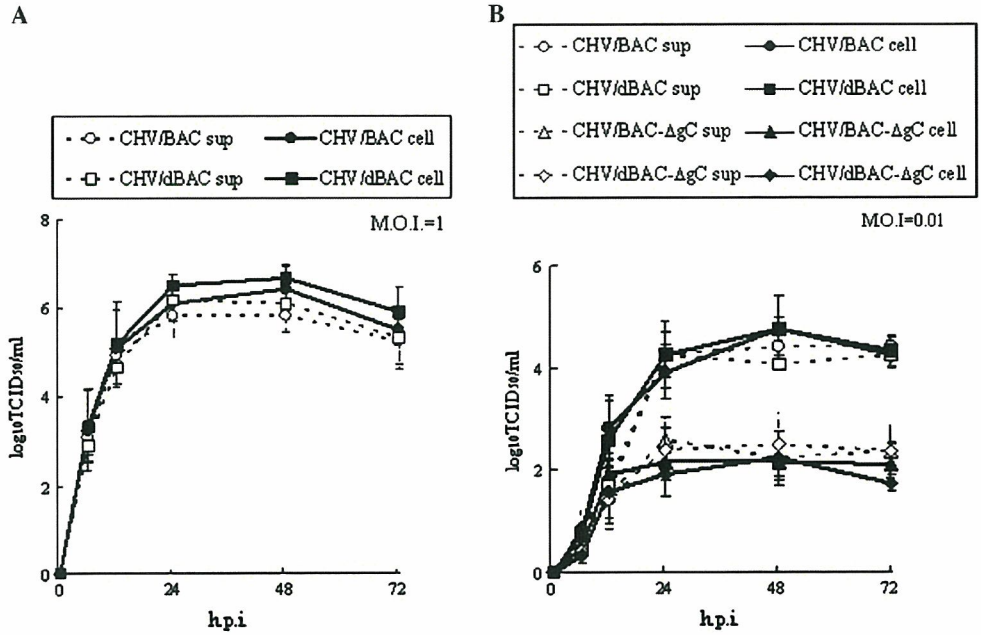

Fig. 5. Growth kinetics of the recombinants. Monolayers of MDCK cells were infected with CHV/BAC, CHV/dBAC, CHV/BAC–ΔgC, or CHV/dBAC–ΔgC. The cells or supernatants were recovered at 6, 12, 24, 48, and 72 h p.i. and $TCID_{50}$ was determined using A-72 cells. The experiments were performed in triplicate. Shown are data on $TCID_{50}$ of recombinant viruses from cells and supernatants with standard errors. (A) Cells were infected at an M.O.I. of 1. (B) Cells were infected at an M.O.I. of 0.01.

many investigators use the GET recombination system for modification of BACs [29,25,41], we used the λ recombination system, which is more efficient than the GET system [37]. Originally, the λ recombination system is sufficiently efficient to introduce a point mutation [42]. However, in this study, we attempted to use this system to accelerate the construction of recombinant viruses. Moreover, the λ recombination system, which is independent of viral replication, permits rapid and precise modification of the CHV genome without the introduction of plasmids that carry recombinases. *E. coli* strain EL250, which carries a prophage that contains the flp gene instead of the tetracycline resistance gene that is present in *E. coli* strain DY380 [37], has been used for the recombination of BACs from other herpesviruses [42,43]. The present study is the first to report the adaptation of the EL250 system to the field of virology. Thus, pCHV/BAC manipulations, which include both the insertion and deletion of specific genes, are feasible in *E. coli*.

CHV/BAC is the first BAC to be constructed for the purpose of clinical treatment of domestic animals. The CHV-based vector used as a recombinant vaccine for companion dogs is expected to confer several benefits, such as low pathogenicity for adult dogs and narrow host specificity. For this reason, the CHV-based vector may be used as a safe vaccine, potentially safe vector of gene therapy for domestic dogs, although much more experiments especially the *in vivo* analysis should be done before its clinical use.

Furthermore, the λ recombination system described above has been used successfully to delete the gene coding for gC. We chose the gC gene as one of the targets for deletion from CHV/BAC in *E. coli* because the gC gene of CHV has already been characterized as a binding target for heparan sulfate [44]. As expected, deletion of the gC sequence changed dramatically the character of CHV and reduced the virus titer. An interesting observation of the relationship between the herpes simplex virus type 1 (HSV-1) gC and MDCK cells has been reported. The exposure of MDCK cells at either the apical or basal surface to HSV-1 wild-type results in infection, while HSV-1 in which the gC is deleted can infect the basal surfaces but not the apical surfaces of MDCK cells [45]. This suggests that MDCK cells are polarized cells that express the receptor for HSV-1 gC at least at the apical surface, and an additional receptor at the basal surface. Further study is necessary to determine whether the observations made for HSV-1 infection also apply to CHV infection.

