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Astrovirus and Breda Virus Infections of Dome Cell Epithelium of Bovine Ileum

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A bovine enteric virus antigenically related to the United Kingdom isolate of bovine astrovirus was isolated from diarrheic feces, also containing rotavirus, of a calf in Florida. The astrovirus infected cell cultures and the epithelial cells of domes in the ileum, and there was cross-immunofluorescence with antiserum to the United Kingdom astrovirus. Calves infected with astrovirus alone did not develop clinical disease, but when astrovirus was mixed with rotavirus or Breda virus 2, the calves developed severe diarrhea and more extensive astrovirus infection of the dome epithelium. The dome epithelial cells showed degeneration associated with astrovirus infection, and a few cells showed degeneration with Breda virus 2 infection. Virions with a 30-nm diameter were seen in astrovirus-infected dome cells, and Breda virus 2 virions were also observed either in separate cells or, on occasion, with both viruses in one cell.

Bovine astrovirus was first recognized in England in 1978 after it was isolated from diarrheic feces of a neonatal calf and experimentally transmitted to two gnotobiotic calves (GC) (22). The authors considered this isolate to be nonpathogenic in calves, as no clinical effect was observed. Bovine Breda virus was recently isolated from neonatal calves in Iowa and shown to cause diarrhea of varying severity both in colostrum-deprived calves and GC (25). A second isolate (Breda virus 2) was shown by immunofluorescence (IF) staining to share antigens with Breda virus 1 (24). The taxonomic classification of Breda virus has yet to be determined, but based on serological data and from data on ultrastructural studies (to be published), it is unlikely to be classified in the family Coronaviridae, to which it has a superficial morphological resemblance. It is antigenically related to an equine virus isolated in Switzerland (Berne virus) (20) which cannot be readily classified into any existing virus family.

Astroviruses have been isolated also from the fecal contents of diarrheic children (12), lambs in which the pathogenesis has been described (16), and piglets (2). No antigenic relationship among bovine, human, ovine, and porcine astroviruses has been demonstrated. From their biochemical properties, it has been proposed that astroviruses should be placed in a separate group of the family Picornaviridae, as they have a polypeptide composition (two major capsid polypetides) intermediate between that of picornavirus and calicivirus (6). Lamb astrovirus was shown to infect only the mature epithelial cells of the villus and subepithelial macrophages of the small intestine, in which it produced partial villus atrophy. Astrovirus-infected cells were observed scattered throughout the apical half of the villi of all regions of the small intestine. However, maximum lesions were observed at 38 h postinfection in the midgut and ileum. In the mid-small intestine, lactase levels were reduced. Lamb astrovirus was not observed in the large intestine (16).

In studies on the pathogenesis of a second serotype of "Breda" virus isolated in Iowa (Breda virus 2), which was serologically related but distinguishable from the original isolate (24, 25), it was observed that the virus was contaminated with a second virus. This virus proved to be antigenically related to the United Kingdom (UK) isolate of bovine astrovirus (22).

This report describes the isolation, identification, and results of experimental transmission of the bovine astrovirus isolate and briefly compares the infection with that of Breda virus 2.

MATERIALS AND METHODS

Animals. GC were produced and reared by the method of Matthews et al. (14).

Viruses. Diarrheic calf fecal samples were obtained locally, with the exception of the rotavirus and astrovirus sample, which was received from R. K. Braun, University of Florida.

Cell culture. Cultures of primary bovine embryo kidney (BEK) cells and secondary and tertiary passages of these cells were grown on glass cover slips in tubes or in 96-well microtiter plates (Costar, Cambridge, Mass.). Monolayers of an embryo bovine kidney cell line, kindly supplied by K. Martin of Beecham Laboratories, were grown also on glass cover slips. Growth medium consisted of Eagle minimum essential medium supplemented with 0.25% lactalbumin (Difco Laboratories, Detroit, Mich.), penicillin (100 IU/ml), streptomycin (100 μg/ml), amphotericin B (5 μg/ml; Fungizone, E. R. Squibb & Sons, Princeton, N.J.), and 10% fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.). Before the cultures were infected with virus, they were washed twice with serum-free medium and incubated with the same medium at 37°C.

Virus isolates. Fecal samples and cecal contents were diluted 1:4 in phosphate-buffered saline (PBS; pH 7.2), centrifuged at 6,000 × *g* for 1 h, and diluted in serum-free medium to a final concentration of 1:10 before inoculation of cell cultures. For animal inoculation, the 25% supernatant solution was filtered through 0.45-μm membrane filters. Breda virus 2 was isolated from the feces of a colostrum-deprived calf which originated from the Iowa State University Veterinary College herd and which spontaneously developed diarrhea. This virus was later shown to be contaminated with rotavirus. Astrovirus was isolated from the feces of a diarrheic calf; the sample contained both rotavirus and astrovirus.

