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Immunological Responses of Mice and Cattle to Baculovirus-Expressed F and H Proteins of Rinderpest Virus: Lack of Protection in the Presence of Neutralizing Antibody

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Rinderpest is a highly contagious viral disease of ruminants and has greater than 95% morbidity and mortality. The etiological agent, rinderpest virus (RPV), is a member of the family *Paramyxoviridae* and the genus *Morbillivirus*. Immune responses to both the hemagglutinin (H) and the fusion (F) antigens of morbilliviruses play an important role in the prevention of infection, and only attenuated live vaccines have been shown to provide protective immunity against the group. The lack of protection with inactivated vaccines has been attributed to the denaturation of the F glycoprotein of the virus. Our previous study, however, demonstrated complete protection of cattle vaccinated with infectious vaccinia virus recombinants expressing the H (vRVH) or F (vRVF) protein alone, even in the presence of only 4 U of serum-neutralizing (SN) antibody to RPV (T. Yilma, D. Hsu, L. Jones, S. Owens, M. Grubman, C. Mebus, M. Yamanaka, and B. Dale, Science 242:1058–1061, 1988). We have constructed recombinant baculoviruses that express the F (F_b) and H (H_b) glycoproteins of RPV. Furthermore, we have analyzed the immune responses of mice and cattle to these antigens. Cattle vaccinated with F_b or H_b or a mixture of both antigens were not protected from challenge inoculation with RPV, even when the SN titer was greater than in cattle vaccinated with vRVF alone. This lack of protection, in the presence of SN antibody, would indicate that live attenuated and recombinant vaccines induce immune responses necessary for protection (e.g., cell-mediated immunity) that are not generated by subunit or inactivated whole-virus vaccines.

Rinderpest is a highly contagious viral disease with greater than 95% morbidity and mortality in cattle and buffalo (15, 16). The disease is characterized by high fever, profuse bloody diarrhea, and necrosis of the mucosa in the digestive tract in infected animals. It poses serious economic, social, and ecological problems in Africa, Asia, and the Middle East. Until recently, rinderpest accounted for a loss of over 2,000,000 cattle and buffalo per year (16).

Rinderpest virus (RPV) is a member of the genus *Morbillivirus* of the family *Paramyxoviridae*; other members of the group include measles virus of humans, canine distemper virus, phocid distemper virus, and peste-des-petits-ruminants virus of goats and sheep (23). These viruses are antigenically related and can confer cross-immunity in experimentally immunized animals (9, 16). Like other members of the paramyxovirus group, RPV has a single-stranded RNA genome with a minus polarity. We have characterized eight proteins of the virus, including the large (L), phosphoprotein (P), hemagglutinin (H), nucleoprotein (N), fusion (F), matrix (M), and C proteins and provided evidence for another protein (V) (6, 23). In addition, we have described the sequences of all the genes that code for these proteins, with the exception of the L protein (8, 11, 23, 24).

There is an effective tissue culture rinderpest vaccine (TCRV) developed by Plowright that provides lifelong immunity to cattle (15). The use of the vaccine is limited, however, because of its high cost of production, heat lability, and difficulty of delivery to animals in the hot and humid regions of Africa and Asia. In contrast, the production and use of a recombinant vaccinia virus (rVV) vaccine does not require refrigeration, trained personnel, expensive tissue culture facilities, or syringes and needles for delivery.

In preparation for the development of an rVV vaccine, we propagated the highly virulent Kabete O strain of RPV in primary bovine kidney cells and made cDNA copies of the H (24) and F (8) genes of RPV. In addition, we constructed rVVs that expressed the F (vRVF) or the H (vRVH) or both (vRVFH) glycoproteins (5, 25). Cattle vaccinated with either the single rVVs (vRVF or vRVH) or the double rVV (vRVFH) were completely protected when challenge inoculated with 10^3 50% tissue culture infective doses ($TCID_{50}$) of RPV, a dose greater than 1,000 times the lethal dose of the virus. Even cattle with serum neutralization (SN) titers as low as 4 were completely protected; this substantiates previous observations that any detectable SN antibody induced by live RPV provides complete protection against the disease (9). However, there is a concern about the safety of using rVVs, because of the report of a generalized disseminated vaccinia virus infection after smallpox vaccination of an individual positive for the human immunodeficiency virus (17). It is, therefore, important that safety considerations be addressed before introducing live recombinant viruses into the environment, particularly in regions of Africa and Asia with a high incidence of human immunodeficiency virus.

