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VACCINES AND ANTIVIRAL AGENTS

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.

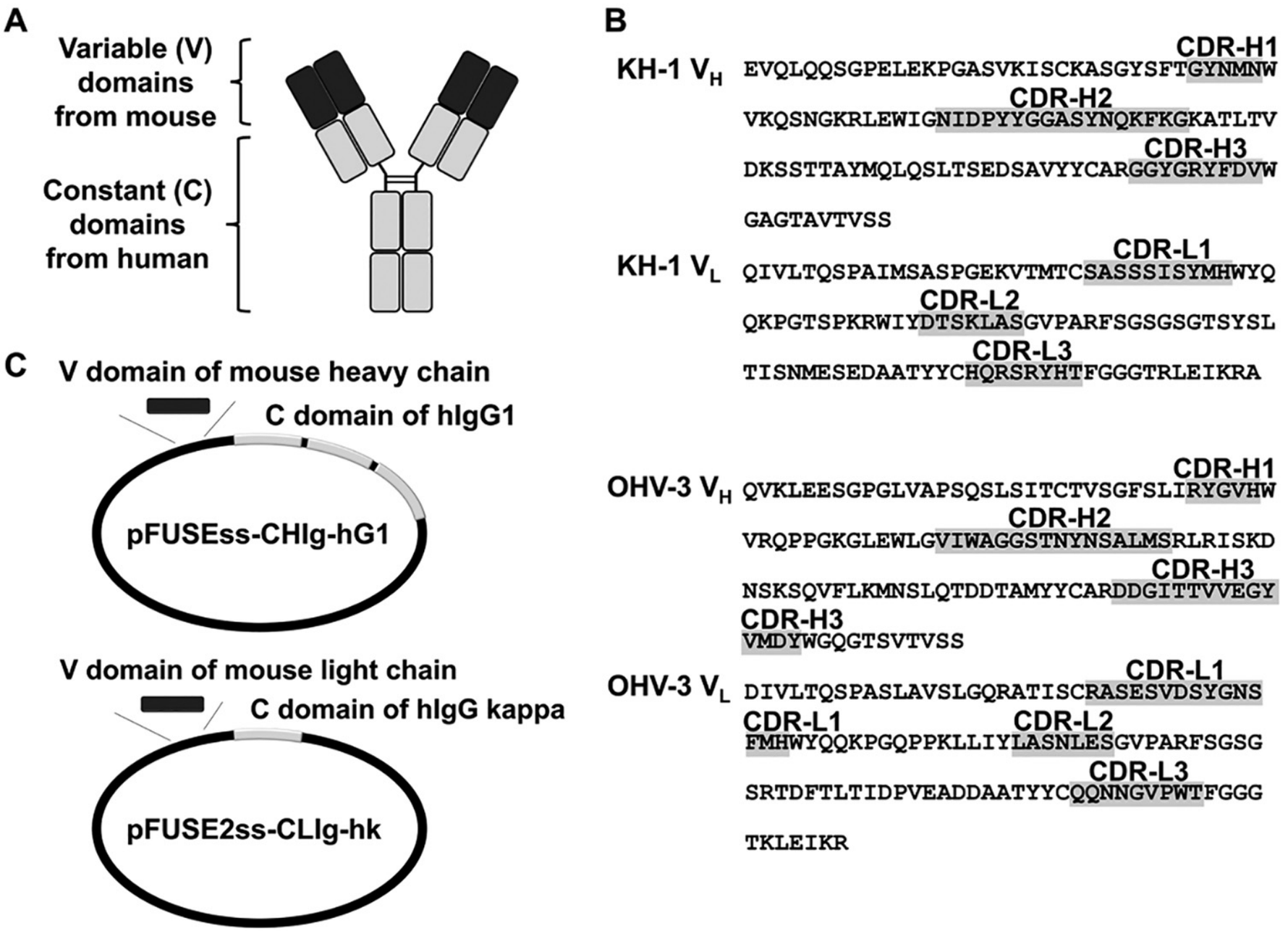

FIG 1 Construction of the chimeric forms of KH-1 and OHV-3. (A) Model of the chimeric form of the antibody. Constant domains of the heavy chain and light chain are shown as shaded rectangles, while variable domains are shown as filled rectangles. (B) Amino acid sequences of the variable domains of MAbs 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.

