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Helper-Independent Live Recombinant Adenovirus Vector Expressing the Hemagglutinin-Esterase Membrane Glycoprotein

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The hemagglutinin-esterase glycoprotein (HE) gene of bovine coronavirus, coupled with a simian virus 40 early promoter and polyadenylation signal, was inserted into a human adenovirus transfer vector. The transfer vector was used to co-transfect 293 cells along with adenovirus genomic DNA. The hemagglutinin-esterase transcription unit was rescued into the adenovirus genome by homologous *in vivo* DNA recombination between the vector plasmid DNA and the adenovirus genomic DNA, and a recombinant adenovirus was isolated by several rounds of plaque assays. Thus the recombinant adenovirus carries the hemagglutinin-esterase gene in the early transcription region 3 (E3) of the adenovirus genome in the parallel orientation to the E3 transcription. The recombinant adenovirus synthesized the HE polypeptide in HeLa cells as demonstrated by immunoprecipitation with anti-coronavirus rabbit antisera. The recombinant HE polypeptide could be labelled by [^{3}H]glucosamine, demonstrating that the recombinant HE was glycosylated. Cells expressing the HE polypeptide exhibited hemadsorption activity when incubated with mouse erythrocytes. The HE was transported to the plasma membrane as shown by the cell surface immunofluorescence, indicating that the recombinant HE polypeptide retained its biological activities. Potential for the use of infectious recombinant adenovirus as a live virus-vectored vaccine candidate for bovine coronavirus disease is discussed.

Vaccination against infectious diseases of man and animals has dramatically reduced the suffering and economic loss resulted from many infectious diseases. Nevertheless, none of the vaccines, with the exception of smallpox, have eliminated a specific viral infection from the population, and many infectious diseases continue to plaque our society. Most of the immunogens currently available are composed of either the inactivated pathogens or live-attenuated pathogens. There are, however, significant disadvantages to both types of vaccines. One important advantage in using live-attenuated vaccines over inactivated vaccines is that they can be delivered to mucosal surfaces and induce local immunity. Since the mucosal surface is the primary site of the entry of many pathogens, immunity at the mucosal surfaces will be much more valuable than systemic immunity in controlling the disease process. The recent advances in recombinant DNA technology have provided the means to dramatically improve the efficacy of vaccination strategies used to ameliorate infectious diseases. Recombinant vaccinia virus has been developed as a live virus vector and has provided a method for delivering antigens to induce protective systemic immune responses (for a review see, 23). However, a major disadvantage of vaccinia virus vectors for animal application is its wide host range, including humans. This may lead to the potential hazard of contamination of human populations with unknown health consequences. In addition, partially emotional but

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significant problems also exist in using vaccinia virus to human since vaccinia virus is related to smallpox disease.

Adenovirus appears to provide a unique alternatives to vaccinia virus antigen delivery (for a recent review see 4, 14). Adenoviruses are ubiquitous in the human and animal populations and replicate in the mucosal surfaces of the respiratory and intestinal tracts. Unattenuated live adenovirus has been used for oral immunization to prevent acute respiratory disease in the US military recruits for over 20 years, and it has proven to be safe and efficacious by producing an excellent mucosal immunity (6, 31). Millions of people have been orally immunized with adenovirus in enteric-coated capsules, and there have been no reports of adverse reactions. Adenoviruses are double-stranded, non-enveloped DNA viruses with the genome size of approximately 35 Kb. The DNA contains short inverted terminal repeats in which one end is associated with a 55 KDa terminal protein (27). During the early phase of infection, four noncontiguous regions of the genome are transcribed, which are designated as early region 1 (E1), E2 E3, and E4 (for a review see, 29). While other regions are essential, the E3 region is non-essential for virus replication, and more than 2.5 Kb can be deleted from this region without interfering with virus growth in culture (5). Recombinant adenoviruses containing inserts in the E3 region are helper-independent and grow to high titre on permissive cells grown in culture. Therefore recombinant adenoviruses containing foreign gene insertions at the E3 locus are ideal candidates for efficacious live recombinant vaccine vectors (14).