The primary objective of this study was to assess the efficacy of the F (F_b) and H (H_b) glycoproteins of RPV expressed by recombinant baculovirus vectors Bac-F and

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Bac-H, respectively, as subunit vaccines for rinderpest. These inexpensively produced subunit vaccines, it was thought, might provide an alternative to rVVs in countries where safety concerns preclude the use of live recombinant vaccines.

A second objective was to determine the reasons for the failure of inactivated, whole-virus RPV vaccines to protectively immunize cattle against rinderpest. Similar observations have been made for other members of the morbillivirus group (13). Previously, this failure was attributed to the denaturation of F glycoprotein during vaccine preparation (13); however, protection by vRVH alone rules out such a hypothesis (25). In this study, we show that most cattle were not protected from rinderpest by immunization with subunit preparations of F_b or H_b or a mixture of both antigens. This lack of protection, even in the presence of SN antibody, provides indirect evidence that cell-mediated immunity plays an essential role in protection against rinderpest and other members of the morbillivirus group.

MATERIALS AND METHODS

Cells and virus. *Spodoptera frugiperda* (Sf9) cells were purchased from American Type Culture Collection. *Autographa californica* nuclear polyhedrosis virus (AcNPV) and the baculovirus plasmid vectors were gifts of Max Summers (Texas A & M University, College Station, Tex.). Sf9 cell cultures were maintained in Ex-Cell 401 tissue culture media (Biosciences, Lenexa, Kans.) supplemented with 5% heat-inactivated fetal bovine serum (Hyclone Laboratories) in the presence of antibiotics (20).

Antisera. Rabbit antisera directed against the carboxy terminus of F and the entire H glycoprotein of measles virus were gifts of C. Richardson (22) and E. Norrby (21), respectively. Polyclonal antisera against both F_b and H_b were also generated in the CB6F1/J strain of mice, primed with vRVF or vRVH (5), and then boosted with the homologous glycoprotein (F_b or H_b).

Construction of baculovirus transfer vectors containing the H or F genes of RPV. The entire coding sequences of H (as a *Bam*HI fragment) and F (as an *Eco*RI fragment) were cloned into pVL1393 at the *Bgl*II and *Eco*RI sites, respectively (Fig. 1). Recombinant plasmids pBac-H and pBac-F were characterized by restriction enzyme analysis.

DNA transfection. To generate Bac-F or Bac-H, Sf9 monolayers grown in T-25 flasks were transfected with a mixture of 30 μg of recombinant plasmids (pBac-F or pBac-H) and 2 μg of wild-type viral DNA (12, 20). Successful transfection was confirmed by the presence of polyhedrin protein (produced by the wild-type virus) within 5 days of transfection. By using serial dilutions of culture supernatant obtained from transfected Sf9 cells, a plaque assay was performed, and recombinant plaques were screened for the absence of polyhedrin protein normally found in wild-type plaques (20). Putative polyhedrin-negative plaques were first amplified in Sf9 cells in 24-well plates, and the presence of H or F genes was identified by DNA dot blot hybridization (18, 20). Recombinant viruses designated Bac-H and Bac-F were separated from wild-type virus by four consecutive plaque purifications.

Dot blot immunoassay. To assess the expression of recombinant proteins by various clones, 3×10^6 Sf9 cells were infected with either recombinant Bac-H or Bac-F at a multiplicity of infection (MOI) of 10. At 60 h postinfection, cells were pelleted at room temperature (RT) for 2 min and resuspended in 100 μl of TBS (50 mM Tris-Cl [pH 7.4], 100 mM NaCl). Samples were then dot blotted on an Immobilon-P (Millipore Co.) transfer membrane and treated for 1 h at RT in blocking solution (5% nonfat dry milk in 1× TBS). The membrane was incubated with the primary antibody (1:1,000 dilution of rabbit anti-H or anti-F in blocking solution) at RT for 1 h. After being washed in 1× TBS, the membrane was incubated with the second antibody (goat anti-rabbit serum conjugated to alkaline phosphatase diluted 1:2,000 in blocking solution) at RT for 1 h. Finally, the antibody-treated membrane was washed and developed by using BCIP (5-bromo-4-chloro-3-indolylphosphate) as the enzyme substrate in alkaline phosphatase buffer.