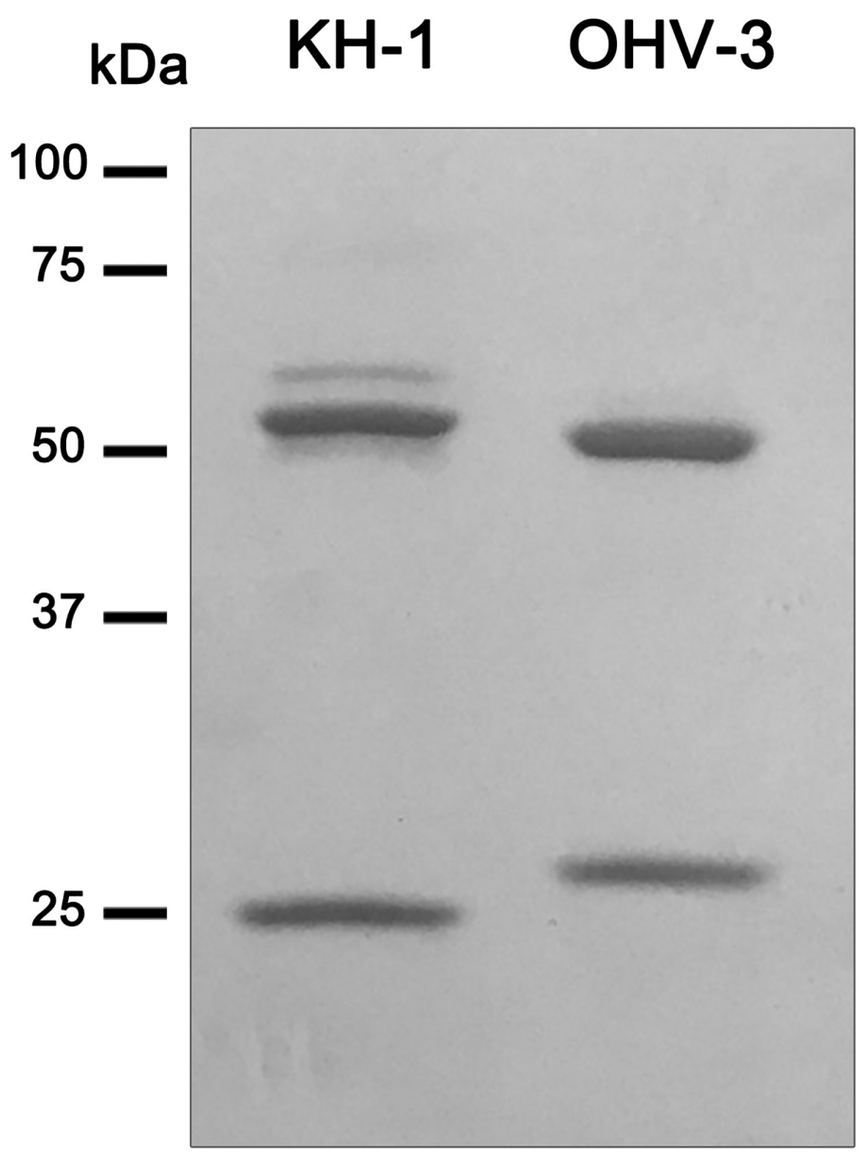

FIG 2 Purified chimeric MAbs. Purified chimeric KH-1 and OHV-3 were analyzed by SDS-PAGE and Coomassie blue staining. Both chimeric MAbs, KH-1 and OHV-3, were prepared by cotransfecting the expression plasmids of both the heavy chain and the light chain into HEK293T cells. The supernatants were collected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

