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Immune Response in the Bovine Mammary Gland After Intestinal, Local, and Systemic Immunization

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The immune response in mammary glands of cattle was measured after intestinal, local, and systemic immunization with T4 bacteriophage. Nonlactating pregnant cows were immunized by infusions into the intestine or mammary gland and by subcutaneous injections in the region of the prescapular or external inguinal lymph nodes. Titers of antibodies of different isotypes were measured in serum and in lacteal secretions by enzyme-linked immunosorbent assay, and numbers of cells producing antibodies of each isotype were determined in lacteal secretions by the Jerne plaque assay. Substantial increases in immunoglobulin G subclass 1 (IgG1) and IgG2 antibody titers were detected in serum and lacteal secretions of animals immunized through an intestinal fistula. IgM and IgA antibody responses were low or undetectable. Low numbers of IgA and IgG1 plaque-forming cells were occasionally detected. It is proposed on the basis of these data that migration of antigen-stimulated IgG lymphoblasts, and perhaps of antigen, to spleen and peripheral lymph nodes may be dominant events after intestinal immunization of ruminants. This is consistent with the predominance of serum-derived IgG antibodies in colostrum and milk. Intramammary infusion of antigen gave rise to increases in antibody titers in all classes which were greater not only in lacteal secretions but also in blood serum than with either systemic route used. There was clear evidence from relative antibody titers for local synthesis of antibodies, principally IgA and IgG1, in the immunized glands. Comparison of IgA titers in secretions from the immunized glands with those in serum also suggested that locally synthesized IgA antibodies may have contributed in some measure to serum titers. Local synthesis in both immunized and nonimmunized glands was also reflected by the presence of increased numbers of IgA and IgG1 plaque-forming cells. It is hypothesized that antibody-forming cells responsible for local synthesis originated in lymphoid tissue within the mammary gland or from peripheral lymph nodes, depending upon the route of immunization.

During the past decade important advances have been made in understanding migration patterns of B lymphoblasts within the secretory immune system. Antibody-forming precursor cells from Peyer's patches give rise in rabbits to plasma cells synthesizing immunoglobulin A (IgA) in the lamina propria of the intestine (7, 8, 34, 35) as well as in the bronchi (34). A reciprocal migration was observed by cells from bronchus-associated lymphoid tissue (34). In several species, including humans, rats, rabbits, and swine, oral immunization during pregnancy has been shown to result in the presence of antibodies, predominantly IgA, in colostrum and milk (3, 13, 24, 25, 36). Oral immunization of women with *Escherichia coli* resulted in a marked increase of cells forming O antibodies of the IgA class in preparturient lacteal secretions and in colostrum (13). Cell transfer experiments in mice have demonstrated the migration of lymphoblasts from mesenteric lymph nodes into the intestine and mammary gland (20, 33, 42), as well as to other secretory organs and to mesenteric and peripheral lymph nodes (20, 42). Most cells migrating into secretory organs and to mesenteric lymph nodes synthesized IgA, but most of those migrating to peripheral lymph nodes produced IgG or IgM (20, 42).

Comparable information is not available for cattle, and certain distinctive functional aspects of the secretory immune system in ruminants prompted us to examine whether the relationships which have been established in other species apply in the same fashion to cattle. In cattle the lack of transplacental transfer of IgG is compensated by a high concentration of IgG1, selectively transferred from the bloodstream, in colostrum (4, 16). There are also reports that in

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cattle IgG1 rather than IgA is the principal isotype synthesized in the lamina propria of the small intestine (9, 19, 27), although contrary findings have been reported (2, 5, 32; J. P. Vaerman, thesis, Catholic University of Louvain, Sintal-Louvain, Belgium, 1970). The main objective of this study was to determine whether the phenomenon of B-cell migration from intestine to mammary gland found in other species also occurs in cattle. Additional experiments were performed to contrast intestinal with local and parenteral immunization on the humoral immune response in the bovine mammary gland.

MATERIALS AND METHODS

Antigen preparation. *E. coli* B (ATCC 11303) was grown at 37°C in a shaking incubator for 4 h in medium containing 8 g of nutrient broth (Difco Laboratories, Detroit, Mich.) and 5 g of NaCl per liter. A 60-ml portion of T4 phage (ATCC 11303-B11) at a concentration of 2×10^{10} plaque-forming units (PFU) per ml was then added, and the mixture was incubated for an additional 8 h. Whole *E. coli* and lysed cell residue were removed by centrifugation at 9,000 × *g* at 4°C for 15 min, and the supernatant was filtered through a membrane filter (0.45-μm pore size; Millipore Corp., Bedford, Mass.). The concentration of PFU was measured in the filtrate (1), which was then concentrated by ultrafiltration (Amicon PM10 membrane) under pressure (20 lb of N_2 per in^2). The phage was pelleted from the concentrated filtrate by centrifugation at 27,000 × *g* and 4°C for 1 h and resuspended in saline at a concentration of 10^{12} PFU/ml. One preparation was subjected to ultrasonic disruption in an ice bath for 30 min (Sonic Dismembrator, Artrex Systems Corp., Farmingdale, N.Y.) and dialyzed against saline. All preparations were stored at −20°C.