Bovine coronavirus is an enteropathogenic virus causing severe diarrhoea in newborne calves (22). The hemagglutinin-esterase (HE) gene of bovine coronavirus (BCV) encodes a polypeptide of 45 KDa (24). The 45 KDa polypeptide is posttranslationally modified by the addition of carbohydrates, resulting in the 65 KDa polypeptide, and the mature HE protein with molecular weight of 130 K is found in BCV virion as a homodimeric form of the 65 KDa polypeptide (10, 19). The HE glycoprotein contains hemagglutination activity (20). This protein also possesses an acetylesterase activity similar to the receptor-destroying enzyme in human influenza virus type C (25, 32, 33). Previous studies have demonstrated that monoclonal antibodies directed against the HE glycoprotein neutralized BCV infectivity *in vitro* (9) and were capable of protecting the intestinal epithelia from virus infection in cattle (11). These reports indicate a signficant function of this protein in the initiation of BCV infection. To examine potentials of using adenovirus as live virus-vectored vaccine for BCV infection, we constructed a helper-independent recombinant adenovirus carrying the hemagglutinin-esterase gene of bovine coronavirus in the E3 locus of human adenovirus, and characterized the recombinant polypeptide produced by this adenovirus vector.

MATERIALS AND METHODS

Cells, Viruses and Antibodies

Cell line 293 derived from human embryonic kidney (15) was used for calcium phosphate transfection. Wild-type human adenovirus serotype 5 (Ad5) and recombinant adenovirus were titrated on 293 cells. Bovine coronavirus was grown in Mardin-Darby bovine kidney (MDBK) cells. MDBK cells were propagated in minimal Eagle's medium (MEM) containing 10% fetal bovine serum (GIBCO) . 293 cells were maintained in MEM containing 10% fetal bovine serum supplemented with 1× vitamins, 1× amino acids and 20 mM glutamine (Flow Laboratories). HeLa cells were grown in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum. The cells were maintained at 37℃ in a humidified, 5% CO_2 incubator. Polyclonal rabbit anti-bovine coronavirus antiserum and mouse ascitic fluid of monoclonal antibody HC10-4 were prepared as described previously (9).

Preparation of Adenovirus DNA

293 cells infected with adenovirus were harvested, and 1/10 vol of 5% sodium deoxycholate was added. The mixture was incubated on ice for 30 min. The cellular DNA was sheared by homogenizing with Waring blender at maximum speed for 5 min to avoid aggregation of viruses and cellular materials during the subsequent CsCl banding step. For 3.1 m*l* of virus suspension, 1.8 m*l* of saturated CsCl in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) was added. The virus suspension was centrifuged for 16 hr at 4℃ and 35,000 rpm using an SW41 rotor (Beckman). The viral band was identified and pooled. The purified virus was digested with pronase (100 μg/m*l*) for 2 hr at 37℃ followed by phenol extraction and ethanol precipitation.

Partial Purification of Radiolabelled Coronavirus

Confluent MDBK cells in flask (150 cm^2, Corning) were washed with MEM and then infected with virus at a multiplicity of infection (m.o.i.) of 1 to 2 plaque-forming-unit (PFU) per cell by adsorption for 1 hr at 37℃. The inoculum was replaced with MEM containing 3% fetal bovine serum. At 12 hr post-infection the medium was replaced with a 10 m*l* of methionine-free MEM. Viral proteins were labelled by addition of [^{35}S]methionine (Amersham) at 50 μCi/m*l* in MEM containing 10% of the normal concentration of methionine. Cells were further incubated and supernatants containing radiolabe-

lled virus were harvested at 36 hr post-infection. Partially purified virions from the supernatant were obtained by a modification of the method described by Deregt *et al.* (10). Culture media from BCV-infected cells were clarified by centrifugation at 3,000 rpm (Sorvall GS-3 rotor) at 4℃ for 20 min. Supernatants were centrifuged in SW27 rotor (Beckman) at 25,000 rpm at 4℃ for 2 hr. The virus pellet was resuspended in a small volume of TNE buffer (20 mM Tris-HCl, pH 7.2, 100 mM NaCl, 1 mM EDTA), and this suspension was used for radioimmunoprecipitation.