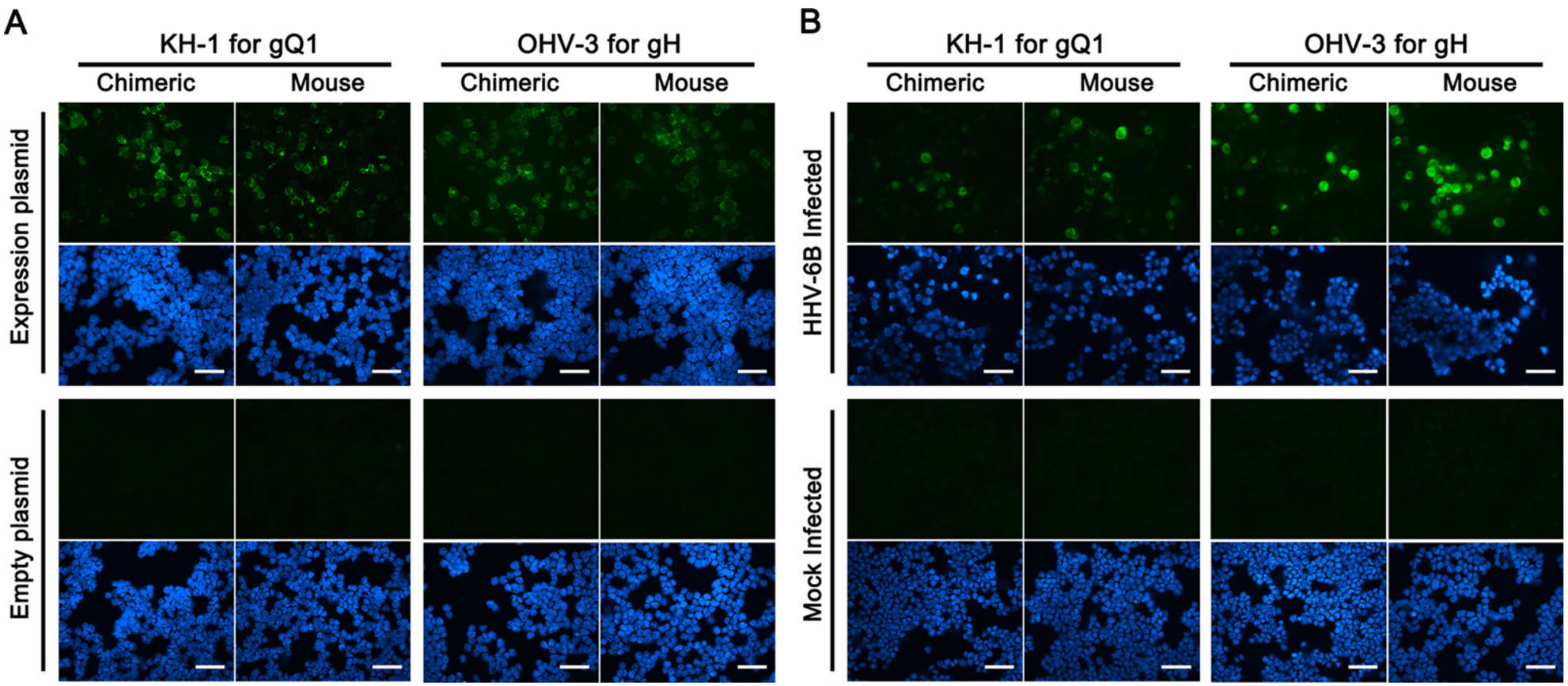

FIG 3 Antigen recognition by the chimeric MAbs. (A) HEK293T cells were transfected with an empty plasmid or a plasmid expressing either gQ1 or gH and were stained with either the mouse MAb or the chimeric MAb (green) along with Hoechst 33258 (blue) 2 days later. (B) MT4 cells either infected with HHV-6B (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 μm.