Animal vaccination. Nonlactating Holstein cows were used. Immunizations were initiated at 7 months of pregnancy, unless stated otherwise.

(i) Intestinal immunization. A one-stage fistula of the jejunum was prepared by placing a 1-in. (2.54-cm) metal cannula (Precision Instrument Co., Lincoln, Neb.) through a stab incision in the flank. A custom-made, tightly fitting Plexiglas cylinder with two partially recessed O rings was inserted into the cannula. The phage suspension was drawn into a 50-ml plastic syringe and injected into the intestine through a 14-gauge, blunt-tipped needle inserted through a hole bored in the Plexiglas. The needle was just long enough to penetrate a tight rubber seal on the inside of the Plexiglas fitting. Instillation of phage suspension was made by this procedure with no loss of fluid.

Immunizations were initiated at about 8 (cows 45 and 67), 6 (cow 33), and 3 (cow 1) weeks before parturition, at which time recovery from surgery was complete. Phage suspensions (10^{13} PFU in 60 ml of saline) were administered daily for 3 weeks. Cow 33 received a second 7-day course of immunization 1 week after the first.

(ii) Intramammary immunization. The right front quarter of each of two cows (1838 and 2353) was infused with a mixture of 2 ml of phage suspension in saline (10^{12} PFU/ml) and 2 ml of saline containing 25,000 U of penicillin four times at 3- to 5-day intervals.

(iii) Prescapular lymph node immunization. Three cows (1789, 2309, and 2326) were given four injections of 2 ml of phage suspension in saline (10^{12} PFU/ml) subcutaneously near the right prescapular lymph node at 3- to 4-day intervals.

(iv) External inguinal lymph node immunization. Three cows were given four injections of 2 ml of phage suspension in saline (10^{12} PFU/ml) subcutaneously close to the right external inguinal lymph node at intervals of 3 to 4 days.

As controls, four cows were sampled over the same span of time as those which had been immunized.

Sampling and sample processing. Samples of blood and lacteal secretions were taken at 4- to 8-day intervals on two occasions just before immunization and throughout the period of the experiment, until 2 to 5 weeks after parturition. A sample of colostrum was taken before the calf was allowed to nurse. The first day of preimmunization sampling has been designated day 0. Equal volumes of secretion from each quarter taken on the same day were pooled for subsequent analysis of antibodies and antibody-forming cells except in animals immunized by intramammary infusion, in which, once immunization had begun, only left front and rear quarter samples were pooled; the right front and right rear quarter samples were kept separate.

Blood samples were taken from either the jugular vein or the tail vein. Sera were separated from the clots by centrifugation and frozen at −70°C until used.

Mammary secretions were obtained by hand milking and kept on ice. A portion of the secretion was assayed within 2 h for antibody-forming cells. The remainder was clarified by centrifugation at 45,000 × *g* for 1 h, stored at −70°C, and thawed immediately before assay for antibody content.

Antiglobulin preparation in guinea pigs. Antisera monospecific for bovine IgG1, IgG2, IgM, and IgA were prepared by the methods of Duncan et al. (11) and Fey et al. (12). Monospecificity was based on the development of a single precipitin line after 2 days at 23°C in a microimmunodiffusion assay, using templates (26). Agar contained 3% polyethylene glycol (molecular weight, 6,000) to enhance detection of limiting quantities of antibodies (14).

Jerne plaque assay. Cells in lacteal secretions were separated and washed three times with Hanks balanced salt solution by centrifugation at 4°C for 10 min at 300 × *g*. Dilutions were made in the same medium, and total cell counts were determined in a hemacytometer. Sonicated phage antigen (from a suspension containing 1.3×10^{11} PFU/ml) was conjugated onto sheep erythrocytes with chromium chloride (39). A 0.1-ml quantity of a 3% suspension of conjugated sheep erythrocytes and 0.1 ml of lacteal cell suspension containing 2×10^5 cells were added to 1 ml of 0.8% Sea-Plaque agarose (Marine Colloids Div., FMC Corp.) in Hanks balanced salt solution at 37°C, mixed, poured over a 2% agar overlay in a petri dish (50 by 9 mm; 1006 petri dish, Falcon Plastics, Oxnard, Calif.), and incubated at 37°C for 2 h. Monospecific antisera

(1 ml of a 1:20 dilution in Hanks balanced salt solution) were then added for detection of IgG and IgA plaque-forming cells (PFC). The plates were incubated for 1 h at 37°C and washed with Hanks balanced salt solution, and 1 ml of a 1:10 dilution of normal guinea pig serum in Hanks balanced salt solution was added as a source of complement. Plaques were examined through an indirect light source after an additional 1 h of incubation. Duplicate assays were performed on unconjugated sheep erythrocytes, and results were corrected to discount PFC specific for sheep erythrocytes.