DNA Cloning

Standard molecular cloning techniques (28) were employed for subcloning and manipulation of bacterial plasmids.

DNA Co-transfection

Subconfluent monolayers of 293 cells were co-transfected with the viral DNA and transfer plasmid using the calcium phosphate precipitation technique (15). Ten μg of the plasmid DNA and 5 μg of viral DNA were added to HEPES-buffered saline (25 mM HEPES, 150 mM NaCl, 5 mM KCl, 0.7 mM $Na_2PO_4 \cdot 2H_2O$, 6 mM glucose, pH 7.1) containing 10 μg/ml of salmon-sperm DNA. To this transfection mix, 2.5 M $CaCl_2$ was slowly added for a final concentration of 125 mM. The mix was then incubated for 30 min to form fine precipitates at room temperature. The suspension was added to the cells without removing the growth medium and incubated at 37℃ in a CO_2 incubator. At 4 hr post-incubation, the medium was removed. The cells were overlayed with 0.5% agarose plus MEM containing 10% bovine serum and further incubated at 37℃ for 5~8 days until viral plaques were developed.

Purification of Recombinant Adenovirus

293 cells were plated on 60 mm dishes a day before the plaque assay. Virus was diluted in phosphate buffered saline in 10-fold dilution, and 1 ml of virus was used to infect 293 cells. Cells were agarose-overlayed and incubated at 37℃. When viral plaques were developed, well-isolated plaques were picked from the transfected culture by punching out agarose using a sterile Pasteur pipet and transferred to 1 ml of phosphate buffered saline. Medium was removed from 293 cell monolayer, and the cells were infected with 0.2 ml of virus suspension. When the cytopathic effect was complete, cells were harvested and digested with 100 μg/ml of pronase in the presence of 0.5% SDS. The viscous lysates was extracted with phenol and ethanol-precipitated. The crude DNA was redissolved in 0.1× SSC (8.75 g NaCl, 4.4 g sodium citrate dihydrate per liter) and digested with *Hin*dIII. The DNA was electrophoresed on a 1% agarose gel with marker DNA of a *Hin*dIII digest of wild type adenovirus DNA. Recombinant adenovirus containing the correct restriction pattern were further purified by two additional plaque assays and amplified for virus stock (13).

Radiolabelling of Intracellular Proteins and Preparation of Cellular Extracts

A monolayer of cells was infected with wild-type or recombinant adenovirus at a multiplicity of infection of 10 PFU per cell. At 12 hr post-infection, cells were washed once with MEM. The cells were incubated with 100 μCi/ml of [^{35}S]methionine (specific activity 3,000 μCi /mmol, Amersham) for 6 hr in methionine-free MEM. For glycosylation studies, virus-infected cells were labelled with 50 μCi/ml of D-[6-^{3}H]glucosamine HCl (Amersham, specific activity 25.4 μCi/mmol) in glucose-free, Hanks' balanced salt solution (GIBCO). For the preparation of cell lysates, cell monolayers were washed once with ice-cold PBS and harvested by scraping with a rubber policeman in PBS. Cells were collected by centrifugation at 2,000 rpm and resuspended in lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.5% Triton X-100, 1 mM EDTA), followed by incubation on ice for 30 min. Cell lysates were centrifuged at 10,000 rpm in a microcentifuge and the supernatant was collected for radioimmunoprecipitation.