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Virus culture and assay. Serial 10-fold dilutions of the fecal or intestinal sample were inoculated onto monolayers and incubated at 37°C for 24 to 72 h. Microtiter plate cultures were fixed at 24 h postinfection with 80% acetone in PBS at −25°C, and the cover slip cultures were fixed at 24 to 72 h with acetone. After fixation for 10 min, the cultures were dried and stored at −25°C or used immediately for IF examination. The number of immunofluorescent cells (ic) was counted in a single field of the ×10 objective lens in each of four wells, and the mean number was calculated. The counts were expressed as the number of ic per field.

Animal experiments. Ten GC of various ages were inoculated orally with 4 ml of virus obtained from fecal samples. A fecal sample was taken immediately before virus inoculation and twice daily thereafter for the duration of each experiment. Rectal temperatures were recorded at the same time each day in the morning and evening; the clinical health and the appetite of the animals were recorded, and the presence of diarrhea was recorded if the feces changed from dark brown with a solid consistency to a greenish yellow or yellow color if they were soft (if stool samples took the shape of the container), or if they had a watery consistency. The animals were anesthetized with pentobarbitone at different times postinfection, and tissues were removed for histopathological examination and IF of cryostat sections.

The ic titer of astrovirus in the preparations used to infect calves GC43, GC44, GC45, and GC49 was $30.5 \pm 4 \times 10^3$ ic/ml; for calves GC37, GC40, and GC46 it was <10 ic/ml, and for calves GC47 and GC52 it was $25 \pm 5 \times 10^2$ ic/ml.

Two uninfected calves (GC38, GC41) served as controls for all the studies. These calves were killed at 6 days of age.

Calf GC21 was inoculated orally with CD15, the original isolate of Breda virus 2. Although rotavirus particles were not observed initially in fecal electron microscopy (EM) preparations, GC21 convalescent antiserum had rotavirus antibodies. Subsequently, after a careful search, rotavirus particles were shown to be present in the feces. To remove the rotavirus from the Breda virus 2 preparation, calf GC32, which was vaccinated at birth with rotavirus and challenged 3 weeks later with a rotavirus for other studies, was inoculated orally with the Breda virus 2 rotavirus preparation 1 week after the rotavirus challenge. This calf excreted Breda virus 2, but not rotavirus, which was confirmed by inoculating calves GC37 and GC46 with the preparation from GC32.

Serology. GC antisera to Breda virus 1 (25), to Breda virus 2 and astrovirus (GC37), to the U.S. isolate of astrovirus (GC43), to the UK isolate of astrovirus (L107), and to rotavirus (GC5) were obtained as 21-day convalescent blood samples after oral inoculation. GC (L107) was carefully studied for the presence of other viruses, but none was detected (21).

(i) IF. Breda virus 2 and astrovirus antigens in gut tissue sections and astrovirus in cell culture were demonstrated by IF by the indirect method previously described (21, 23) or with serum GC43 conjugated with fluorescein isothiocyanate (GC43 conjugate). To differentiate between cells infected with Breda virus 2 and astrovirus, antibody to Breda virus 1 was used (SB219). Replicate sections or cell cultures were tested with the various antisera, SB219, GC5, GC37, GC43, L107, and also with rabbit antibovine immunoglobulin G serum conjugated with fluorescein isothiocyanate. Rabbit antibovine serum alone immunofluoresces with cells seen in the lamina propria, in Peyer's patches, and occasionally as discrete IF of crypt epithelial cells. Few or no cells of the large intestine early in GC life are observed to fluoresce with rabbit antibovine serum. The nonspecific IF exhibited by eosinophilic cells was prevented by pretreatment of the acetone-fixed sections with diaminobenzidine and hydrogen peroxide by the method of Valnes and Brandtzaeg (19).

For a serological survey, bovine serum samples taken from various calves and adult cattle in Iowa were diluted 1:10 and 1:40. Primary BEK cells in 96-well microtiter plates were infected with a GC fecal preparation of astrovirus at a 1:100 dilution and incubated overnight at 37°C. After fixation with cold 80% acetone in PBS (vol/vol) for 10 min, the plates were rehydrated with PBS (2 to 5 min) and then the indirect IF test was performed, with each serum tested in two wells. Results were recorded as positive or negative.

For rotavirus antibody determination, the IF test on cell cultures infected with canine rotavirus was used (5).

(ii) Hemagglutination. The hemagglutination and hemagglutination inhibition tests for identifying the presence of Breda virus antigens in feces have been described previously (25).