Enzyme-linked immunosorbent assay. The enzyme-linked immunosorbent assay was done essentially according to procedures formulated by K. W. Walls (40). The antigen was a sonicated phage preparation obtained from a suspension containing 2×10^{11} PFU of phage per ml. Titrations were performed on serial twofold dilutions of serum or secretions in microtiter plates (1-223-29 Cooke microtiter system, Dynatech Laboratories, Alexandria, Va.). Intermediate layers of monospecific guinea pig anti-bovine sera (1:1,280 dilutions) were followed by a 1:1,000 dilution of peroxidase-conjugated rabbit anti-guinea pig antiserum (Cappel Laboratories, Inc., Cochranville, Pa.). The substrate used was 2,2'-azino-di(3-ethyl benzthiazoline sulfonic acid). Results were read at 490 nm in an enzyme-linked immunosorbent assay reader (Dynatech Laboratories), and the dilution having an absorbance closest to 0.5 was taken as the titer.

Experimental design. (i) Selection of antigen. T4 bacteriophage was chosen as a strong antigen for which sensitive assay systems were available and with which cattle probably had minimal contact. Preimmunization titers and low levels of PFC in unimmunized glands (see Table 2) were found consistently, however, suggesting that cattle may be exposed naturally to this phage. Experimental immunizations probably induced secondary responses which could account for the relatively diminished formation of IgM antibodies.

(ii) Rationale of immunization procedures. Intestinal immunization was performed directly into the jejunum because the forestomachs of ruminants preclude reliable intragastric introduction of antigen. Delivery of the antigen into the jejunum ensured effective contact with Peyer's patches, which are relatively abundant in cattle (37). Studies in mice have indicated that homing of B lymphoblasts into the mammary gland occurs only during the latest stages of pregnancy (33). For this reason immunization schedules in cows 33 and 1 were modified to provide exposure to antigen within a week of, or until the time of, parturition.

Systemic administration of antigen to cattle for prevention of mastitis has been performed by various routes, including at the external inguinal lymph node (29), the principal node draining the mammary gland. We wished to make a critical comparison, not heretofore available, of immunization at the external inguinal node with that at another node.

(iii) Rationale for pooling of lacteal secretions. In animals immunized in one mammary gland, we wished to analyze the response in the immunized gland separately and to compare responses in unimmunized glands on the same and opposite sides of those immunized. If direct penetration of antigen into unimmunized glands occurred at all, it would probably have been restricted to the homolateral gland due to the very strict anatomical separation between contralateral glands (37).

(iv) Tabulation of data. An outstanding characteristic of precolostral secretions is the extremely high concentration of immunoglobulins, principally IgG1 (38). Within 4 days before parturition, there is a voluminous increase of fluids in the mammary gland (38) and a concurrent reduction in concentrations of immunoglobulins or specific antibodies (Fig. 1 through 3) in colostrum (a term we reserve for the first secretion after parturition) and in milk. Data in Table 1 were therefore tabulated separately for precolostral secretions and for colostrum. Values for milk, which would have been ≤ 0, were not included.

In Table 1 antibody titers are expressed in log 2 increments. An increase in antibody titer of less than fourfold in serum and eightfold in secretions was considered inconsequential. Therefore, a serum titer 2 logs above the preimmunization level was assigned a value of 1 unit; a titer 3 logs higher, a value of 2 units; etc. In lacteal secretions a titer 3 logs above the preimmunization level was assigned a unit value of 1. Negligible values for the control group (Table 1) support this interpretation.

For each animal, units of antibody levels in serum and lacteal secretions were calculated on a weekly basis. In weeks that two samples were taken the values were averaged. Weekly units were then averaged between the time of the first immunization and parturition, providing an overall mean weekly unit value. In this manner quantitative comparisons in antibody responses in precolostral secretions and in serum were possible among animals. Values for antibody levels in colostrum are expressed in the same fashion, except that only one sample per animal was tested.

In Table 2 absolute numbers of PFC are tabulated. Average weekly levels of PFC in preparturient secretions and in milk were calculated in the manner described for antibody levels.

RESULTS

Titers of antibodies and numbers of PFC are presented for representative animals (Fig. 1 through 3). PFC are shown only for cells producing IgA and IgG1 antibodies, because those of IgM and IgG2 isotypes were almost always negligibly low (Table 2).

Intestinal immunization. Titers of IgG1 and IgG2 antibodies in serum and lacteal secretions increased in all animals 1 to 2 weeks after immunization was initiated and peaked or plateaued 2 to 5 weeks later (Fig. 1). With two exceptions serum IgG2 antibody titers persisted at relatively high levels throughout the experiment and IgG1 titers tended to decline. This is reflected in slightly higher unit values for serum IgG2 antibodies (Table 1). Substantial increases in IgG1 and IgG2 antibody levels in lacteal secretions occurred in all cows except one, immunized closest to parturition, and accounts for the broad range of values in Table 1.

Table 1. Average weekly increases in antibody levels in preparturient and colostral secretions and in blood serum of cows immunized with T4 phage by different routes. Antibody levels were measured by ELISA and values are expressed as increases in titers converted to log 2 (see Materials and Methods).

