Bovine Venereal Vibriosis: Antigenic Variation of the Bacterium During Infection

L. B. CORBEIL, G. G. D. SCHURIG, P. J. BIER, AND A. J. WINTER*

New York State Veterinary College, Cornell University, Ithaca, New York, 14850

Host parasite relationships in the female genital tract were studied in bovine venereal vibriosis by investigating agglutinin production and alterations in superficial antigens of the bacterium during the course of infection in two heifers. Cervicovaginal mucus (CVM) steamed cell agglutinins were shown to appear earlier and remain at consistently higher levels than whole-cell agglutinins. Whole-cell agglutinin titers fluctuated much more than steamed cell titers, suggesting possible changes in whole-cell antigens. Marked antigenic variation was demonstrated in successive monthly CVM isolates from the two heifers by agglutination tests with rabbit antisera of various specificities. Some changes in CVM antibody specificity during the infection were noted also. Antigenic variation in the bacterium was proposed as a mechanism for maintenance of the asymptomatic cervicovaginal carrier state in the presence of antibody.

Bovine vibriosis is a naturally occurring venereal disease caused by *Campylobacter* (*Vibrio*) *fetus venerealis* (19). It is characterized by transient infertility and mild endometritis which is often followed by persistent cervicovaginal infection in the presence of antibody (18). The latter feature presents a problem in control since asymptomatic animals may transmit the infection at coitus. In this respect it is similar to the asymptomatic carrier state of gonorrhea (13).

How the etiological agents of these two venereal diseases maintain themselves in the presence of host immune responses is not known. However, it has been suggested that microbial antigenic variation may be responsible for survival of pathogenic organisms in the face of host defenses (1). Such antigenic variation is known to occur in some viral and protozoal diseases (3, 8) but has not been well studied in bacterial infections.

The present investigation was undertaken because *C. fetus* does persist on a mucous surface in the presence of antibody and an in vivo transition in serotype had been observed in an earlier study of isolates from persistent genital infections with an intestinal strain (16). On this basis, it was decided to determine the antigenic makeup of cervicovaginal mucus (CVM) isolates taken at intervals throughout infection with a venereal strain of *C. fetus* and to compare any antigenic changes with changes in CVM antibody specificity.

MATERIALS AND METHODS

Treatment of animals. Two-year-old virgin Holstein heifers were infected cervicovaginally at estrus with 1.0 ml of suspension containing 10^6 to 2×10^6 *C. fetus venerealis* organisms (0.5 ml deposited in the caudal cervix and 0.5 ml in the cranial vagina). A cloned population of cells (1016-43b3) was used for infection as described in the companion paper (17). Samples of CVM were collected weekly by aspiration into uterine infusion pipettes and divided for bacterial culture procedures (21) and antibody extraction (16). The total amount of CVM remaining after culture was extracted in 5 ml of saline rather than on a weight-volume basis, because weight changes in CVM are due largely to changes in hydration at various stages of the estrus cycle and may not reflect changes in amount of antibody protein.

Cultured mucus was incubated for 3 days on blood plates with antibiotics in an atmosphere of 10% CO_2, 2.5% O_2, and 87.5% N (21). Then, sweeps of *C. fetus* colonies were streaked heavily on fresh plates, harvested in sterilized skimmed milk after 3 days of incubation, and frozen at −70 C for subsequent antigen production.

Agglutination tests. Bacterial cells were recovered in formolized saline after 3 days of incubation on blood plates and adjusted to an optical density at 525 nm of 0.6. Cells were not washed. Equal volumes of cell suspensions and doubling dilutions of serum or CVM extracts were incubated overnight at 50 C. The titer was taken as the reciprocal of the last dilution which gave 50% clearing. In initial tests, antigens consisted of whole (W) or steamed (O) cells (16) of an uncloned population of *C. fetus venerealis* strain 1016-43b. Antigenic variation was studied by testing whole cells of the cloned infecting strain (1016-43b3)

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and of monthly isolates against various rabbit antisera. The sera against heat-labile antigens 1 to 7 (2) and postgrowth broth antigens (12) were provided by R. Berg (Veterinary Research Laboratory, Montana State University, Bozeman, Mont.). Serum pool 331-332 was obtained from two rabbits immunized with whole 1016-43b cells and pool 333-334 (O antiserum) from two rabbits immunized with steamed 1016-43b cells. Serum 321 was produced with an immunoprecipitate developed with a heat-labile antigen removed from *C. fetus* cells by extraction with pH 2.2 glycine-hydrochloride buffer. Removal of this substance permits agglutination of whole cells in O antiserum, so serum 321 was taken to be directed against a superficial somatic antigen (antigen [a]), possibly the microcapsule.

W antigens from monthly isolates and the infecting strain were also tested against selected CVM extracts from the two heifers to determine whether changes in antibody specificity followed antigenic changes.

RESULTS

CVM from heifer 55 was culturally positive for the first 4 months of the experiment. No further isolations of *C. fetus* were made from CVM or from the whole tract at necropsy. In contrast, CVM from heifer 20 was culturally positive throughout the 10-month experimental period with the exception of 2 consecutive weeks during the 6th month.

