle medium (DMEM) containing 8% fetal bovine serum, 4 mM L-glutamine, and 20 µg/ml gentamicin. Serum-free CD 293 medium (Thermo Fisher Scientific) supplemented with 4 mM L-glutamine and 20 µg/ml gentamicin was used after transfections. The human T-cell line MT4 was cultured in RPMI 1640 medium with 8% fetal bovine serum containing 20 µg/ml gentamicin. HHV-6B strain HST was prepared as described previously (15). For virus propagation, umbilical cord blood mononuclear cells (CBMCs) were prepared as described previously (26). CBMCs were purchased from Riken (Institute of Physical and Chemical Research, Japan). With regard to the use of CBMCs, this study was approved by the ethical committee of the Kobe University Graduate School of Medicine.

Antibodies. The neutralizing MAb KH-1 and OHV-3 have been described in previous studies (18, 19). The mouse MAb against the HHV-6B tegument protein U14 has been described in previous work (20). An anti-α-tubulin MAb was purchased from Sigma-Aldrich. Alexa Fluor 488-conjugated goat anti-human IgG, donkey anti-mouse IgG, and donkey anti-rabbit IgG for immunofluorescence assays were purchased from Invitrogen.

Sequence determination. Total RNAs of the hybridoma for KH-1 and OHV-3 were extracted with TRIzol reagent (Thermo Fisher Scientific) by following the manufacturer's protocol. The cDNA of each MAb was constructed by reverse transcription-PCR (RT-PCR) using an oligo(dT)₂₀ primer and the SuperScript III first-strand synthesis system (Invitrogen). The cDNAs of the heavy-chain variable domains were amplified by PCR with high-degeneracy primers for the mouse heavy-chain constant region and mouse heavy-chain framework 1 (FR1) region (27). For amplification of the light-chain variable domains, the mouse kappa chain constant-region primer and the mouse kappa chain FR1 region universal degenerate primer were used (27).

The PCR products were cloned into a pCR4 TOPO vector using a TOPO TA cloning kit (Invitrogen), and the vectors were used to transform *Escherichia coli* strain DH5α. Positive colonies were selected by PCR using the M13 forward and M13 reverse primers (Table 1). The DNA fragments were sequenced with a 3130 Genetic Analyzer (Applied Biosystems). For purposes of analysis, the sequences were divided into the frame region and the complementarity-determining region (CDR), as described by Abhinandan and Martin and by Kabat et al. (28, 29), using the Web service Abnum.

Plasmids. The pFUSE-CHlg-hG1 and pFUSE2-CLlg-hK expression vectors were purchased from InvivoGen. New primers were designed according to the sequences of the variable domains of the mouse MAb as shown in Table 1 and were used for amplifying DNA fragments by PCR. The variable domains of heavy chains were subcloned into a pFUSE-CHlg-hG1 vector using EcoRI and XhoI sites. For the variable domains of light chains, EcoRI and BsiWI sites were used for subcloning into the pFUSE2-CLlg-hK vector. In each construct, a vector-derived interleukin 2 (IL-2) signal sequence was inserted in place of the original signal sequences of mouse IgG. The gQ1 and gH expression plasmids have been described in previous work (25).

Expression of chimeric MAb. For the expression of each chimeric MAb, vectors for the heavy chain and the light chain were cotransfected to HEK293T cells by the calcium phosphate method, as described previously (30). The medium was changed to conditioned serum-free CD 293 medium (Invitrogen) 6 to 8 h after the transfections. The cells were cultured for 4 days, and the supernatants were collected by centrifugation at $5,000 \times g$ for 5 min at 4°C.

Antibody purification. For antibody purification, rProtein A Sepharose (GE Healthcare) was added to the supernatant, and the mixture was gently rocked at 4°C for 10 to 12 h. Then the protein A Sepharose was spun down by centrifugation at $500 \times g$ for 5 min at 4°C, and the mixture was washed five times with cold phosphate-buffered saline (PBS). Antibodies were eluted with sodium citrate buffer (40 mM trisodium citrate [pH 3.4]), and the pH was adjusted to near 7.0 by Tris buffer (1.0 M Tris-HCl [pH 8.0]) immediately after the elution. The purity of the antibodies was assessed by SDS-PAGE, and the concentrations of antibodies were determined by a NanoDrop spectrophotometer (Thermo Fisher Scientific). Mouse MAb were purified as described previously (18).

Immunofluorescence assays. Indirect immunofluorescence assays (IFAs) were performed as described previously (26). Briefly, mock-infected or HHV-6B-infected MT4 cells were collected at 3 days postinfection (dpi) and were fixed with a cold acetone and methanol solution (acetone/methanol ratio, 7:3). To check antigen recognition, the cells were incubated with purified mouse or chimeric antibodies as the primary antibodies and by fluorescence-conjugated antibodies against mouse or human IgG as the secondary antibodies.

Immunoblotting. MT4 cells were lysed by TNE buffer (10 mM Tris-HCl [pH 7.4], 200 mM NaCl, 1 mM EDTA, 0.5% Tween) 3 days after infection. The soluble fraction was collected after 1 h by centrifugation at $45,000 \times g$ and was resolved by SDS-PAGE. Proteins were electrotransferred to polyvinylidene difluoride (PVDF) membranes for immunoblotting. After blocking, viral antigens were detected by a MAb or antiserum and were visualized using a horseradish peroxidase-linked secondary conjugate and enhanced chemiluminescence detection reagents (Nacalai Tesque).

Virus neutralization assay. The 50% tissue culture infective dose (TCID₅₀) was measured to determine the virus titers (31). Each purified mouse or chimeric KH-1 or OHV-3 MAb was serially diluted in PBS, and 50 µl of each solution was incubated with 100 µl of an HHV-6B solution (3.2×10^4 TCID₅₀/ml) at 37°C for 30 min. For infection, 5×10^4 MT4 cells suspended in 50 µl RPMI medium were added to each virus solution, and the solutions were centrifuged at 37°C for 1 h using a flat-bottom 96-well plate. The

cells were washed twice with RPMI medium and were cultured for 3 days. The infection rate was detected by IFAs using a rabbit antiserum against HHV-6B immediate early protein 1 (IE1) (16). For calculation, both the nuclei and the IE1-positive cells were counted from six random visual fields at $\times 400$ magnification for one sample. The infection rate was calculated as (number of IE1-positive cells)/(number of nuclei) $\times 100\%$. The relative infection rate was expressed as a percentage of the infection rate of cells infected with the virus solution incubated without any MAbs. The inhibition rate was defined as $100 - \text{relative infection rate}$.

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We report no conflict of interest.

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