Immunization	No. of Animals in Group	Source of Antibodies	Mean Weekly Increase of Antibodies in Different Immunoglobulin Classes			
			IgG_1	IgG_2	IgA	IgM
Intestinal	4	Preparturient secretions	2.0(1.0-2.8)[a,b]	1.4(0.0-2.3)	0.1(0.0-0.3)	0.2(0.0-0.5)
		Colostrum	2.5(0.0-4.0)[b,c]	2.0(0.0-3.0)	0.3(0.0-1.0)	0.3(0.0-1.0)
		Blood serum	1.5(1.2-2.0)[a,d]	2.2(1.6-2.9)	0.2(0.0-0.6)	0.0(0.0-0.2)
Intramammary	2	Immunized gland: Preparturient secretions	9.3(8.3-10.3)	4.7(3.1-6.3)	6.3(4.4-8.1)	3.1(0.9-5.3)
		Colostrum	8.0(8.0-8.0)	2.5(1.0-4.0)	6.0(5.0-7.0)	1.5(0.0-3.0)
		Unimmunized glands[e]: Preparturient secretions	7.3(6.9-7.8)	4.2(3.6-4.8)	2.8(1.4-4.1)	3.0(1.9-4.0)
		Colostrum	5.0(4.0-6.0)	3.0(2.0-4.0)	1.0(0.0-2.0)	1.5(0.0-3.0)
		Blood serum	5.1(4.3-5.9)	3.9(3.4-4.4)	3.4(2.8-4.0)	2.2(1.8-2.6)
Systemic: Prescapular Lymph Node	3	Preparturient secretions	3.9(3.7-4.3)	2.3(1.8-2.7)	0.4(0.0-0.8)	1.3(1.2-1.5)
		Colostrum	4.0(3.0-6.0)	3.3(2.0-5.0)	0.3(0.0-1.0)	0.7(0.0-1.0)
		Blood serum	2.6(2.4-2.8)	1.2(1.0-1.5)	2.5(1.0-3.3)	1.2(0.6-1.6)
Systemic: External Inguinal Lymph Node	3	Preparturient secretions	2.8(1.8-5.0)	2.7(2.5-2.8)	1.0(0.5-1.8)	0.7(0.5-1.0)
		Colostrum	2.2(1.0-5.0)	2.7(2.0-3.0)	0.5(0.0-2.0)	0.0(0.0-0.0)
		Blood serum	2.3(1.6-3.4)	3.0(2.0-3.5)	1.7(1.0-3.0)	0.5(0.2-0.8)
None	4	Preparturient secretions[f]	0.2(0.1-0.3)	0.1(0.0-0.1)	0.1(0.0-0.1)	0.0(0.0-0.1)
		Colostrum	0.0(0.0-0.0)	0.0(0.0-0.0)	0.0(0.0-0.0)	0.0(0.0-0.0)
		Blood serum	0.0(0.0-0.1)	0.0(0.0-0.1)	0.0(0.0-0.1)	0.0(0.0-0.0)

[a] Number outside parentheses represents the grand mean, and numbers within parentheses the range of means, of weekly increases in antibody levels.

[b] One unit represents a titer increase of 3 logs (log 2) over the preimmunization level.

[c] Number outside parentheses represents the mean and numbers within parentheses the range of increases in antibody levels. A single sample was tested from each cow.

[d] One unit represents a titer increase of 2 logs (log 2) over the preimmunization level.

[e] Pooled secretions from contralateral glands.

[f] Antibody levels during the last 7 to 10 weeks of pregnancy were compared with those just prior to that time.

Increases in titers of IgA and IgM antibodies were negligible (Table 1), except for small increases of IgA antibodies in serum and secretions of cow 33 and of IgM antibodies in secretions of cow 45 (Fig. 1).

Slightly increased numbers of PFC producing IgG1 and IgA antibodies were detected in preparturient secretions, colostrum, and milk (Table 2). Responses were variable, the greatest being the increase of IgG1 PFC in cow 45 during immunization (Fig. 1).

Intramammary immunization. Immunization was initiated on day 6 and repeated on days 9, 14, and 18. Increased antibody titers in all isotypes occurred in serum and in secretions by day 14 in cow 2353 (Fig. 2) and by day 18 in cow 1838. Titers in secretions peaked or plateaued by day 48, later than did serum titers (Fig. 2).

Before parturition titers of antibodies in all classes were higher in the immunized quarter (after day 30) in cow 2353 (Fig. 2); this was so only for IgG1 and IgA antibodies in cow 1838. Levels of antibodies in the unimmunized homolateral glands were almost identical to those in the contralateral glands (data not shown). Increases of IgA antibodies in preparturient secretions and in colostrum from immunized glands were markedly superior to those in unimmunized glands; distinctions in IgG1 antibodies between immunized and unimmunized glands were less striking, and distinctions in IgG2 and IgM antibodies were inapparent (Table 1). Overall increases of antibodies in mammary glands, unimmunized as well as immunized, and in blood serum, were substantially greater in all immunoglobulin classes after intramammary immunization than with any other method used (Table 1).