EM. Negatively stained preparations of fecal and intestinal samples were examined for the presence of virus particles following methods previously described (22, 25). Three milliliters of a 1:4 dilution of feces in PBS was centrifuged at 80,000 × *g* for 1.5 h. The pellet was reconstituted in PBS and centrifuged at 80,000 × *g* through 40% sucrose and resuspended in PBS.

Histopathology and tissue ultrastructural studies. At necropsy, two 5-cm loops were taken from each of seven small intestine and three large intestine sites. One loop from each site was intraluminally fixed with 3% cold glutaraldehyde in 0.1 M cacodylate buffer, and the other loop was fixed in 10% buffered Formalin. The glutaraldehyde-fixed material was postfixed in 1% buffered osmium tetroxide, dehydrated, and embedded in epoxy resin. Intestine samples from each site were snap-frozen in liquid nitrogen and later stored at −70°C for IF studies. The intestinal sites taken were as follows: duodenum at 15 cm behind the pyloric sphincter; jejunum at 100, 320, 450, and 700 cm posterior to the duodenum site; ileum at 120 and 50 cm before the ileal-cecal junction, and in animals GC44, GC45, GC46, GC49, and GC52, additional sections from 25 and 10 cm before the ileal-cecal junction; cecum, spiral colon at ansa centralis; and descending colon at 20 cm before the anus. The frozen sections were stained with hematoxylin and eosin to correlate histopathology with IF. The Formalin-fixed sections were stained with hematoxylin and eosin, and for ultrastructural studies, thin sections were stained with 2% methanolic uranyl acetate and Reynolds lead citrate. Determination of size of the virus particles was made after calibration of the microscope with tobacco mosaic virus (18 by 300 nm) and by determining the mean size of 20 particles in thin sections.

RESULTS

Cell culture isolation of astrovirus. In attempts to isolate Breda virus 2 in cell culture, primary BEK cells were infected with the cecal contents of GC40. At 24, 48, and 72 h postinfection, the cultures were tested by IF with the Breda virus 2 convalescent calf antiserum (GC37). In the islands of epithelial-type cells, 25 to 50% of the cells were positive. As the IF appearance of the cells was similar to that observed for the UK astrovirus (22), antiserum to this latter virus was used and shown to produce an identical IF reaction with the GC40 cecal contents. This astrovirus was shown by tissue culture isolation and serology to be present in the bovine rotavirus preparation used to infect calves GC32, GC47, and GC52 (Table 1). It was not present in the original isolate of

Breda virus 2 (CD15). Thus, at different times GC32 was excreting astrovirus alone, which was used to infect calves GC43, GC44, GC45, GC49, or Breda virus 2 plus astrovirus, which were used to infect calves GC37, GC40, and GC46.

Cultures infected with astrovirus did not react by IF with antisera to bovine coronavirus, bovine rotavirus, and bovine Breda viruses 1 and 2, which were possible contaminants of the fecal preparations. Antiserum to bovine astrovirus (GC43) did not cross-react with bovine coronavirus, bovine rotavirus, and Breda viruses 1 and 2, and this serum did not contain antibodies to bovine pestivirus (BVD virus) as tested by M. Coria, National Animal Disease Center, Ames, Iowa.

The embryo bovine kidney cell line could not be infected with astrovirus. Secondary and tertiary BEK cells showed a decline in the number of cells infected by astrovirus when compared with primary BEK cells. For all further isolations of astrovirus, primary BEK cells were used. No cytopathic effect was detected in astrovirus-infected cells over a period of 7 to 10 days. Immunofluorescent cells were observed in cells fixed at 24 to 72 h postinfection. On passing to secondary cell cultures tissue culture medium containing infected cells, only occasionally were a few IF-positive cells observed. This failure probably was due to a combination of the relative lack of susceptible cells in secondary BEK cells and the lack of trypsin in the medium, which has been shown to be necessary for subcultivation of human astrovirus (11).

Serological survey. Serum samples from calves and a few cows in Iowa were examined for the presence of IF antibody to astrovirus at 1:10 and 1:40 dilutions, and 72 and 230 were positive.

Animal experiments. The astrovirus-infected calves remained clinically normal, although the feces became yellow in color and slightly soft. This change coincided with excretion of astrovirus. The calves infected with Breda virus 2 together with astrovirus developed severe greenish-yellow watery diarrhea and were depressed and anorexic. The scours persisted for 3 to 5 days or until the animal was killed for postmortem examination. A similar effect was observed in two calves (GC58, GC60) inoculated with Breda virus 2 uncontaminated with astrovirus or rotavirus. The calf infected with rotavirus plus astrovirus (GC52) developed profuse yellow diarrhea and excreted both viruses. In contrast, calf GC47, 3 weeks convalescent from rotavirus, did not develop diarrhea or excrete rotavirus but did excrete astrovirus (Table 1).