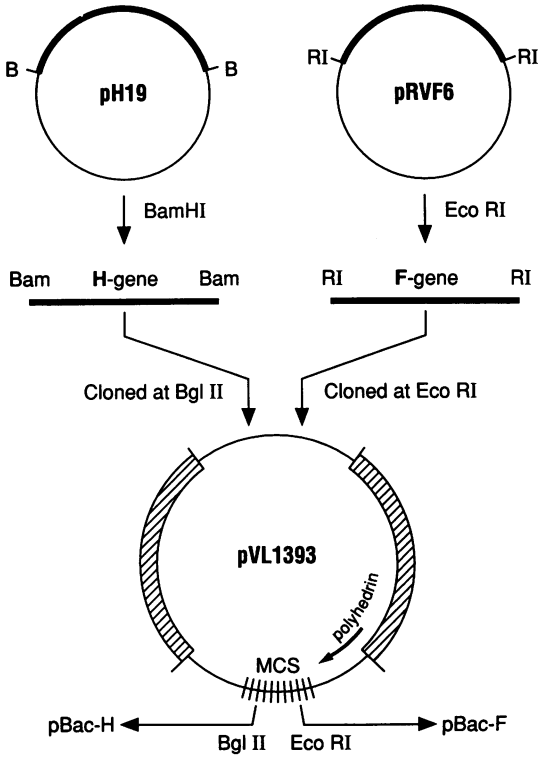

FIG. 1. Strategy for the cloning of the H and F genes of RPV in baculovirus plasmid vectors. DNA sequences derived from pH19 and pRVF6 are shown as solid single lines as *Bam*HI (B or Bam) and *Eco*RI (RI) fragments for the H and F genes, respectively, of RPV. DNA sequences shown in dashed lines in pVL1393 are the homologous viral (polyhedrin) genomic sequences. MCS, multiple cloning sites. The arrow represents the direction of transcription.

Western blot (immunoblot) analysis of recombinant proteins. For immunological analysis of recombinant proteins, 1.5×10^6 Sf9 cells were infected with wild-type or recombinant baculoviruses and then harvested at 12-h intervals over a 72-h period. Cells were suspended in 500 μl of phosphate-buffered saline (PBS), and 10 μl of each sample (approximately 3×10^4 cells) was lysed by boiling in electrophoresis sample buffer (0.06 M Tris-Cl [pH 6.8], 2% sodium dodecyl sulfate [SDS], 10% glycerol, 2% β-mercaptoethanol, 0.005% bromophenol blue) for 5 min. The supernatants were then resolved by SDS–8% polyacrylamide gel electrophoresis (PAGE) (10). After electrophoresis, proteins were transferred onto an Immobilon-P membrane in Tris-glycine buffer containing 20% methanol (20). The membrane was probed with primary and secondary antibodies and developed in alkaline phosphatase buffer as described for the dot blot immunoassay.

TABLE 1. Immune responses of mice vaccinated with various immunogens

Primary immunogen	Secondary immunogen	SN titer[a]
Wild-type baculovirus		<2
F_b		<2
F_b	vRVF	20
H_b		160
H_b	vRVH	2,400
Wild-type vaccinia virus		<2
vRVF		80
vRVF	F_b	600
vRVH		400
vRVH	H_b	3,200

[a] SN titer is expressed as the reciprocal of the highest serum dilution that completely neutralized 100 $TCID_{50}$ of RPV in Vero cells.

Radioimmunoprecipitation. The authenticity of the recombinant glycoproteins was confirmed by immunoprecipitation and SDS-PAGE analysis employing specific antibodies to F and H glycoproteins, and the variability in glycosylation was determined by comparing with the glycoproteins expressed by rVVs in human 143 thymidine kinase-negative (TK^-) cells. Previously, we have demonstrated that glycoproteins H and F expressed in rinderpest virus-infected cells and those expressed by rVVs were indistinguishable by radioimmunoprecipitation (5). Briefly, monolayers of 10^6 Sf9 cells were infected with Bac-F or Bac-H at an MOI of 10, and the virus was adsorbed for 1 h at 28°C. After propagation of the virus for 48 h, the cells were rinsed with 1 ml of methionine and cysteine-free medium and then labelled with 200 μCi of ^{35}S-Translabel (Amersham Co.) per ml in the same medium for 2 h. The metabolically labelled cells were pelleted at 4°C, washed two times with ice-cold PBS, and lysed in 500 μl of lysis buffer (10 mM Tris-Cl [pH 7.5], 100 mM NaCl, 1% Nonidet P-40, 0.3% deoxycholate, 1 mg of bovine serum albumin per ml) on ice for 20 min. Lysed cells were centrifuged at 4°C for 5 min to pellet the nuclei and cell debris. Immunoprecipitations were performed on ice in a total volume of 50 μl of lysis buffer by incubating extracts with rabbit polyclonal anti-F- or anti-H-specific antibodies at a 1:40 or 1:4 dilution, respectively. Immune complexes were mixed with 10 μl of protein A-Sepharose in lysis buffer and incubated on ice for 30 min while shaking. The immunoprecipitates were washed five times with 1 ml of buffer (10 mM Tris-Cl [pH 7.5], 500 mM NaCl, 1% Nonidet P-40, 0.1% SDS) and one time with distilled water. Samples were disrupted by boiling for 5 min in protein sample buffer and then resolved by SDS–8% PAGE (10).