Agglutinin titers of CVM from both heifers against the uncloned strain 1016-43b varied from week to week. O agglutinins appeared first and remained relatively higher than W agglutinins throughout both infections. O titers persisted for 3 months after termination of infection in heifer 55 (Fig. 1). W titers appeared several weeks later and several antibody peaks, followed by drops to very low levels, were noted in each animal (Fig. 1).

Antigenic analysis of CVM isolates from heifers 20 and 55 (Table 1) revealed many changes in superficial antigens. Reactions to antisera specific for factors 2, 3, 4, 5, and 6, as well as antigen [a] (antiserum 321), developed or disappeared with great differences in titer. Reactions to antisera directed against mixtures of superficial antigens (postgrowth broth; 331–332) revealed 10- to 100-fold fluctuations in titer, implying substantial changes in quantities of these antigens on the cell surface. Isolates periodically became slightly agglutinable with O antiserum, further indicating losses of superficial components. These results were reproducible (with each of six isolates) with second lots of antigen produced from the original frozen isolates.

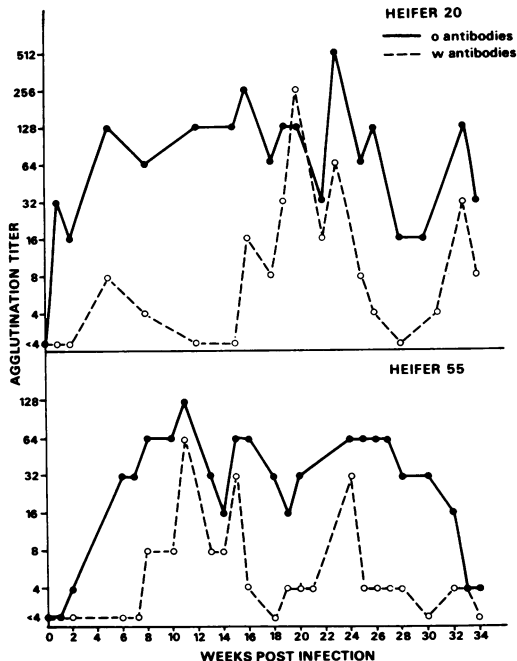Since it was hypothesized that antigenic variation in vivo would bring about later

FIG. 1. *Agglutination titers of CVM antibody extracts tested against strain 1016-43b O and W antigens (O, steamed C. fetus cells; W, whole C. fetus cells). Heifer 20 CVM was culturally positive throughout the experimental period except for weeks 26 and 27. Heifer 55 CVM was culturally positive until week 18 and negative thereafter.*

changes in specificity of mucosal antibodies, the CVM isolates were tested against CVM antibody extracts from selected times throughout infection. Agglutination titers for heifer 55 CVM isolates and extracts are recorded in Fig. 2. No overall pattern emerged. W agglutinins did not appear for several weeks, but when they were detected they reacted best with the infecting strain. Isolates obtained late in the infection (e.g., days 98 and 120) did not react well with CVM extracts taken at the time of isolation, but reactivity increased with later extracts.

DISCUSSION

Antigenic variation is known to be an adaptive feature of several agents pathogenic for man and animals. Immunological drift has been associated classically with influenza virus and appears to be an evolutionary mechanism for survival in the host population. Fenner et al. (5) suggest that any viral infection of mucosal surfaces in vertebrates probably has such an evolutionary mechanism since presence of small amounts of local antibody on the secretory surface of susceptible cells would provide an

TABLE 1. *Agglutination titers of rabbit antisera tested against C. fetus strains isolated from CVM of two heifers at successive stages of infection*

Specificity of rabbit antisera[a]	Source of whole cell antigens														
	I[b]	Isolates from heifer 20								I[b]	Isolates from heifer 55				
		28[c]	60	99	127	155	169	197	219		21	35	67	98	120
[a]	—	—	—	—	—	—	—	—	—	—	640	640	160	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	40[d]	—	—	—	—	—	—	—	—	40	—	40	—	—	—
3	—	—	320	—	640	—	—	—	1,280	—	—	—	80	80	—
4	320	—	—	—	40	2,560	80	1,280	40	320	—	—	—	1,280	2,560
5	—	—	—	—	—	—	—	—	—	—	320	320	160	—	—
6	—	—	—	1,280	40	40	—	—	—	—	640	640	1,280	—	40
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PGB	320	80	—	640	2,560	80	2,560	160	1,280	320	80	40	—	2,560	2,560
W	2,560	1,280	5,120	1,280	640	640	320	2,560	640	2,560	640	640	2,560	320	640
O	—	—	40	40	40	—	40	40	—	—	—	40	40	40	—

[a] Antigens [a], 1, 2, 3, 4, 5, 6, and 7 are specific heat-labile surface antigens of *C. fetus*. Mixed antigens include postgrowth broth (PGB), 1016-43b whole cell antigens (W), and O antigens (O).

[b] I, Infecting strain 1016-43b3.