The incubation time between astrovirus infection and the first detectable excretion of virus in feces varied considerably, despite the fact that the calves were inoculated with the same preparation of astrovirus or astrovirus with Breda virus 2, respectively (Table 1). This variation was independent of age of the calf and dose of virus inoculated and suggested individual animal variation. The highest titer of astrovirus excreted in the feces varied among calves by as much as 50-fold but was not dependent on the dose or presence or absence of other viruses. Calf GC52 commenced excreting astrovirus at 48 h postinfection and rotavirus at 96 h postinfection.

With the exception of calf GC43, which was maintained for antiserum production, the calves continued fecal excretion of astrovirus, which was infectious for tissue culture cells, until the respective experiment was terminated. Thus, GC47 and GC52 excreted astrovirus in the feces for 1 week postinfection, and the virus was present in the cecal contents taken at autopsy. Calf GC43 excreted astrovirus until 9 days postinfection.

IF of intestinal sections. Astrovirus infection was observed by the indirect and direct methods only in the dome epithelial cells of the ileum over Peyer's patches and in cross-sections of dome crypts involving some or all of the cells (Table 1; Fig. 1A and B). Astrovirus IF was not seen at any other site in the small or large intestine. Where there was fusion of dome epithelium with neighboring villus epithelium near the dome base, two to four cells of the villus also showed astrovirus IF. In those calves infected with astrovirus alone, there were three to six cells of the dome epithelium which were IF positive, whereas in those calves infected with

TABLE 1. Breda virus 2 and astrovirus infection of GC

Calf no.	Virus[a]	Age inoculated (h)	Virus excreted (postinfection)		Peak virus excretion (ic titer/ml)	Age autopsied (h)	Astrovirus IF-positive cells in dome epithelium[b]
			Breda virus	Astro-virus			
GC32	A BR2	336 (14 days)	NA[c]	72	12×10^4	NA	NA
GC43	A	48	NA	72	75×10^3	NA	NA
GC44	A	72	NA	48	60×10^4	120	++
GC45	A	72	NA	24	12×10^5	120	++
GC49	A	0	NA	72	66×10^4	72	++
GC37	A BR2	0	48	96	ND[c]	NA	NA
GC40	A BR2	0	48	48	32×10^5 (cecal contents)	72	+++++
GC46	A BR2	0	48	48	58×10^2	72	++
GC47	A R	528 (22 days)	NA	24	12×10^5	696 (29 days)	+++++
GC52	A R	528 (22 days)	NA	48	$>10^3$	696 (29 days)	+++++

[a] Abbreviations: A, astrovirus; BR2, Breda virus 2; R, rotavirus.
[b] IF score is + to +++++, indicating the proportion of cells IF positive.
[c] NA, Not applicable.
[d] ND, Not done.

Breda virus 2 or rotavirus plus astrovirus, most of the dome epithelial cells were IF positive, and these cells appeared to be necrotic.

The astrovirus-infected cells were either flattened or rounded and extruded from the tip; some were free in the lumen (Fig. 1C). The indirect IF with rabbit antibovine serum had the advantage over the direct conjugate (GC43) in that the lymphoid tissues of the Peyer's patches were more easily identified due to the lymphocytic IF, and thus, the domes could be distinguished from short villi. Both the direct and indirect methods gave IF of cells in the lamina propria. As this effect was removed by pretreatment with diaminobenzidine and hydrogen peroxide, these cells were probably eosinophilic cells reacting nonspecifically with fluorescein (19). After this treatment, the conjugate reacted with only the epithelial cells, and no IF was observed in the lymphoid tissues of the Peyer's patches. Thus, it was concluded that astrovirus infection was restricted to the epithelium of the domes and occasionally the bases of neighboring villi.