rVVs. The construction of rVVs expressing either F (vRVF) or H (vRVH), immunological characterization of recombinants, and protective studies in cattle have been described elsewhere (5, 25).

Immunization studies with mice. The immunogenicity of F_b and H_b were evaluated by using CB6F1/J mice (Table 1). A group of five mice were vaccinated intramuscularly with lysates of 10^6 Sf9 cells infected with either Bac-F or Bac-H at an MOI of 10 and harvested at 72 h postinfection. To evaluate the priming effect of rVV, five groups of mice (five per group) received a primary vaccination of 10^7 PFU of vRVF or vRVH by intradermal inoculation and were boosted 21 days later with the homologous F_b or H_b by intramuscular injection in incomplete Freund's adjuvant. For a control, mice were first vaccinated with H_b or F_b and then boosted with vRVH or vRVF, respectively. SN titers were determined on pooled sera for each group of mice.

Immunization studies with cattle. Protective immunity was evaluated by using cattle vaccinated with F_b and H_b. Three groups of cattle (three animals per group) were vaccinated with crude cell lysates of F_b or H_b or a mixture of both. Each animal received approximately 6×10^6 cells infected with Bac-H or Bac-F and emulsified in incomplete Freund's adjuvant in a single intramuscular injection. Four weeks later, all cattle, including two control animals, were challenge inoculated with 10^3 $TCID_{50}$ of RPV. Temperatures were recorded, and animals were examined for clinical signs of rinderpest daily up to 14 days postchallenge. The serum antibody titers were determined by SN and enzyme-linked immunosorbent (ELISA) assays.

SN assay. Immune responses of both cattle and mice to recombinant F_b and H_b were determined by SN assay. Sera were run in triplicate in 96-well microtiter plates. An equal volume of TCRV (50 μl) containing 100 $TCID_{50}$ was added to each well. The plates were incubated for 1 h at 37°C, 100 μl of Vero cells (10^4 cells) was added to each well, and the plates were incubated at 37°C for 7 days in a CO_2 incubator. The plates were read by examining unfixed and unstained cell monolayers under an inverted-phase light microscope. SN titers were expressed as the reciprocal of the highest serum dilution that completely neutralized 100 $TCID_{50}$ of RPV in Vero cells.

Indirect ELISA. In addition to SN antibodies, anti-F and anti-H antibodies were measured by ELISA as previously described (1). Briefly, microtiter plates were coated with sucrose gradient-purified RPV (100 μl per well) in carbonate buffer (pH 9.6) overnight at 4°C. After being washed four times in PBS containing 0.5% Tween 20 (PBST), duplicate serial twofold dilutions of sera in PBST containing 5% fetal bovine serum as a blocking reagent were added, and plates were incubated at 37°C for 1 h. After another wash, horseradish peroxidase-conjugated rabbit anti-bovine immunoglobulin G (Sigma Chemical Co.) in PBST-fetal bovine serum was added, and plates were incubated at 37°C for 1 h. Plates were then washed, and substrate (tetramethylbenzidine and 0.01% H_2O_2 in phosphate-citrate buffer [pH 5.0]) was added. The plates were incubated at room temperature for 25 min, and 2 M H_2SO_4 was added to stop color development. The optical density at 450 nm (OD_{450}) was determined. ELISA titers were expressed as the reciprocal of the highest serum dilution that gave an OD reading of at least two times the OD for negative serum.