Radioimmunoprecipitation and SDS Polyacrylamide Gel Electrophoresis

Immunoprecipitation was performed as described (34). Radiolabelled cytoplasmic fractions were incubated with antibody at room temperature for 2 hr, and 10 mg of Protein A Sepharose beads (Pharmacia) were added. The mixtures in RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 10 mM EDTA) containing 0.5% SDS were incubated overnight at 4℃ with continuous shaking. Immune complexes bound to Sepharose beads were washed two times with RIPA buffer containing 0.5% SDS, and once with RIPA buffer. Immune complexes were dissociated by boiling for 5 min in 1% SDS, 25% glycerol, 1% mercaptoethanol, 0.25% bromophenol blue, 10 mM Tris-HCl, pH 6.8. The Sepharose beads were spun down and the supernatants were analyzed on 10% SDS polyacrylamide gels at 180 volts for 4 hr. The gels were fixed in 10% acetic acids and 40% methanol for 20 min and treated with Amplify (Amersham) for 30 min. The gel was then dried under vacuum and exposed to X-ray film (Du Pont Cronex Lightening Plus) at −70℃.

Immunofluorescence Assay

For determination of antigen distribution on the cell surface, cells were grown on a Lab-Tek chamber slide (Miles Laboratories, Inc.) and infected with virus at an m.o.i. of 5 PFU/cell. At 16 hr post-infection, cells were washed with cold PBS without fixing and incubated for 1 hr with anti-BCV rabbit antibody. Cells were washed

with PBS and reacted with a 1:100 dilution of fluorescence isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Boehringer Mannheim) for 1 hr. Cells were washed again with PBS and examined for fluorescence (Zeiss microscope Model IM35).

Hemadsorption Assay

Balb/C mouse erythrocytes were taken and washed twice by centrifugation at 1,500 rpm for 5 min with Alsevers solution (100 mM glucose, 20 mM sodium citrate, 70 mM NaCl, 2 mM citric acid) Erythrocytes were resuspended in Alsevers solution to 20% of erythrocytes. Virus-infected cell monlayers at 18 hr post-infection were rinsed twice with PBS and incubated with 0.5% mouse erythrocytes for 10 min at room temperature. Cell monolayers were washed twice with PBS, and the binding of erythrocytes to virus-infected cells was monitored by microscopic examination.

RESULTS

Rescue of the E3 Insert

A sequence containing the entire open reading frame of the BCV HE gene (24) was obtained by *Bam*HI digestion. The HE gene was subcloned into the *Bam*HI site of expression vector pSV2X3 (26), so that the BCV HE gene was positioned between simian virus 40 (SV40) promoter and SV40 polyadenylation signal. Plasmids containing the desired orientation of the HE gene were identified by restriction analysis. Insertion of the HE gene into the E3 region of the adenovirus genome was facilitated by subcloning of the expression cassette into an adenovirus transfer vector. Transfer vector pFGdX1 contains a segment representing map units 59.5-100 with a deletion of map units 78.5-84.7 (18). The expression