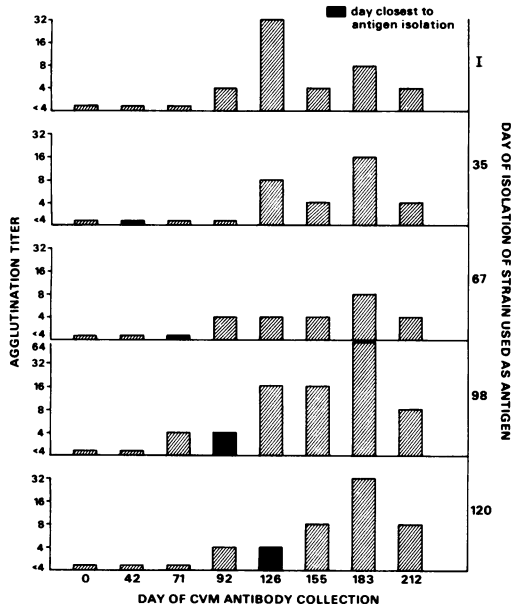[c] Day of isolation.

[d] Lowest dilution tested was 1/40.

FIG. 2. *Agglutination titers of heifer 55 CVM antibody extracts tested against heifer 55 CVM isolates and infecting strain (I).*

excellent environment for selecting among antigenic variants. It was proposed (5) that such variants could bypass antibody directed against the infecting strain and multiply sufficiently to be transmissible to a fully susceptible host.

There are also several chronic systemic infections where the infecting agent is known to evade the individual host's immune response by

successive antigenic transitions. For example, in the protozoan blood-borne infections of malaria (3) and trypanosomiasis (7), periodic fluctuations of parasitemia (with associated remissions and exacerbations of symptoms) have been shown to parallel the proliferation of new serotypes which are not susceptible to host antibodies. However, production of antibody specific for the variant begins within a few days and initiates another cycle. Recently, such antigenic variation was reported to be responsible for the persistent viremia and recurrent fever characteristic of equine infectious anemia (8).

Evidence is scarce that antigenic transitions in vivo are operative in persistent bacterial infections, even though antigenic polymorphism in pathogens such as *Escherichia coli*, *Diplococcus pneumoniae*, and *Salmonella* is well known (1). Changes in antigens of *Vibrio cholera* strains isolated during infection of gnotobiotic mice have been documented (10, 15). The earlier report from this laboratory of antigenic transition in an intestinal strain of *C. fetus* during a prolonged genital infection (16) and the present observations of a series of antigenic changes during infection with a venereal strain indicate that such adaptive mechanisms do occur in this infection. Many changes in surface antigens during infection were revealed by the data in Table 1. Drops in agglutination titers with standard rabbit antisera indicated that antigens had been lost or were no longer exposed on the cell surface. Increases in titer were seen also and suggested that altered populations of organisms with new surface anti-

gens, or with preexisting antigens displayed on the surface in differing proportions, had proliferated as the original population was being selected against by the immune response. The prolonged presence of the organism in the caudal reproductive tract in the presence of mucosal antibody may be explained by antigenic variations of this kind. A similar mechanism could account for the troublesome carrier state in gonorrhea.

Mechanisms which cause the emergence of new variants are unknown but the immune response is undoubtedly a factor. Selection of organisms resistant to antibody from a population of varying susceptibilities is a possibility. Antiserum has been shown to suppress development of selected antigens with trypanosomes (6), viruses (9), and bacteria (4, 10, 11, 14). In the present study, however, a cloned population of organisms was used for infection so that a simple selection from variants existing in the inoculum does not appear likely. The fact that clones of trypanosomes produce many antigenic variants (6) supports this conclusion. Other possible mechanisms responsible for antigenic polymorphism in bacteria include highly mutable genes, phase changes, episomal control (1), or other intracellular control systems. The serologic reactions of uncloned strains of C. fetus remain stable through many in vitro passages, although a low proportion of antigenic variants have been noted among individual colonies of a single strain (E. McCoy, personal communication). Therefore, we hypothesize that organisms change by one or more of the above mechanisms and that population changes occur in vivo because of selective pressures due to the immune response.

In malaria (3) and equine infectious anemia (8), serum taken at the time of isolation of the agents did not react well with the isolate but subsequent serum samples gave much higher titers. In vibriosis, a similar pattern was observed with some CVM isolates and antibody extracts (Fig. 2). These observations may account for the ability of new variants to evade the host's immune response. The less consistent pattern in vibriosis may be due to the fact that each C. fetus isolate had several superficial antigens. Since it is not known which antigens are important virulence factors nor which immune responses are most effective in protection, the agglutination titers may not reflect completely host-parasite interactions in selection of variants. However, the agglutination data demonstrate clearly that antigenic changes in C. fetus do occur during infection. Such antigenic changes must be taken into account in produc-

tion of vaccines since variants may survive in the presence of antibodies to vaccine antigens as has been shown by Schurig et al. (17). This variation should be considered in choosing antigens for diagnostic CVM agglutination tests also because W antibodies present in CVM may be missed if inappropriate W antigens are used. The simplest solution to the last problem is the use of O antigens or strains agglutinable by O antiserum such as UM (20) in diagnostic tests.

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