After parturition antibody levels in all classes were higher in immunized than in unimmunized quarters. Concentrations of serum antibodies exceeded those in lacteal secretions, except for those of the IgA class (Fig. 2).

Table 2. Average weekly numbers of antigen specific PFC in preparturient and colostral secretions and in milk of cows immunized with T4 phage.

Immunization	No. of Animals in Group	Secretion	Mean Weekly Numbers of Antigen Specific PFC Producing Antibodies of Different Immunoglobulin Classes			
			IgG_1	IgG_2	IgA	IgM
Intestinal	4	Preparturient	5.2(0.4-11.3)[a]	0.8(0.0-1.3)	5.8(1.6-10.0)	2.0(0.8-5.0)
		Colostrum	1.5(0.0-2.0)[b]	0.0(0.0-0.0)	3.5(1.0-7.0)	0.5(0.0-1.0)
		Milk	5.0(1.0-8.7)[a]	0.4(0.0-1.0)	4.9(0.4-13.8)	1.0(0.2-1.5)
Intramammary	2	Immunized gland: Preparturient	46.4(44.3-48.4)	2.9(1.9-3.9)	66.0(45.4-86.6)	2.4(0.9-3.8)
		Colostrum	1.0(0.0-2.0)	0.0(0.0-0.0)	1.0(1.0-1.0)	0.5(0.0-1.0)
		Milk	1.3(0.5-2.0)	0.3(0.0-0.5)	0.8(0.5-1.0)	0.0(0.0-0.0)
		Unimmunized glands[c]: Preparturient	26.0(21.8-30.1)	0.9(0.8-0.9)	4.1(3.8-4.4)	0.5(0.3-0.6)
		Colostrum	0.0(0.0-0.0)	0.0(0.0-0.0)	1.0(1.0-1.0)	0.5(0.0-1.0)
		Milk	0.8(0.0-1.5)	1.0(1.0-1.0)	0.5(0.5-0.5)	0.5(0.5-0.5)
Systemic: Prescapular Lymph Node	3	Preparturient	23.1(0.7-37.5)	1.4(0.5-2.0)	27.9(0.8-43.8)	1.4(0.8-2.5)
		Colostrum	3.0(3.0-3.0)	3.0(3.0-3.0)	0.0(0.0-0.0)	1.0(1.0-1.0)
		Milk	0.5(0.5-0.5)	0.5(0.0-1.5)	1.0(0.5-1.5)	0.8(0.5-1.5)
Systemic: External Inguinal Lymph Node	3	Preparturient	13.5(5.7-19.2)	1.7(0.7-3.0)	28.7(3.0-109.5)	1.8(0.8-3.0)
		Colostrum	3.0(2.0-4.0)	1.0(1.0-1.0)	0.5(0.0-1.0)	1.0(0.0-2.0)
		Milk	0.8(0.0-2.5)	0.1(0.0-0.3)	1.1(0.5-2.5)	0.1(0.0-0.3)
None	4	Preparturient	1.9(1.3-3.0)	0.6(0.0-1.1)	3.1(2.2-4.5)	0.5(0.4-0.7)
		Colostrum	1.0(0.0-2.0)	0.7(0.0-2.0)	0.7(0.0-2.0)	0.3(0.0-1.0)
		Milk	1.2(1.0-1.7)	0.2(0.0-0.7)	1.0(0.5-1.5)	0.1(0.0-0.3)

[a] Number outside parentheses represents the grand mean, and numbers within parentheses the range of means, of weekly numbers of antigen specific PFC per 2×10^5 cells.

[b] Number outside parentheses represents the mean and numbers within parentheses the range of antigen specific PFC per 2×10^5 cells. A single sample was tested from each cow.

[c] Pooled secretions from contralateral glands.

An increase in PFC producing IgA and IgG1 was detected by day 14 to 21. In preparturient secretions of immunized glands IgA PFC exceeded (Fig. 2) or were equivalent in number to IgG1 PFC. In unimmunized glands numbers of IgG1 PFC greatly exceeded those of the IgA type (Fig. 2; Table 2). In preparturient secretions increases in IgA and IgG1 PFC generally reflected increases of antibodies of those classes in immunized glands but diminished greatly before parturition (Fig. 2). Antigen-specific PFC in colostrum and in milk were also present in extremely low numbers (Table 2) despite continued presence of antibodies, particularly of IgG1 and IgA isotypes in colostrum of immunized glands (Table 1).

Systemic immunizations. Subcutaneous immunizations in the region of the prescapular and external inguinal lymph nodes produced generally comparable results. Increased antibody titers in all isotypes were apparent in blood serum and lacteal secretions within a week after the first immunization (Fig. 3). In lacteal secretions overall increases in IgG1 and IgG2 antibodies predominated, a feature less evident in serum (Table 1).

Increased IgA and IgG1 PFC in comparison with unimmunized animals were present in preparturient secretions in all but one animal of these groups. PFC were detected within a few days after the first immunization, attained peak numbers 1 to 3 weeks thereafter, and then declined (Fig. 3). The magnitude of these responses varied greatly, however (Table 2).