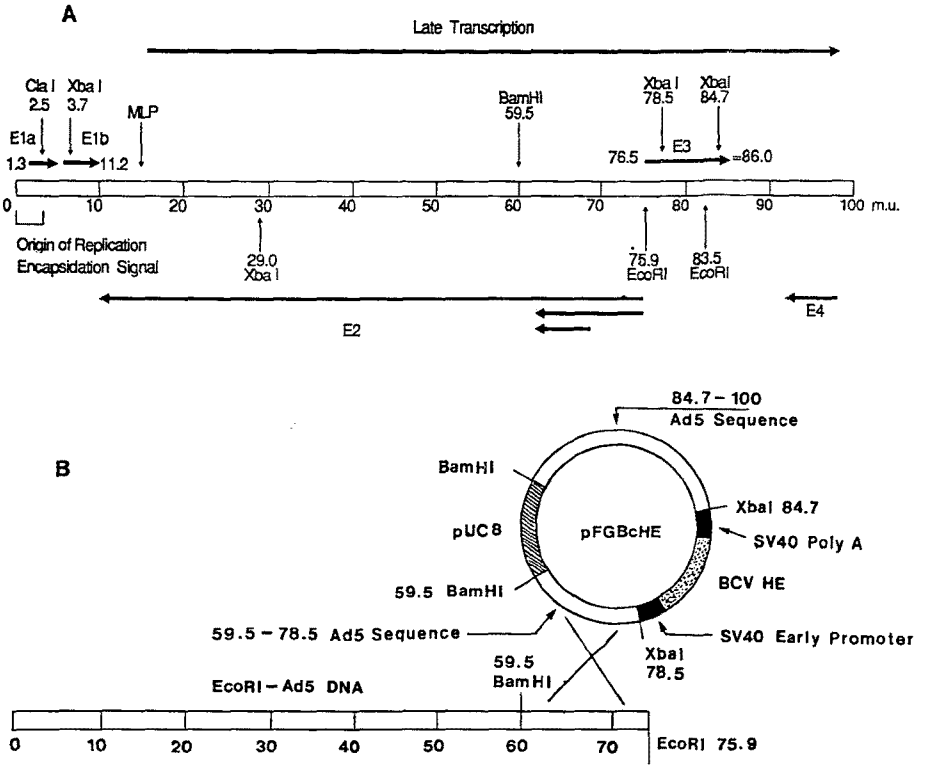

Fig. 1. Adenovirus transcription regions (A) and strategy for construction of recombinant adenovirus with the BCV HE insertion in the E3 region (B).

(A), Arrows indicate transcription units, and numbers represent map units (m.u.). A 100 map units corresponds to the full length of the genome of 36 kilobases; (B), An expression cassette of the BCV HE gene flanked by upstream SV40 early promoter and downstream polyadenylation signal was cloned into the *Xba*I site of adenovirus transfer vector pFGdX1. The resulting plasmid, pFGBcHE, was used to co-transfect 293 cells along with adenovirus DNA that had been digested with *Eco*RI. The BCV HE transcription unit is recued by homologous *in vivo* DNA recombination into the E3 transciption region in the E3 parallel orientation (left to right).

cassette consisting of the SV40 promoter and SV40 polyadenylation signal flanking the HE gene was isolated from the pSV2X3 by *Xba*I digestion, and the resulting fragment was subcloned into the unique *Xba*I site of pFGdX1 in the parallel orientation to the E3 transcription. This vector was used to co-transfect 293 cells with viral DNA that had been resticted with *Eco*RI at map units 76.0-83.5, tending to inhibit the infectivity of viral DNA and select for intracellular recombination (Fig. 1). The transfected 293 cells were agarose-overlayed and further incubated. At 5-7 days post-incubation putative recombinant adenovirus plaques were developed (Fig. 2). These plaques were picked with sterile Pasteur pipetts and used to infect 293 cells. Crude DNA was prepared from virus-infected cells and recombinant progeny viruses were screened by DNA analysis on agarose gel after *Hin*dIII restriction endonuclease digestion. A recombinant adenovirus carrying the HE gene in the E3 region, AdBcHE, was isolated and further purified. The recombinant adenovirus was amplified on 293 cells. AdBcHE grew to titre of $>10^9$/ml and used for subsequent studies.

Synthesis and Glycosylation of the Recombinant HE Polypeptide

Expression of the HE gene inserted into adenovirus genome was determined by immunoprecipitation and gel electrophoresis of the metabolically radiolabelled polypeptides from cells infected with recombinant adenovirus AdBcHE. HeLa cells were infected with approximately 10 PFU/cell of recombinant adenovirus and were radiolabelled with [^{35}S]methionine at 12 hr post-infection for 6 hr. In the presence of reducing agent, a 65 KDa polypeptide was identified with anti-bovine rabbit antisera from cell lysates infected with AdBcHE (Fig. 3, lane 3), which was not present in wild-type adenovirus-infected cell lysates (Fig. 3, lane 1). This polypeptide corresponded, in molecular migration, to the HE protein immunopreciptiated from purified BCV virions (Fig. 3, lane 2). Since the HE gene is capable of encoding a 45 KDa polypeptide (24), the difference between the predicted molecular weight and the observed molecular weight was due to the posttranslational modification (19, 10). The identical migration of the recombinant HE polypeptide with the authentic viral HE protein suggests appropriate glycosylation of the recombinant HE.