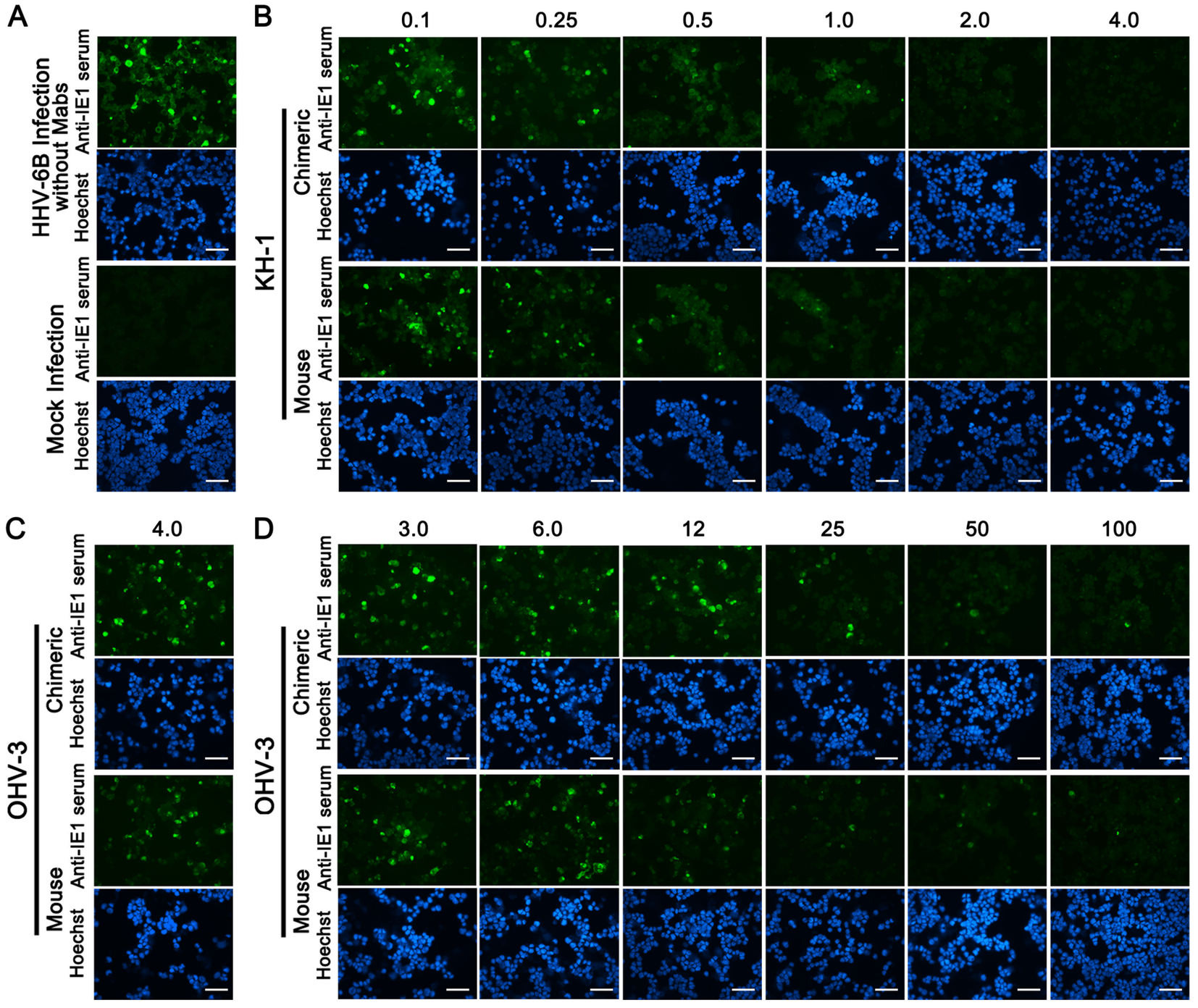

FIG 4 Neutralizing activities of chimeric antibodies analyzed by immunofluorescence staining. MT4 cells were either left untreated or preincubated with a serial dilution of the indicated antibodies using the centrifuge method and were then infected with HHV-6B (strain HST) stock (3.2×10^4 $TCID_{50}$/ml). 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.