DISCUSSION

The immune response of cows to a viral antigen instilled in the jejunum differed from those in other species in which oral immunization has been performed (3, 23–25, 42) in respect to the prominence of the IgG response and the paucity of IgA PFC in lacteal secretions. Generalizations

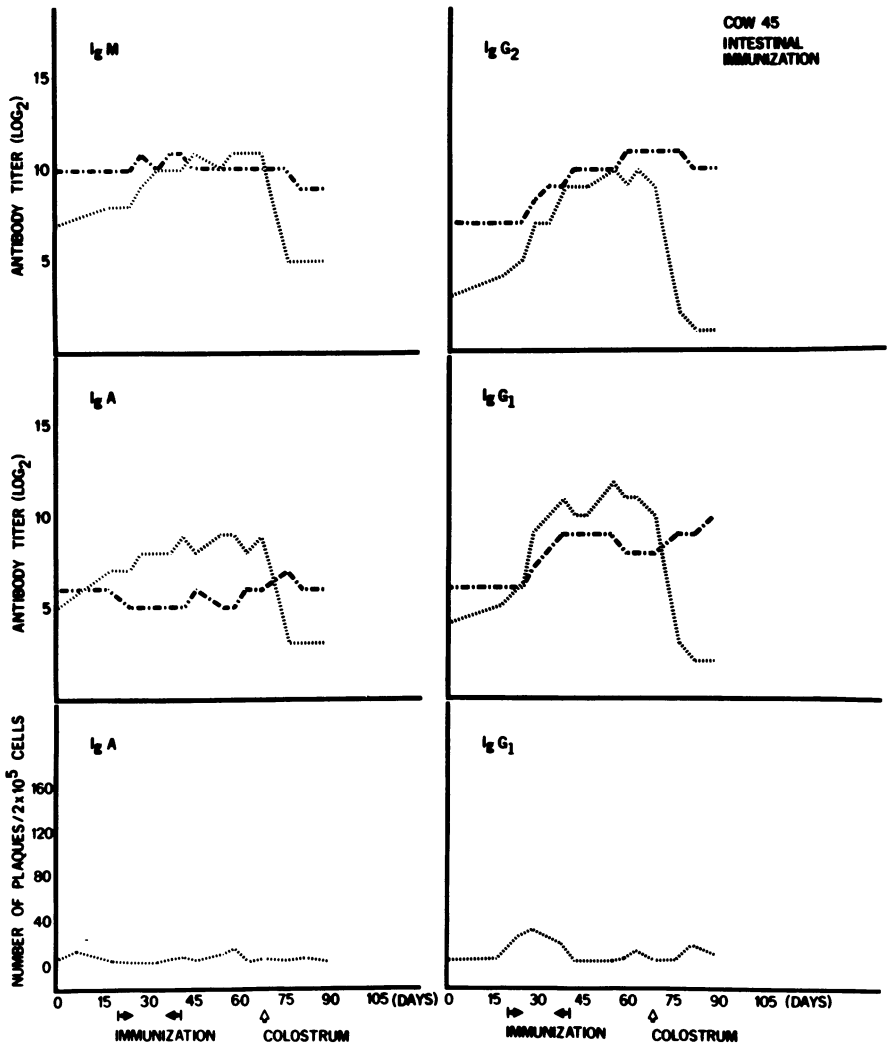

FIG. 1. *Levels of antibodies and numbers of antibody-forming cells after immunization through a jejunal fistula. Symbols:* (·—·—·) *serum;* (·····) *pooled lacteal secretions.*

from these data must be cautious since only a single antigen has been tested and the route of immunization in cattle differed from that used in monogastric species. Furthermore, we did not establish the identity of PFC and cannot rule out the suggestion of Crago et al. (6) that some of these may have been macrophages with passively adsorbed antibody. It is probable, however, that most PFC were B cells since antibodies in lacteal secretions were very frequently detected in the absence of PFC and the percentage of PFC in the total cell population never exceeded 0.1%.

If the response of cattle to intestinal immunization proves distinctive, it may be explained by the maternal relationship to fetus and neonate singular to ruminant species. Colostral antibodies in ungulates, predominantly IgG, are crucial for conferring to the newborn immunity to intestinal pathogens. In swine (31) and horses (22) IgA quickly becomes the dominant antibody in milk, and there is evidence in swine that milk IgA antibodies exert an important continuing protective role (31) and are produced in the mammary gland by cells which have migrated from Peyer's patches (24, 25). In ruminants, in contrast to swine and horses (10, 22), selective transport of IgG1 from serum is initiated before parturition and continues throughout lactation so that IgG1 is the principal immunoglobulin in colostrum and milk (4, 16, 17). Serum IgG1 antibodies specific for intestinal pathogens