Fig. 2. Development of recombinant adenovirus plaque.
293 cells were co-transfected with the adenovirus genomic DNA digested with *Eco*RI and the transfer vector pFGB-cHE. The co-transfected cells were agarose-overlayed and incubated at 37℃ for 8 days.

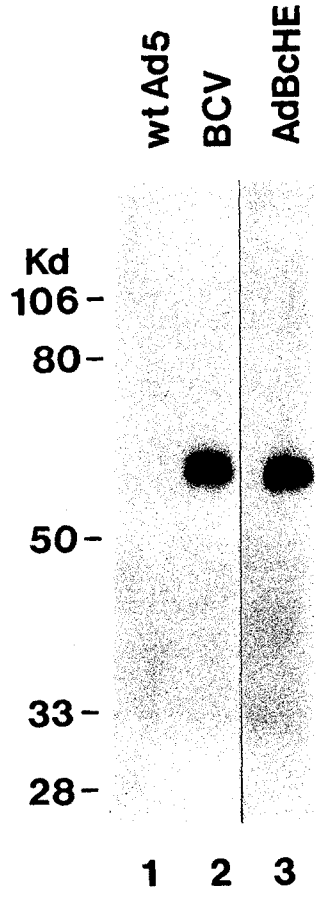

Fig. 3. Immunoprecipitation of the recombinant HE polypeptide.
HeLa cells were infected with wild-type adenovirus type 5 or recombinant adenovirus AdBcHE at an m.o.i. of 10, and labelled with 50 μCi/ml of [^{35}S]methionine from 12-18 hr post-infection. Radiolabelled cell-lysates were extracted and the cytoplasmic fractions were immunoprecipitated with anti-BCV rabbit antiserum using Protein A-coupled Sepharose beads. The immune complex was dissociated by boiling for 5 min in the presence of β-mercaptoethanol and resolved by electrophoresis on a 10% SDS polyacrylamide gel followed by autoradiography; lanes: (1), wild type adenovirus-infected cell lysate. (2), BCV virions purified from BCV-infected MDBK cells. (3), AdBcHE recombinant adenovirus-infected cell lysates.

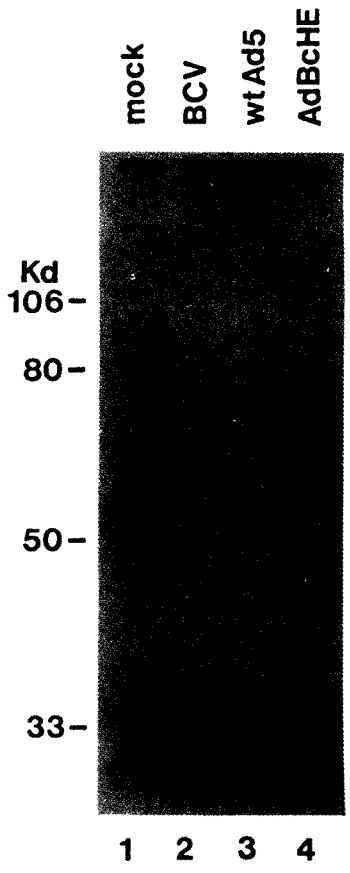

Fig. 4. Glucosamine radiolabelling of the recombinant HE polypeptide.
Cells were infected with BCV or AdBcHE and radiolabelled for 6 hr with 50 μCi/ml of [^{3}H]glucosamine at 12 hr post-infection. Cell lysates were extracted and immunoprecipitated with anti-BCV rabbit antibody for AdBcHE-infected cell lysate, or with anti-HE monoclonal antibody HC10-4 for BCV-infected MDBK cell lysate. The polypeptide labelled with [^{3}H]glucosamine were dissociated from Sepharose beads. In the presence of β-mercaptoethanol, samples were resolved by polyacrylamide gel electrophoresis and autoradiography. lanes: (1), mock-infected MDBK cells; (2), BCV-infected MDBK cells; (3), wild-type adenovirus-infected HeLa cells; (4), recombinant adenovirus AdBcHE-infected HeLa cells.