RESULTS

Construction of recombinant baculoviruses. The cloning strategies used for the generation of Bac-F and Bac-H are described in Fig. 1. The H gene was excised as a *Bam*HI fragment from the pH19 plasmid and then cloned in the *Bgl*II site of the baculovirus transfer plasmid vector pVL1393 to generate recombinant plasmid pBac-H. Similarly, the F gene was obtained as an *Eco*RI fragment from pRVF6 and cloned in the *Eco*RI site of the pVL1393 to generate pBac-F.

Recombinant baculoviruses were generated by homologous recombination with pBac-H or pBac-F and wild-type baculovirus DNA in Sf9 cells. Recombinant viruses (Bac-H and Bac-F) were identified by the absence of polyhedrin inclusion bodies and by DNA dot blot assay with H- or F-specific nucleic acid probes (data not shown). Three recombinant clones from each type were selected for further analysis.

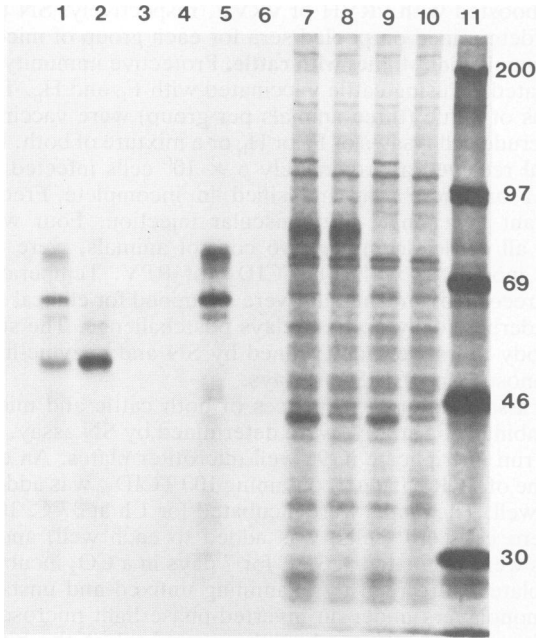

FIG. 2. Characterization of fusion and hemagglutinin proteins of RPV expressed by recombinant baculovirus in Sf9 cells and rVV in human 143 TK^- cells. Sf9 cells were infected with Bac-F or Bac-H at an MOI of 10. At 48 h of infection, the infected cells were starved of Met and Cys for 1 h and then labelled with [^{35}S]Met and [^{35}S]Cys for 2 h. The radioactive labelling of human 143 TK^- cells infected with rVVs has been described previously (5). Cell lysates were immunoprecipitated with anti-H- or anti-F-specific antibodies and analyzed on an 8% by SDS–PAGE. Lane 1, mixed H_b and F_b cell lysates immunoprecipitated with both anti-H and anti-F sera; lane 2, F_b lysate with anti-F; lane 3, F_b lysate with anti-H; lane 4, H_b lysate with anti-F; lane 5, H_b lysate with anti-H; lane 6, cell lysate from AcNPV-infected Sf9 cells with anti-H and anti-F sera; lane 7, cells infected with vRVFH with anti-H and anti-F sera; lane 8, cells infected with vRVH with anti-H; lane 9, cells infected with vRVF with anti-F; lane 10, cells infected with wild-type vaccinia virus (Wyeth) with anti-H and anti-F sera; lane 11, molecular size markers (in kilodaltons).