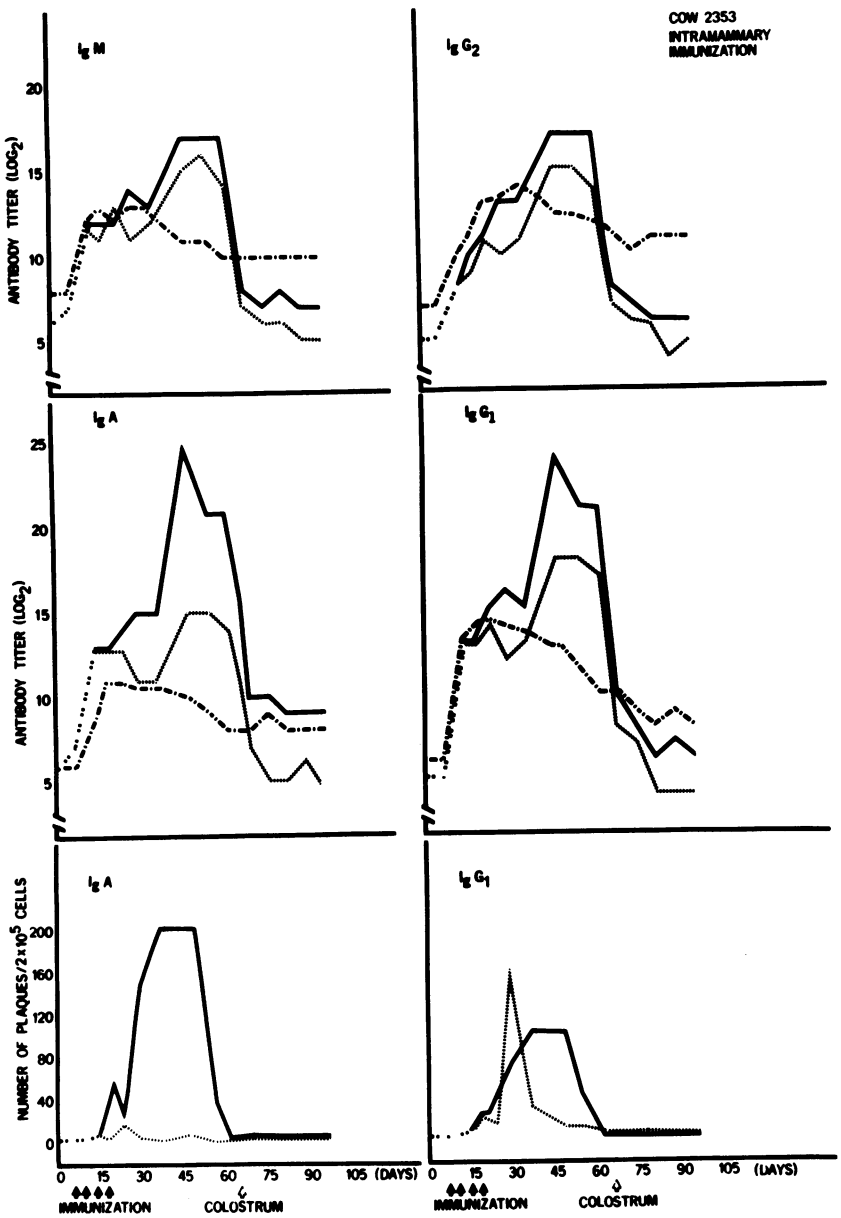

FIG. 2. *Levels of antibodies and numbers of antibody-forming cells after immunization in the right front (RF) quarter. Symbols:* (—·—·) *serum;* (·· ··) *pooled lacteal secretions;* (——) *lacteal secretions, RF;* (·····) *lacteal secretions, left front and left rear.*

would logically have an important role in conferring protection to neonates of these species and may be supplied principally by plasma cells of intestinal origin.

Thus, the dominant migratory pattern of antigen-sensitized B cells from Peyer's patches in ruminants may differ because the majority of cells involved is committed to synthesize IgG and, after stimulation by antigen, migrates principally to spleen, peripheral lymph nodes, and intestinal lamina propria. IgG antibodies in serum would then be accounted for by synthesis of cells which had emigrated to spleen and peripheral lymph nodes, and IgG antibodies in lacteal secretions would result almost entirely from transport of serum antibodies. There is now direct evidence from studies in mice (20, 42) that IgG- and IgM-producing cells from mesen-

