Neutralizing Antibody Response in Bovine Serum and Nasal and Salivary Secretions After Immunization with Live or Inactivated Foot-and-Mouth Disease Virus

FELIPE FIGUEROA, ADELA OHLBAUM, AND GUILLERMO CONTRERAS

Unidad de Virología, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad de Chile, Santiago, Chile

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Calves develop nasal and salivary neutralizing antibodies against foot-and-mouth disease virus after nasal inoculation with live virus. Nasal and salivary antibody was not detected after exposure to inactive virus. Serum antibodies were induced by live and inactive virus given subcutaneously. Passively acquired antibodies were detected as long as 7 months after birth.

Local immunity seems to play a major role in the resistance to infection by rhinoviruses (10). Since foot-and-mouth disease virus (FMDV) shows many of the features of a rhinovirus (8), local immunity in cattle could provide resistance against the disease (7), and it would be possible to appraise it by searching for neutralizing antibodies in nasal secretions. The present work is an investigation of neutralizing antibodies in serum and in nasal and salivary secretions of calves that had been inoculated either with inactive or active type O, FMDV.

The calves selected for this study came from a virus-free farm, and were born from dams vaccinated with commercial, inactive FMDV polyvalent vaccine. The animals had a negative history for FMDV, were negative for intestinal parasites, and had normal hemograms. The calves were isolated when they received live virus. They were examined daily, and the desired specimens were collected at selected intervals after inoculation. The virus, type O1, maintained as a prototype at the Instituto Bacteriológico de Chile, was kindly provided by Aldo Gaggero. For over 2 years we have grown and assayed the virus in primary cultures of fetal calf kidney cells or in BHK-21 cell cultures, or both. Vaccine was prepared by inactivation of a viral suspension (10⁶ plaque-forming units [PFU]/ml) with 0.05% Formalin at 32°C for 44 h (3). Inactivation was tested by intraperitoneal inoculation of suckling mice, and potency, as determined by complement fixation test (5), was adjusted to a 1:4 titer. Neutralization was measured either as plaque reductions or as 50% neutralization end point tests (5). Serum was obtained from the blood specimens by centrifugation, heated at 56°C for 30 min, and stored at −20°C. The nasal and salivary secretions were obtained by washing the nasal or the buccal cavities with 20 ml of saline; the washings were then centrifuged at 600 x g for 20 min, and the supernatant fluid was heated at 56°C for 30 min and then concentrated by lyophilization or by concentration with polyethylene glycol. Protein was measured by the method of Lowry (6) and was adjusted to 2 mg/ml. When necessary, specimens were dialyzed against buffered saline.

Prior to viral exposure, neutralizing antibodies were detected in every one of the 16 sera tested, and a correlation between their titer and the age of the calf is evident (Fig. 1). Since nasal specimens were devoid of neutralizing ability at a 1:2 dilution, only nine were tested. The same day that the specimens were obtained, the animals were divided into four groups and given the corresponding viral preparation. Six calves of one group received 5 ml of inactive FMDV subcutaneously. A second group of four animals received the same inactive virus preparation as a nasopharyngeal spray. The third group of five calves received 10⁶ PFU of live FMDV as a
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Fluid formed in the FMDV groups of nasopharyngeal spray in 1 ml of Hanks saline. The fourth group of two calves was not inoculated. All animals were observed daily; as expected, the only calves to develop the disease were the ones receiving live virus. Two days later their rectal temperature rose to 40 C for 1 to 2 days, and they showed abundant salivation for another two days and typical blisters in the mouth and on the feet. The mild disease lasted 7 to 10 days with full recovery. Examination of the hearts revealed normal findings, they did not show prostration at any time, and except for the two initial days, their appetites were good. Newborn mice inoculated with vesicular fluid died with the typical signs of FMD.

The results of the neutralization tests performed with the specimens from the three groups of inoculated animals are shown in Fig. 2. All five calves receiving live virus acquired neutralizing capacity in their nasal secretions, and in three it was also possible to detect neutralizing activity in saliva; all showed a rapid increase in their serum neutralizing antibodies. It should be noted that the nasal and salivary neutralizing capacity was measured for specimens adjusted to a protein content of 2 mg/ml. No neutralizing capacity was detected at a 1:2 dilution in the 20 nasal specimens tested from the two groups of calves receiving inactive virus. Furthermore, the nasopharyngeal exposure to inactive virus did not induce local or systemic immunological response, although the same inactive virus preparation given subcutaneously was able to induce serum immunoglobulin. The two uninoculated calves remained healthy, their nasal secretions were devoid of neutralizing capacity, and their serum titer remained unmodified.

The presence of FMDV neutralizing antibodies in the sera of young unexposed calves up to 7 months of age and their titer-age relationship (Fig. 1) strongly suggest that these immunoglobulins were received from the actively immunized mothers as shown by Graves (4) and that their half-life is longer than human maternal antibodies (2). Human colostral immunoglobulin seems to play a role in the local protection of the gastrointestinal tract and has been implicated in the partial failure of newborns' response to poliovirus attenuated vaccine (1). However, the immunoglobulin in the cow's colostrum is predominantly immunoglobulin g and passes the intestinal mucosa. It is found in the serum (Fig. 1; reference 7) and does not seem to interfere, as shown here, with the immune response to live virus.

![Graph](image-url)

**FIG. 1.** Neutralizing antibodies against type O FMDV in the sera of 16 healthy nonvaccinated calves under 7 months of age. Sera were treated and tested as described in the text.

![Graph](image-url)

**FIG. 2.** Neutralization detected in nasal and salivary secretions and in serum after exposure of calves to FMDV. The specimens were treated and tested as described in the text. The results plotted correspond to the three following experimental groups: •••• live virus nasopharyngeal vaccination; O—O, inactive virus nasopharyngeal vaccination; x---x inactive virus parenteral vaccination.
The nasal neutralizing capacity we report here, resulting from exposure to live virus, could in fact be secretory immunoglobulins. They could play an important role in the resistance to infection by FMDV since it has been shown that secretory antibody provides resistance at the portal of entry against many respiratory viruses, the rhinoviruses among them (9, 10). It is worth noting that we were not able to detect nasal "antibodies" after a local exposure to inactive FMDV, although such observation has been made in other virus-cell systems (9, 11).

Future experiments will be to challenge calves immunized in the different ways reported here with live "wild" FMDV and to score the protection afforded by each method. Also, it would be worthwhile to look for FMDV temperature-sensitive mutants that could multiply at the lower temperature of nasal and perinasal cavities and not at the animal's internal temperature. Such mutants, if found, could provide a firm basis on which to develop an effective FMDV vaccine.

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