Glycosylation of the 65 KDa recombinant polypeptide was determined by glucosamine labelling. Virus-infected cells were radiolabelled at 16 hr post-infection with [^{3}H] glucosamine for 6 hr. Cell lysates were prepared and immunopreciptiated followed by polyacrylamide gel electrophoresis. A 65 KDa polypeptide was identified by the radioactive glucosamine labelling (Fig. 4, lane 4), and this polypeptide was identical to the authentic HE protein of bovine coronavirus (Fig. 4, lane 2). This confirms that the recombinant HE polypeptide was glycosylated similar to the authentic counterpart.

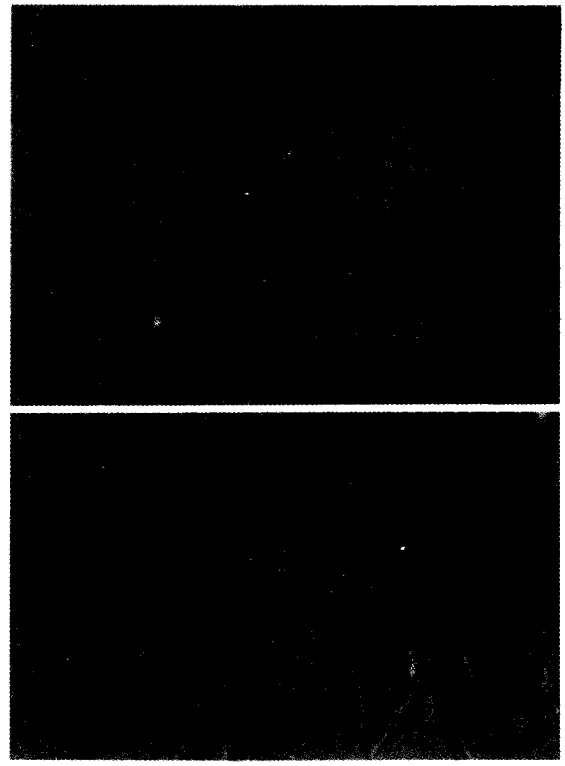

Fig. 5. Hemadsorption activity of the recombinant HE polypeptide.
Balb/c mouse erythrocytes were washed two times Alsevers solution. At 16 hr post-infection cell monolayers were incubated with 0.5% erythrocytes for 10 min at room temperature, and unbound erythrocytes were washed with PBS. Binding of the erythrocytes to virus-infected cells was photographed with a phase contrast microscope (Zeiss model IM35) at 100×. panels: (A), HeLa cells infected with AdBcHE; (B), MDBK cells infected with BCV.

Hemagglutination Activity of the Recombinant HE Polypeptide

The HE protein of BCV is capable of agglutinating red blood cells (20). In order to examine if the recombinant polypeptide retained hemagglutination activity, hemadsorption activity of the cells infected with recombinant adenovirus was measured. At 12 hr post-infection, cell monolayers were washed with phosphate buffered saline. Mouse erythrocytes were added to the monolayers, and the cells were incubated with erythrocytes for 10 min. Unbound erythrocytes were washed off, and the attachment of erythrocytes to the cell surface was examined with a microscope. Both BCV-infected MDBK cells (Fig. 5, panel B) and recombinant adenovirus-infected HeLa cells (Fig. 5, panel A) bound erythrocytes, while mock-infected or wild-type adenovirus-infected cells did not bind to erythrocytes (dat not shown), indicating that the recombinant HE polypeptide retained hemagglutina-

tion activity. The result of hemadsorption assay also suggested that the recombinant HE polypeptide was expressed on the surface of the cells infected with adenovirus.