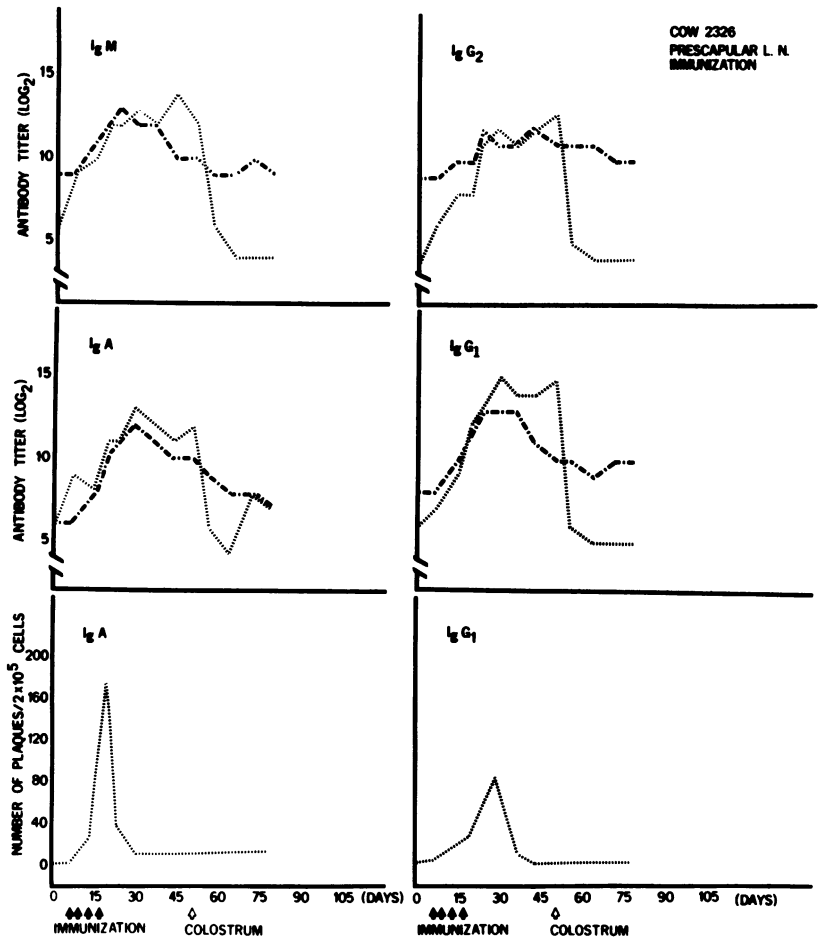

FIG. 3. *Levels of antibodies and numbers of antibody-forming cells after immunization subcutaneously at the right prescapular lymph node. Symbols:* (—·—·) *serum;* (·····) *pooled lacteal secretions.*

teric lymph nodes do migrate preferentially to peripheral lymph nodes. Some antigen may also have been absorbed systemically and induced responses of resident lymphocytes in spleen and peripheral lymph nodes. The relative contributions of migrant and resident lymphocytes to the synthesis of circulating IgG antibodies cannot be distinguished in these experiments. In either fashion the imperatives of neonatal survival would be satisfied.

Local antibody synthesis in immunized mammary glands, demonstrated previously in cattle (28, 30, 43, 44) and sheep (17), has been confirmed in this study. In unimmunized glands of cows 1838 and 2353 local synthesis of antibody before parturition cannot be inferred from comparisons of antibody titers but is indicated by the presence of a few IgA and much larger numbers of IgG1 PFC in secretions (Fig. 2; Table 2). The possibility that immune responses in unimmunized glands resulted through transfer of antigen from the immunized gland seems remote, especially since the magnitude of the response in homolateral and contralateral glands was almost identical. Alternatively, local immune responses in both vaccinated and unvaccinated glands may have resulted from migration into the glands of antigen-stimulated lymphoblasts. Rudzik et al. (34) have proposed that secretory organs other than intestinal and respiratory tracts may possess lymphoid follicles analagous functionally to gut- and bronchus-associated lymphoid tissue, so that migration of antigen-sensitized B cells might be a general consequence of immunization at mucosal surfaces. To some extent lymphocyte localization is believed to be unaffected by the presence in a site of specific antigen, but specific antigen augments homing (15, 16). Higher levels of antibody and larger numbers of PFC in immunized glands (Fig. 2; Tables 1 and 2) would be in accord with these observations, although to explain the dis-

proportionate concentrations of IgG1 and IgA PFC in immunized and unimmunized glands (Table 2), it would be necessary to postulate that the migration of IgG-forming cells is less influenced by specific antigen.

It is difficult to explain why identical protocols of immunization resulted in higher levels of circulating antibodies of all classes after intramammary immunization than with either systemic route (Table 1). Systemic absorption of some antigen infused into the mammary gland doubtlessly occurred, and systemically produced antibodies would have been transferred into mammary secretions by transudation or selective transport from the bloodstream. Conversely, circulating antibodies, particularly of the IgA class, may have been derived in part from the mammary gland. In the sheep and dog and most likely in other species, including cattle, in which serum IgA occurs solely as a dimer, a large proportion of serum IgA is derived from the intestine (16). In like manner, IgA antibodies locally synthesized in the mammary gland may have accounted in some measure for those in serum. This is supported by an earlier rise in IgA titers in lacteal secretions and consistently higher levels of IgA in the immunized glands, even during early lactation (Fig. 2), when daily milk volume in Holstein cows may approach blood volume. The source of PFC in lacteal secretions of systemically immunized cows is uncertain. Circulating antigen may have entered the mammary gland and elicited a local immune response, although this seems most unlikely. Alternatively, antigen-stimulated lymphoblasts may have migrated into the mammary gland, a possibility favored by Lascelles and co-workers from studies in sheep (18, 21) and cattle (41).

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