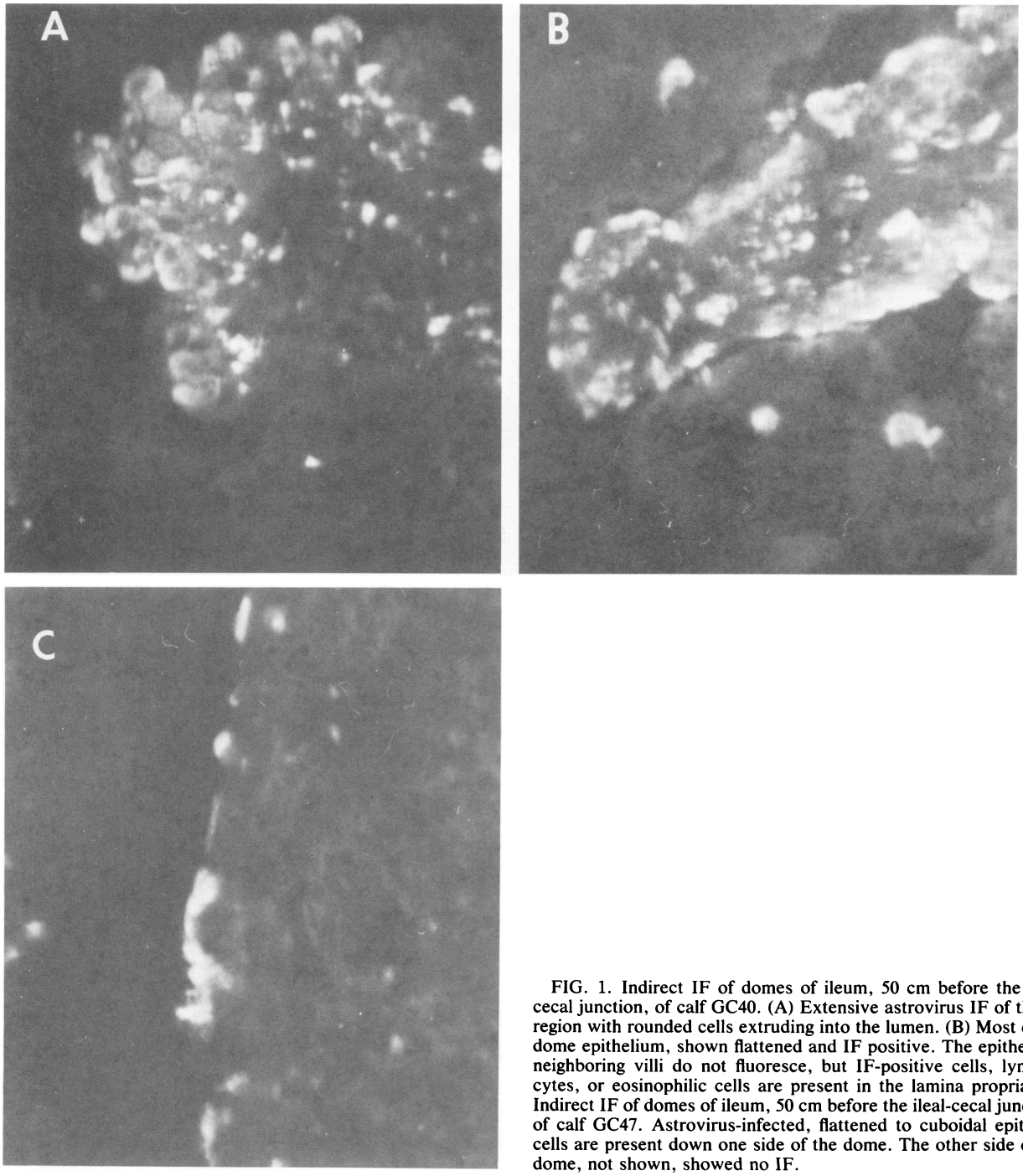

FIG. 1. Indirect IF of domes of ileum, 50 cm before the ileal-cecal junction, of calf GC40. (A) Extensive astrovirus IF of the tip region with rounded cells extruding into the lumen. (B) Most of the dome epithelium, shown flattened and IF positive. The epithelia of neighboring villi do not fluoresce, but IF-positive cells, lymphocytes, or eosinophilic cells are present in the lamina propria. (C) Indirect IF of domes of ileum, 50 cm before the ileal-cecal junction, of calf GC47. Astrovirus-infected, flattened to cuboidal epithelial cells are present down one side of the dome. The other side of the dome, not shown, showed no IF.