In order to determine the cell surface transport of the recombinant HE polypeptide, cell surface immunofluorescence was measured. Cells infected with recombinant adenovirus were grown on a chamber slide for 12 hr. The cells were unfixed and reacted with anti-BCV polyclonal rabbit antibody, followed by incubation with FITC-conjugated anti-rabbit IgG. When examined with a fluorescence microscope, immunofluorescence was evident on the surface of the cells expressing the HE polypeptide (Fig. 6).

DISCUSSION

We demonstrated the expression of the HE polypeptide of bovine coronavirus using a helper-independent live recombinant human adenovirus vector. The recombinant adenovirus was constructed to carry BCV HE gene in the adenovirus E3 transcription region. The HE gene was flanked by SV40 early promoter and polyadenylation signal, and the entire expression cassette was placed in the parallel orientation to the E3 transcription. Even though adenovirus has been widely utilized to express various sources of eukaryotic genes, in most cases foreign genes have been inserted into the E1 region of the adenovirus genome (2, 4, 8, 21, 30). Since the E1 region is essential for virus replication (17), recombinant adenoviruses constructed so were replication-defective and required helper virus. Alternatively, the defective recombiants could be grown in 293 cells in which the E1-encoded proteins were constitutively expressed (1, 3, 15). Since the E3-replaced recombinant adenovirus would be infectious, we chose HeLa cells to study the synthesis of recombinant polypeptides. The recombinant AdBcHE synthesized the HE polypeptide in HeLa cells, proving that it was truly helper-independent. The recombinant HE synthesized in HeLa cells was glycosylated and retained its biological activities such as hemadsorption activity (Fig. 5) and immunoreactivity (Figs. 3, 4). The HE polypeptide was also properly transported to the plasma membrane of the cell (Fig. 6).

Immunization with live adenovirus vector has several advantages over subunit protein immunization. These include oral administration, low cost of preparation, and stability of the vaccine preparation during transport and storage. One important advantage of using adenovirus as a live vaccine is the induction of mucosal immunity. Adenovirus replicates in the mucosal surfaces of the respiratory and intestinal tracts. Human adneovirus types 4 and 7 have been orally used to prevent respiratory disease (6, 31). Recently Czerkinsky *et al.* (7) demonstrated a common mucosal immune system in humans. The gastrointestinal tract includes a large amount of specialized mucosal lymphoid tissue, containing early precursor of B and T lymphocytes that can migrate selectively to other regions of the intestine and distant mucosal sites. Here these cells express their immunological effector functions, the synthesis of secretory IgA (12). Thus oral immunization with live recombinant adenovirus carrying genes of other pathogens should induce secretory

Fig. 6. Surface immunofluorescence of the cells infected with recombinant AdBcHE.

HeLa cells grown on a chamber slide were infected with AdBcHE. At 18 hr post-infection, cells were incubated with rabbit antibody for 1 hr on ice followed by subsequent incubation with anti-rabbit goat antibody. Cells were washed with PBS, and the cell surface immunofluorescence was examined by Zeiss fluorescence microscope. panels: (A), AdBcHE-infected; (B), wild-type Ad5-infected.

immune responses at distant mucosal surfaces and humoral immune response as well, to protect against infection by the corresponding pathogens. It is also possible that antigen presentation in adenovirus infected cells might induce a better cell mediated immune (CMI) response than that induced by purified subunit protein immunization which require an adjuvant and repeated exposures.

Recent studies in our laboratory revealed that the recombinant HE polypeptide synthesized by this recombinant adenovirus was immunoreactive with a series of BCV neutralizing monoclonal antibodies (35). Therefore, it is of interest to determine if the immune responses mediated by the recombinant adenovirus neutralize BCV infectivity and protect animals from the disease. Such studies are currently in progress.

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