To clone the infectious CHV genome as a BAC, we selected the TK gene for insertion of the BAC–GFP sequence. To date, our laboratory has produced several recombinant vaccines based on feline herpesvirus type 1 (FHV-1) or CHV with homologous recombination that targets the TK gene as a position for the insertion of exogenous genes. These results show that the TK loci of herpesviruses, which include FHV-1 and CHV, are suitable for the insertion of antigen genes from other pathogens, leading to the generation of recombinant and attenuated vaccines for companion animals [11,12,46–49]. CHV/BAC or CHV/dBAC would be a real basal vector for polyvalent or live attenuated vaccines by virtue of predicted low-level pathogenicity as we can see in other TK deleted mutant. However, TK is the target of drugs, such as acyclovir and gancyclovir, which are directed against human herpesviruses [50], although the effects of these drugs on CHV remain to be elucidated. Drugs of this type could be used if CHV vectors are exploited

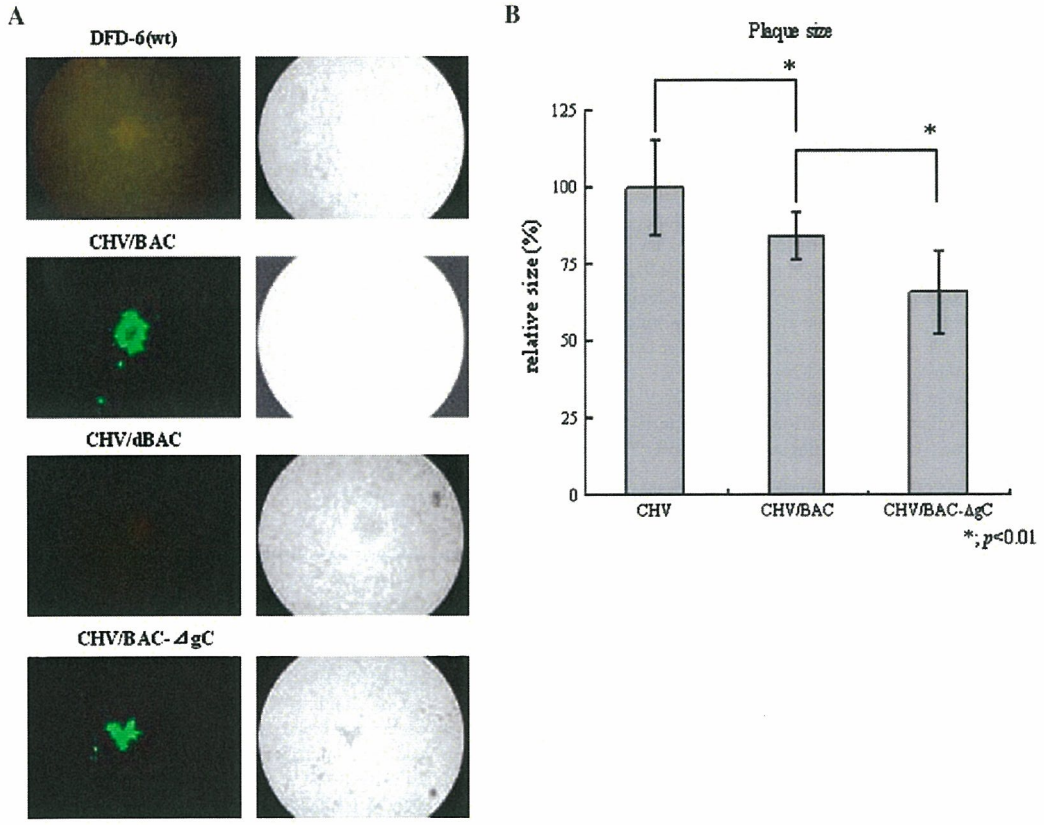

Fig. 6. Plaque morphology and plaque size of the recombinant viruses. (A) MDCK cells were infected with DFD-6, CHV/BAC, CHV/dBAC, or CHV/BAC–ΔgC in maintenance medium that contained methylcellulose and DEAE-dextran. Three days later, the plaques were observed under a fluorescent microscope (left panel) or a light microscope (right panel). (B) Relative sizes of 50 randomly selected plaques of viruses with standard errors. The plaques formed by CHV/BAC were smaller than that of DFD-6 ($p < 0.01$), but were larger than that of CHV/BAC–ΔgC ($p < 0.01$).

in the future for animal gene therapy but cause side effects. In terms of using CHV vectors for gene therapy applications in companion animals in the veterinary field, the next infectious clone of CHV that contains the TK gene could be constructed.

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