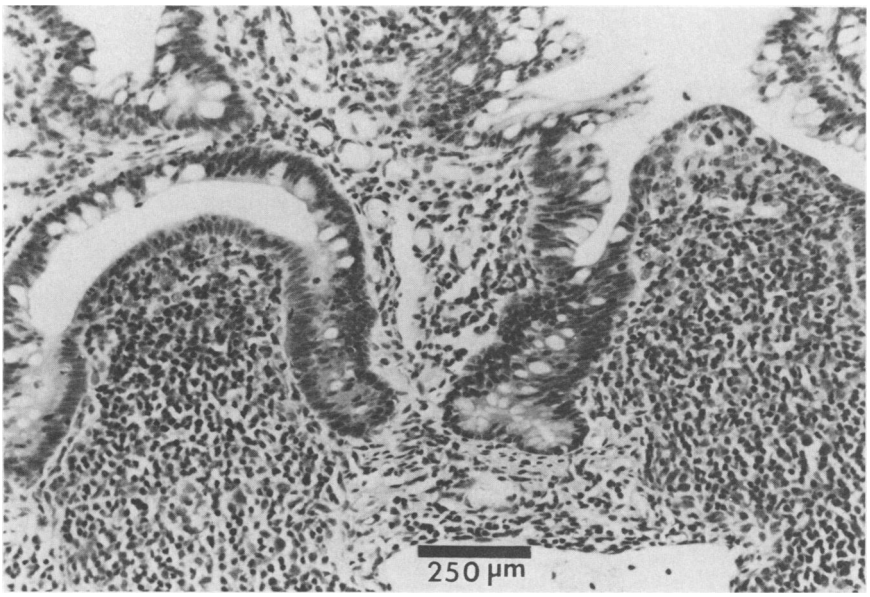

FIG. 2. Section of domes from ileum, 50 cm before the ileal-cecal junction, of calf GC40. Dome on right shows loss of columnar epithelium of tip with focal necrosis and replacement by flat to cuboidal cells. Dome on left shows irregularity of the epithelium and replacement with cuboidal cells. Hematoxylin and eosin stain was used.

Those calves infected with Breda virus 2 had extensive Breda virus IF of the epithelium of the villus and crypt of the mid-jejunum, ileum, and the folds of the large intestine. Two to five cells of the dome epithelium also were IF positive for Breda virus. Rotavirus IF was not observed in calf GC47, probably because autopsy was performed 7 days postinfection.

Histopathology. In GC44 and GC49 (infected with astrovirus alone) and GC47, intestinal lesions were seen only in domes of the ileum. However, in GC45 (also infected with astrovirus only), dome-like structures in the jejunum, as well as ileal domes, had lesions as described below (Fig. 2). In animals GC40, GC47, and GC52, in which extensive IF of cells was seen, most domes were covered by cell debris consisting of sloughing epithelial cells, mononuclear cells, and eosinophilic cells. The eosinophilic cells contained many eosinophilic granules, but it was not possible to distinguish them from globule leukocytes or other granulocytes. The tips of the domes were covered by flat to cuboidal cells which progressively changed to columnar cells at the dome base. In several sections there was bridging between dome cells and cells of adjacent intestinal villi. Many intraepithelial eosinophilic cells and mononuclear cells were present in these domes. In subepithelial layers there were increased numbers of neutrophils and pyknotic nuclei scattered in the lymphoblastic cell population. The Peyer's patches associated with these domes were centrally depleted and contained large cells with dark nuclei and eosinophilic cytoplasm. These macrophage-like cells were less prominent in centers of Peyer's patches in the other animals (GC44, GC45, GC46, GC49). In these animals, in which a few cells with specific fluorescence were scattered throughout the dome epithelium, less than half of the domes were affected, and these showed identical lesions as described above. These lesions were not observed in two 5-day-old, noninfected GC used as controls.

Ultrastructure. EM of thin-sectioned material showed virus particles with a mean diameter of 30 nm (27 to 35 nm) to be present in sections of ileum from GC40, GC47, and GC49. In GC40 both M cells and absorptive enterocyte cells of the dome epithelium contained virus, and in one dome both astrovirus and Breda virus 2 were detected in one M cell (Fig. 3). In GC47 astrovirus was seen in dome M cells only. In contrast, in GC49 astrovirus was seen in one absorptive cell only, but it was not possible to conclude that this cell was of dome origin. Microvilli of infected M cells often were severely stunted, leaving very short stubs on the cell surface. Virions were arranged in two patterns: large masses of virions in an electron-dense matrix clustered around the nucleus and smaller aggregates of virions packed between numerous apical tubules and vesicles. Virions were not observed in thin sections of the ileum from calves GC44 and GC45.

EM by negative staining. Routine EM examination of fecal samples diluted 1:4 did not show the presence of astrovirus. However, when 50 g of feces was diluted 1:4 and pelleted through 40% sucrose, a few clumps of virus with the typical appearance of astrovirus (?2) were observed.

DISCUSSION

The U.S. isolate of bovine astrovirus appears to be morphologically and antigenically related to the UK isolate (22). A second isolate, isolated by us from a calf in the U.S., shares common IF antigens with the UK and U.S. 1 isolate but does not cross-neutralize (unpublished data). It is not clear why the astrovirus particle is difficult to demonstrate by EM when there is such a good tissue culture infectivity. Presumably, astrovirus has a much lower ratio of particle count to infectivity than is required for rotavirus (3). It was interesting to observe that Breda virus 2 also showed a tropism for dome epithelial cells, although it is not known at present whether this can occur in the absence of astrovirus. The dome epithelial cells showed degenerative effects, whether infected with both Breda virus 2 and astrovirus, with astrovirus alone, or with rotavirus and astrovirus. The failure to observe the astrovirus virions in dome cells by ultrastructural studies of calves GC44 and GC45 again may