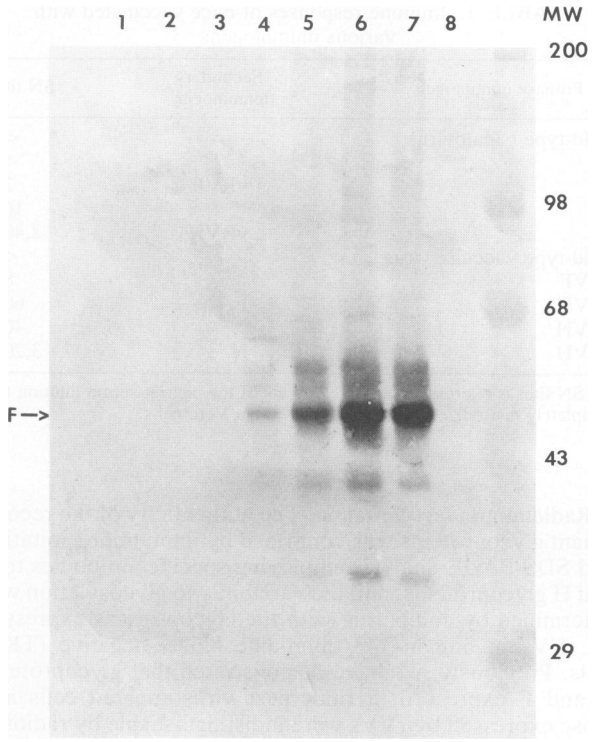

FIG. 3. Western blot analysis of Sf9 cells infected with Bac-F. Cells were harvested at various times postinfection, and samples were lysed in lysis buffer, fractionated through 8% SDS–PAGE, transferred to Millipore Immobilon-P membrane, and incubated with measles anti-F antibodies. Lane 1, 0 h; lane 2, 12 h; lane 3, 24 h; lane 4, 36 h; lane 5, 48 h; lane 6, 60 h; lane 7, 72 h; lane 8, Sf9 cells infected with wild-type AcNPV for 72 h.

Immunological characterization of recombinant H and F proteins. Recombinant glycoproteins expressed by Bac-H and Bac-F were analyzed by immunoprecipitation with H- and F-specific polyclonal antibodies (Fig. 2). A 78-kDa H protein and a 51-kDa F protein, respectively, were identified in Bac-H- and Bac-F-infected Sf9 cells (Fig. 2). H_b was of a lower molecular mass (78 kDa) compared with that expressed by vRVH (87 kDa) in human 143 TK^- cells. Similarly, F_b was of lower molecular mass (51 kDa) compared with that of F_0 (62-kDa) glycoprotein expressed in rinderpest virus- or vRVF-infected cells (6). In addition, in rinderpest virus- or vRVF-infected cells, there is cleavage of F_0 to F_1 and F_2; however, similar cleavage of F_b was not observed (5, 6). Further characterization of recombinant proteins was performed by Western blot analysis on samples obtained from 12 to 72 h postinfection. F_b was first detected at 36 h postinfection and continued to be expressed at high levels up to 72 h postinfection (Fig. 3). Similar results were obtained with the expression of recombinant H protein (data not shown). In contrast, the wild-type polyhedrin protein was detected as early as 12 h postinfection.

Immunogenicity of H_b and F_b in mice. The SN titers of mice vaccinated with F_b and H_b are presented in Table 1. The priming effect of rVVs was evaluated by first vaccinating mice with vRVF or vRVH and then boosting with F_b or H_b, respectively. Immunization of mice with F_b alone failed to induce any detectable SN antibody. In contrast, the H_b-vaccinated group had an average SN titer of 160. Priming with vRVF and then boosting with F_b produced an average SN titer of 600. Moreover, priming with vRVH and boosting with H_b induced an average SN titer of 3,200 (the highest of all observed titers), and primary vaccination with H_b followed by a booster vaccination with vRVH induced a titer of 2,400 SN units.

Immunization and challenge studies with cattle. The SN and ELISA titers of cattle vaccinated with F_b and H_b or a mixture of both are summarized in Table 2 and Table 3, respectively. Although no SN antibodies could be detected in the group of animals vaccinated with F_b, they all had ELISA titers (8 to 16) to RPV. The group vaccinated with H_b had both SN (8 to 32) and ELISA (8 to 16) titers, and similarly, the group vaccinated with the mixture of H_b and F_b had SN (8 to 32) and ELISA (16 to 64) titers to RPV before challenge. All groups of animals were challenge inoculated with 10^3 $TCID_{50}$ of RPV 30 days postimmunization. Only one animal (number 038), a member of the group vaccinated with both proteins, was completely protected and experienced no clinical symptoms. A second animal (number 037) vaccinated with H_b alone suffered from severe clinical rinderpest, including fever and diarrhea, but recovered from the disease 13 days post-challenge inoculation (Table 2). Both animals had high levels of SN (≥4,096) and ELISA

TABLE 2. SN titers of cattle vaccinated with F_b and H_b of RPV

Cow[a]	Antigen	SN titer[b] on day:							Protection[c]
		0	7	14	30	38	45	52	
C1		ND	ND	ND	<2	32	D		−
C2		ND	ND	ND	<2	D			−
032	F_b	<2	<2	<2	<2	D			−
033	F_b	<2	<2	<2	<2	D			−
034	F_b	<2	<2	<2	<2	D			−
035	H_b	<2	8	32	32	ND	D		−
036	H_b	<2	16	8	8	ND	D		−
037	H_b	<2	16	16	16	1,024	≥4,096	≥4,096	±
038	H_b + F_b	<2	16	32	32	32	≥4,096	≥4,096	+
039	H_b + F_b	<2	16	16	16	128	D		−
031	H_b + F_b	<2	8	8	8	64	D		−