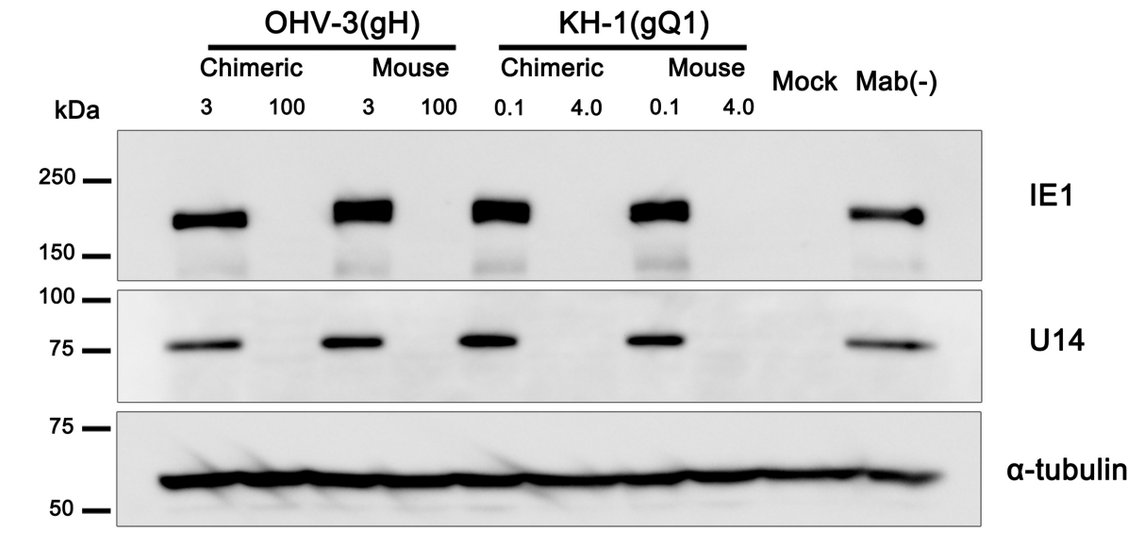

FIG 5 Neutralizing activities of chimeric antibodies analyzed by immunoblotting. MT4 cells were either left untreated or preincubated either with mouse or chimeric OHV-3 at a concentration of 100 or 3 μg/ml or with mouse or chimeric KH-1 at a concentration of 4.0 or 0.1 μg/ml. Then the cells were infected with HHV-6B (strain HST) stock (3.2×10^4 $TCID_{50}$/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.

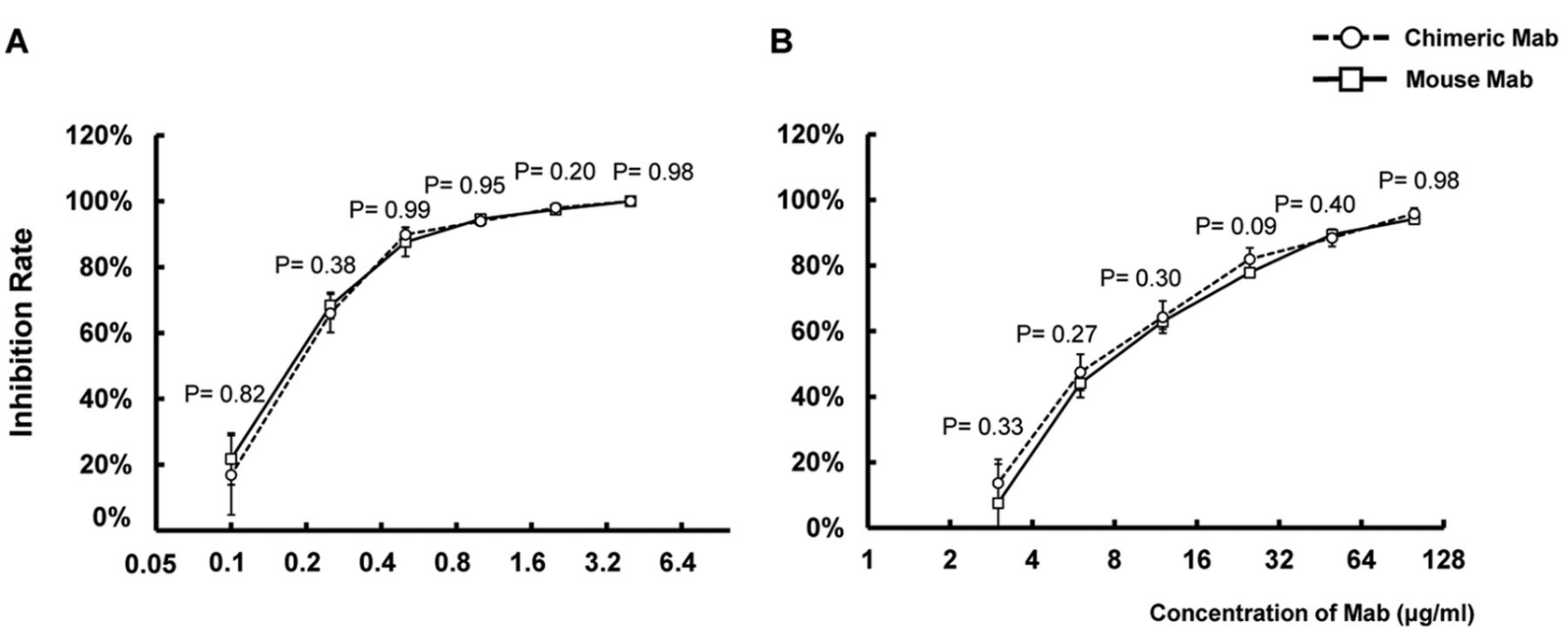

FIG 6 Determination of neutralizing titers. The inhibition rates of MAbs KH-1 and OHV-3 for this study were defined as 100 – relative infection rate. The relative infection rates were determined from the numbers of infected cells and total cells used in the neutralizing assays. The inhibition rates and 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.