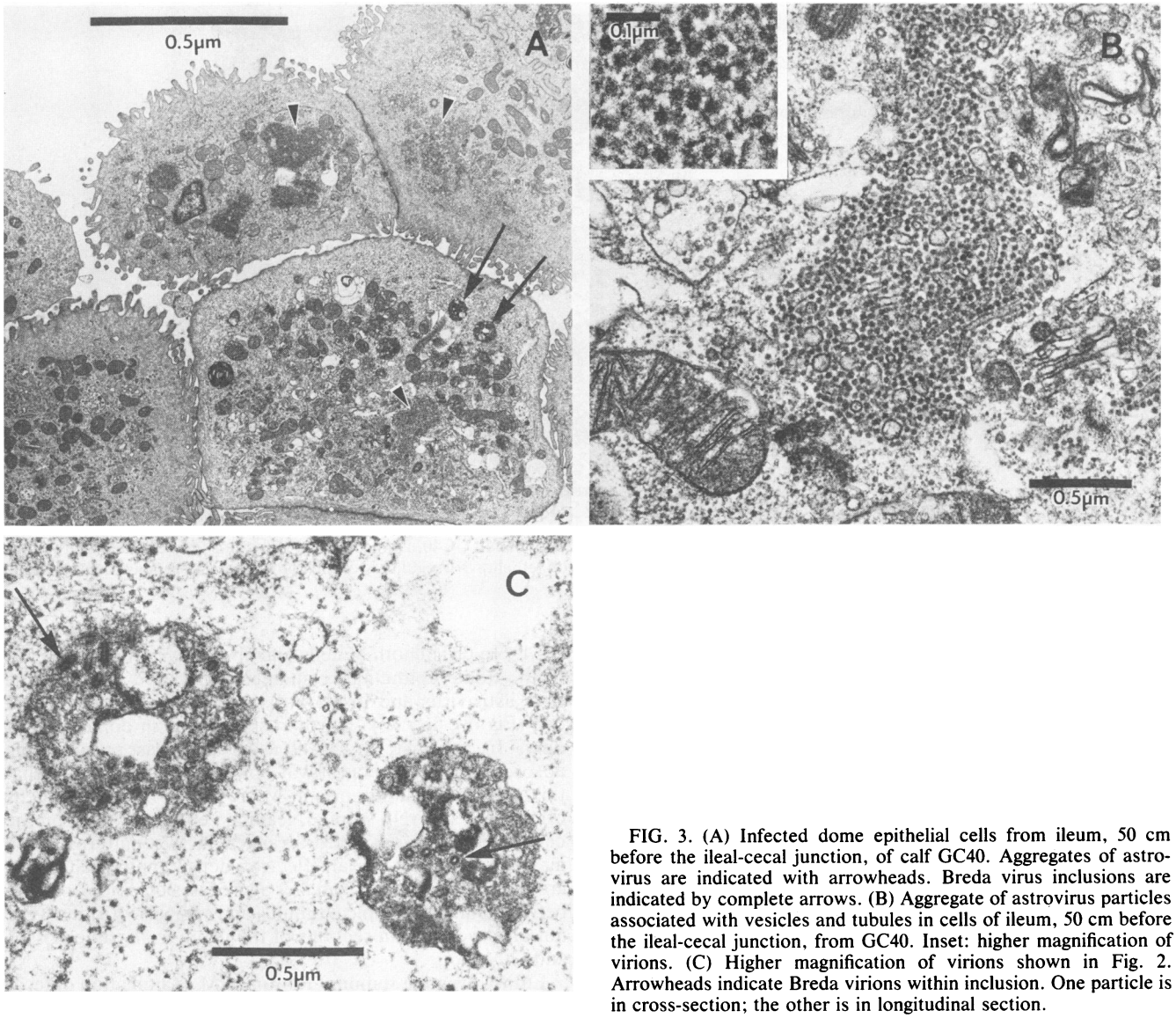

FIG. 3. (A) Infected dome epithelial cells from ileum, 50 cm before the ileal-cecal junction, of calf GC40. Aggregates of astrovirus are indicated with arrowheads. Breda virus inclusions are indicated by complete arrows. (B) Aggregate of astrovirus particles associated with vesicles and tubules in cells of ileum, 50 cm before the ileal-cecal junction, from GC40. Inset: higher magnification of virions. (C) Higher magnification of virions shown in Fig. 2. Arrowheads indicate Breda virions within inclusion. One particle is in cross-section; the other is in longitudinal section.

have been due to sampling error, as IF cells were not observed in all domes and only in a few cells in some domes of these calves.