[a] C1 and C2 are unvaccinated controls.
[b] SN titer is expressed as the reciprocal of the highest serum dilution that completely neutralized 100 $TCID_{50}$ of RPV in Vero cells. D, dead; ND, not done. Animals were vaccinated on day 0 and challenged on day 30.
[c] Animals that died from challenge inoculation (−) and those protected (+) are indicated. Animal 037 (±) recovered after experiencing severe clinical rinderpest.

(2,048) titers after challenge inoculation with RPV, which is indicative of viral replication and a lack of sterilizing immunity. All other animals died from severe clinical rinderpest.

DISCUSSION

Rinderpest, a highly contagious viral disease of cattle and buffalo, inflicts serious economic, social, and ecological damage in Africa, Asia, and the Middle East. The use of TCRV has significantly reduced the annual mortality from the disease. However, its high cost of production, heat lability, and difficulty of administration have limited the use of TCRV in the hot and arid regions of Africa and Asia (16).

To address the economic and logistical problems associated with the use of TCRV, we developed a number of rVV vaccines for rinderpest that provided complete protection to immunized cattle when challenge inoculated with 1,000 times the lethal dose of the virus. rVVs are heat stable, easy to produce, and simple to administer. With the recent spread of the HIV epidemic in Africa and Asia, however, there are safety concerns with the use of infectious vaccinia virus. As an alternative, we have evaluated the potential use of subunit vaccines of viral glycoproteins inexpensively produced in baculovirus expression vectors. In addition, we have investigated the reasons for the ineffectiveness of classical inactivated vaccines to provide protection against morbilliviruses by analyzing the immune responses of cattle vaccinated with F_b and H_b.

TABLE 3. ELISA titers of cattle vaccinated with F_b and H_b of RPV[a]

Cow	Antigen	ELISA titer[b] on day:					Protection
		0	30	38	45	52	
C1		ND	<2	16	D		−
C2		ND	<2	D			−
032	F_b	<2	8	D			−
033	F_b	<2	16	D			−
034	F_b	<2	16	D			−
035	H_b	<2	16	4	D		−
036	H_b	<2	8	ND	D		−
037	H_b	<2	8	32	128	2,048	±
038	H_b + F_b	<2	16	8	2,048	2,048	+
039	H_b + F_b	<2	64	16	D		−
031	H_b + F_b	<2	16	ND	D		−

[a] See footnotes *a*, *c*, and *d* to Table 2 for abbreviations and conditions.
[b] ELISA titer is expressed as the reciprocal of the highest serum dilution that gave an OD reading of at least two times the OD for negative serum. Animals were vaccinated on day 0 and challenged on day 30.

In these studies, we used the virulent strain of RPV (Kabete O) to obtain clones of the H and F genes (25). The genes were then expressed under the control of the polyhedrin promoter of AcNPV. The H and F glycoproteins expressed in recombinant baculovirus-infected Sf9 cells were demonstrated by Western blot and immunoprecipitation analyses (Fig. 2 and 3).

The kinetics of F_b synthesis were followed by a Western blot analysis of samples obtained at different times postinfection. There appears to be an exponential increase in the level of recombinant protein synthesis between 36 and 60 h postinfection, with a plateau at 72 h postinfection. These results are similar to those seen with baculovirus recombinants expressing the F gene of human measles virus (22). However, these findings are in sharp contrast to the time of initial synthesis of polyhedrin protein, which is detected as early as 12 h postinfection.

The multiple bands detected by autoradiography of immunoprecipitated H_b and F_b represent intermediate and processed forms of these proteins, depending on the degree of glycosylation. The 65-kDa protein is the nonglycosylated precursor of the higher-molecular-mass (78-kDa) species of the glycosylated form of H_b. H_b was of a lower molecular mass (78 kDa) compared with that (87 kDa) of H expressed in vRVH-infected cells. The lower molecular mass of H_b may be attributed to partial glycosylation of the glycoprotein due to the deficiency of galactosyl and sialyl transferases in Sf9 cells (3). The 51-kDa band in Bac-F-infected Sf9 cells is the unprocessed F_0, and no cleavage products of the molecule to F_1 and F_2 were observed. Since multiple bands of the same molecular masses also appear in Western blot analysis of F_b, these observations prove that the heterogeneity represents authentic but varying forms of the glycosylated molecules of the same protein. In contrast, the F protein from vRVF-infected TK^- cells was efficiently cleaved to F_1 that appeared as 42-kDa protein. A heterogeneity in F glycoproteins was reported in RPV- and parainfluenza virus-infected cells (2, 7) and in measles virus H and F glycoproteins expressed by recombinant baculovirus expression vectors (22).