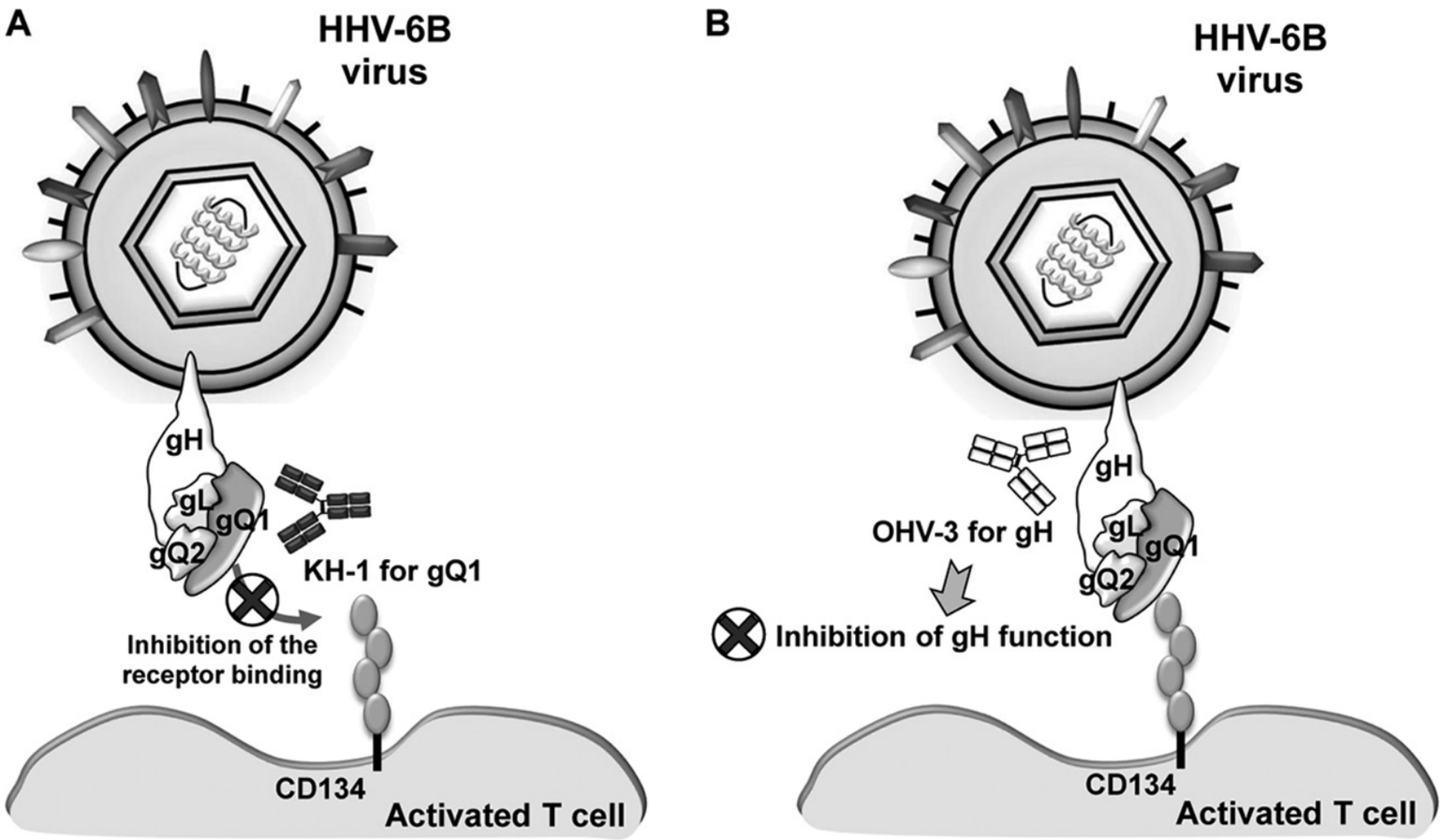

FIG 7 Model of the possible mechanisms of neutralization by MAbs KH-1 and OHV-3. For HHV-6B entry, the gH/gL/gQ1/gQ2 glycoprotein complex, especially gQ1, binds directly to the cell receptor CD134. (A) KH-1, which interacts with gQ1, could directly inhibit receptor binding. (B) The interaction of gH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