With the exception of the description of Landsverk in conventional calves (10), dome epithelial cell changes (i.e., flattening of epithelial cells) have not been described previously in calves used to investigate the cause of enteric diseases. These cells appear to be susceptible to astrovirus infection in the very early stages and within the first 3 to 4 weeks of life. Astrovirus particles were found in M cells and in enteroabsorptive-type cells which are located at the dome base or in adjacent villi. In contrast to the findings of Torres-Medina (18), who studied 2-day-old GC, we found both M cells with microfolds and enteroabsorptive-type cells in the dome epithelium of noninfected control calves aged 6 days and infected calves of various ages. Changes in dome cell population with age and diet may explain this discrepancy, but such investigations have not yet been made in the bovine species.

Further studies are planned for studying the significance of the observations in GC40, GC47, and GC52, in which gross infection with degenerative changes were observed in dome epithelia. This effect appears to be due primarily to the astrovirus, as GC40 and GC52 were also infected with Breda virus 2 or rotavirus, respectively, but GC47 was infected only with astrovirus. Most of the cells of calves GC40, GC47, and GC52 immunofluoresced for astrovirus, but a few cells of GC40 were also IF positive for Breda virus 2. The severe rotavirus diarrhea in GC47 2.5 to 3 weeks earlier may have resulted in an increased susceptibility to astrovirus, although rotavirus antigen could not be demonstrated in the epithelial cells of the intestine at autopsy, and no rotavirus excretion was demonstrated after rotavirus challenge. Calf GC46 was inoculated at the same age and with the same inoculum and was autopsied at the same time postinfection as GC40, but GC46 did not show by IF the same extensive infection of the domes. The variation of incubation times was considerable (24 to 72 h) between calves inoculated with similar preparations. These results suggest considerable animal variation in susceptibility to astrovirus, despite the

fact that they were gnotobiotically derived, and create problems for studying the pathogenesis and development of infection by sequential killing. Thus, the different results in the calves may have been the consequence of performing autopsies at different stages of infection of each animal.

The pathogenesis of this isolate of bovine astrovirus differs from that of the UK isolate of lamb astrovirus (16), in which there was infection of all regions of the small intestine and partial villus atrophy of the jejunum and ileum. No mention was made of infection in dome epithelia. However, the bovine and lamb astroviruses are not antigenically related by IF and may well differ in characteristics other than their superficial similarity, as judged by negatively stained particle morphology. The U.S. and UK isolates of bovine astrovirus may have similar sites of replication, however, as neither isolate caused diarrhea, and the UK astrovirus was judged to be of low virulence; consequently, no autopsies on infected calves were performed.

It is reasonable to assume that the viral tropism for domes with degenerative effects on the epithelium of these important structures could have a significant effect on the gut immune function (18). However, rotavirus- and astrovirus-infected calves produced a good rotavirus serological response which was similar to that produced by astrovirus-negative, rotavirus-infected calves. The results in calves GC9, GC15, and GC17, which were infected with rotavirus and astrovirus, have been published previously (23). Similarly, the calves produced a good serological response to astrovirus. These responses may be related to the respective antigens that reach the local lymph nodes and even the spleen, an event which may be enhanced by M-cell damage in the dome epithelium. The persistence of the astrovirus in dome epithelia of GC47 and GC52 7 days after infection is interesting when compared with studies by one of us (G. N. Woode), who observed a 3-week persistent excretion of the UK isolate of astrovirus by a GC. This may reflect damage to the local immune system, preventing control of the virus in the gut.

In a study of 12 2- to 5-week-old diarrheic calves, Landsverk (10) has reported that the dome epithelia were atrophic and that there was a morphometric reduction of lymphoid follicle length. A number of organisms seem to have a predilection for Peyer's patches, including chlamydia in calves (4), typhoid fever in chimpanzees (17), salmonella enterocolitis in rats (13), salmonella infections in mice (7), and *Escherichia coli* infections in rabbits (8); and reovirus has been shown to have a predilection for dome epithelium (21).

In conclusion, it may be said that the dome epithelium has a special function, probably for rapid presentation of intestinal antigens to be underlying lymphoid tissue, and that a variety of organisms can locate at this site and possibly cause damage. Landsverk (10) has reported on the role of rotavirus but not astrovirus in his study of diarrheic calves with dome cell atrophy, but it is possible that astrovirus may have been involved, as the virus is not readily detected. From the studies in both the UK (22) and United States, infection with this virus is probably common. Of three U.S. isolates of bovine rotavirus studied by this laboratory to date, two of them were found to be contaminated by astrovirus. As this isolate of astrovirus appears to be strictly restricted to the dome epithelium and its crypt cells, it may prove to be a powerful tool for studying the function of bovine dome cells. Whether the astrovirus reduces or enhances the function of Peyer's patches is not known, but it is reasonable to conclude that loss of normal function will result in, at least temporarily, a reduced local immune response. Studies on mixed infection with astrovirus and other viruses and cryptosporidia are planned.

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