H_b was antigenic in both mice and cattle, and anti-H antibodies had neutralizing activities against RPV. Unlike H_b, F_b did not induce any detectable neutralizing antibody in mice or cattle (Table 1 and 2). Therefore, we included an ELISA to detect nonneutralizing antibodies that might be generated to RPV and found equivalent ELISA titers in cattle vaccinated with either F_b or H_b (Table 3). The high levels of SN antibody in H_b but not F_b vaccinates is consistent with the fact that H, not F, is the antireceptor of the virus. In comparison, cattle vaccinated with vRVF had low titers (4 to 16) of neutralizing antibodies, while those vaccinated with vRVH had SN titers ranging from 48 to 256 (25). The SN titers of H_b-immunized mice, although low, were comparable to the SN titers of mice immunized with vRVH (Table 1). Unlike H_b, vRVH is expected to induce both humoral and cell-mediated immune responses. In contrast, a subunit antigen is usually presented in the context of class II major histocompatibility complex proteins and elicits mainly humoral immunity.

To assess the potential use of baculovirus-expressed F and

H glycoproteins as subunit vaccines, protective immune studies were conducted with cattle. Groups of three cattle were vaccinated with a preparation of H_b or F_b or a mixture of F_b and H_b in incomplete Freund's adjuvant. Only one animal in the group vaccinated with the mixture of F_b and H_b was completely protected; another animal vaccinated with the H_b preparation suffered clinical rinderpest, experiencing high fever and severe diarrhea, but recovered by day 13 post-challenge inoculation (Table 2). The remaining seven animals died from rinderpest. The fact that only two animals survived indicates that protection against rinderpest did not correlate with SN or ELISA titers, and cell-mediated immunity may play an important role in protection against rinderpest.

We have not evaluated the protective immune response to RPV in cattle receiving more than a single immunization with F_b and/or H_b. Since rinderpest is a devastating disease of ruminants, particularly affecting the herds of nomadic people in the hot and arid regions of Africa and Asia, employment of a vaccine requiring more than a single inoculation is unacceptable to developing countries as well as to international organizations such as the Office International des Epizooties (OIE) regulating the large-scale eradication programs of rinderpest (14). The logistics of gathering and vaccinating cattle in these regions is the major reason for the failure to eradicate the disease with the heat-labile Plowright tissue culture vaccine.

Although inactivated whole virus vaccines of morbilliviruses induce SN antibodies, it has been previously demonstrated that such vaccines fail to provide protective immunity (13). Similarly, we have demonstrated the induction of SN antibodies and the lack of protection in cattle immunized with subunit antigens expressed in baculovirus. The lack of protection with inactivated, conventional vaccines for measles virus has been attributed to denaturation of the F protein during the inactivation process (13). We have clearly demonstrated, however, that a rVV expressing only the H protein (vRVH) provides complete protection against a challenge inoculation of 1,000 times the lethal dose of RPV (5, 25). Complete protection against measles was demonstrated in mice vaccinated with rVVs expressing either the H or the F of measles virus glycoprotein (4). We have demonstrated that animals vaccinated with rVVs expressing either F or H antigen were completely protected even when SN titers were as low as 4 (25). Others have succeeded, however, in inducing protective immunity in mice vaccinated with subunit preparations of H and F measles glycoprotein when presented in immune-stimulating complexes, presumably as a result of more effective presentation of these antigens to the immune system (21). Since subunit and heat-inactivated morbillivirus antigens are ineffective as vaccines, live attenuated classical vaccines or recombinant vectors (e.g., vaccinia virus) must induce immune responses, such as cell-mediated immunity, not generated by subunit antigens. Currently, we are investigating the role of cell-mediated immunity in protection against rinderpest by analyzing the comparative immune responses of animals vaccinated with subunit antigens (F_b or H_b) or live recombinant vaccines (vRVF or vRVH).

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