Effect of different routes of immunization with bovine rotavirus on lactogenic antibody response in mice

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Summary

The effect of different routes of immunization with either live or killed bovine rotavirus (BRV) on the production of lactogenic antibody response in mice was evaluated. The routes of immunization were intramuscular (IM), oral (O) or intradermal in the mammary region (IMam). Following immunization, serum antibody responses were monitored by an enzyme linked immunosorbent assay (ELISA). Following whelping, the mice were allowed to stay with their mother until sacrificed on alternate days post-parturition from day 1–11. Milk from their stomach was collected for antibody titration by ELISA and virus neutralization test. Regardless of the routes of immunization, a rapid increase in serum anti-rotavirus antibody titers was observed for the first 5 wk after immunization followed by a gradual decline. After parturition, the mean antibody titer of lacteal secretions, as determined by ELISA, increased gradually for 7 days with the greatest increase on day 9, followed by a decrease in anti-rotavirus antibody. These titers also correlated with antibody titers in milk as measured by virus neutralization test. The best lactogenic antibody response was observed when IMam × IM × 2 route of immunization was used with live BRV as the antigen. Interestingly, immunization via the oral route with killed BRV also resulted in good antibody responses. In contrast, in the group where killed BRV was used, animals receiving 3× orally had the highest antibody titer. The distribution of different antibody subtypes in milk samples revealed IgG to be the predominant antibody followed by IgM and IgA. Irrespective of the route of administration, there was an increase in IgA on day 9

as compared to day 1 in most of the groups. The significant role played by mucosal immunity in passive protection and the possible ways to modulate subtype specific lactogenic immune response are discussed.

Animals models; Lactogenic immunity; Rotaviruses

Introduction

Rotavirus has been identified as the single most important causative agent of acute neonatal enteritis in a variety of domestic and laboratory animals and humans. Consequently, a world-wide effort is being made to develop an appropriate vaccine against this pathogen (Babiuk et al., 1985; Flewett and Babiuk, 1984; Hanson et al., 1983). Although previous studies have indicated no correlation between protection from rotavirus infection and the titers of passively acquired antibodies in the serum of newborns (Saif and Smith, 1983; M.K. Ijaz et al., unpublished data), it has been shown that the continuous presence of anti-rotavirus antibodies in the intestine of neonates is important in preventing clinical disease associated with rotavirus infections (Offit and Clark, 1985; Saif and Smith, 1983; Snodgrass et al., 1982; M.K. Ijaz et al., unpublished data).

In several animal species, including humans, immunization during pregnancy has been shown to result in the presence of antibodies in colostrum and milk (Goldblum et al., 1975; Saif and Smith, 1983). In addition to the humoral immune response, the role played by the cellular component of immunity in the production of lactogenic immune response has also been documented (Parmerly and Beer, 1977; Ripenhoff-Talty et al., 1983). Cell transfer experiments in mice have revealed the migration of lymphoblasts from mesenteric lymph nodes into the intestine and mammary gland and other secretory organs and peripheral lymph nodes (McDermott et al., 1980; McDowell and Lascells, 1971). Most cells migrating into secretory organs and mesenteric lymph nodes synthesized IgA, but most of those migrating to peripheral lymph nodes produced IgG or IgM (Bohl et al., 1972; McDermott et al., 1980; Roux et al., 1977). Experiments carried out in porcine and bovine models have revealed the predominance of IgA and IgG subtypes in porcine and bovine milk, respectively, with the decrease of these subtypes occurring during the post-parturition period (Saif and Bohl, 1979; Saif and Smith, 1983).

Thus it becomes imperative to direct efforts in order to stimulate the lactogenic anti-rotavirus antibody response to higher titers for longer periods after parturition to ensure protection against rotavirus infection in neonates receiving these antibodies in mother's milk. Earlier experiments conducted to determine the kinetics of BRV replication in a mouse model revealed that the neonates are most susceptible to infection at the age of seven days (Ijaz et al., in preparation). In our protection studies the neonates are challenged on day seven. Taking this into consideration, we have utilized the murine model to study the influence of different routes of immunization with either live or killed BRV on the rate of decline of antibody

titers and different subtypes in the lacteal secretions of the dam following parturition up to eleven days. This report forms the basis of our protection studies which are currently under way.

Materials and Methods

Animals

Rotavirus-free mice were obtained from Charles River Breeding Laboratories (Wilmington, MA). In order to ensure that they were free of anti-rotavirus antibodies, they were bled on arrival from the coccigial vein. The sera were tested for anti-rotavirus antibodies using an ELISA assay (Bidwell et al., 1977) and all were found to be seronegative. To maintain them rotavirus free, the animals were housed in isolation units throughout the experiment.

Antigen preparation and immunization of dams

Bovine rotavirus (BRV) isolate C-486 was grown in MA-104 cells in the presence of 10 μg of trypsin/ml (Difco Laboratories, Detroit, MI) (Babiuk et al., 1977). After 24 h of incubation at 37°C, supernatant containing the virus was harvested and cell debris removed by centrifugation at 1500 rpm for 20 min. The virus was concentrated from the clarified supernatant fluids by pelleting through a 40% sucrose cushion at 25000 rpm in an SW28 rotor (Beckman Model L5-65) for 2 1/2 h at 15°C. The virus pellet was resuspended in double distilled water and the amount of virus protein measured spectophotometrically by using the formula: $183(A_{230})$ $- 75.8 \ (A)_{260}) \times 100 = \mu$g/ml where A_{230} and A_{260} represents adsorbance at 230 nm and 260 nm, respectively.

When live BRV was used as the antigen, each dam was given 50 μg (2 $\times$ 10^5 plaque forming units) of purified virus contained in adjuvant (Freund's incomplete adjuvant [FIA]) or double distilled water, according to the immunization protocol (Table 1).

Killed BRV was prepared as described above. In order to inactivate the virus, it was subjected to ultraviolet light (2 ergs) for 2 h and the extent of inactivation was tested in vitro. In no instance was active bovine rotavirus found after this inactivation procedure. Each dam was given 50 μg of purified virus contained in adjuvant or double distilled water, according to the immunization protocol (Table 1). For oral immunization, mice were inoculated with 50 μg of BRV followed 3 and 6 wk later with the same viral dose inoculated orally. One week before the final booster inoculation, mice were bred in a female-to-male ratio of 3:1. All the dams gave birth naturally and the pups remained with their dams throughout the experiment.

Sampling and sample processing

All mice were bled prior to immunization and their sera were tested for the presence of rotavirus antibody by both an ELISA and virus neutralization test. All of the mice were seronegative for rotavirus on initiation of the study. Thereafter,

TABLE 1

Schedule for mice immunized with bovine rotavirus using different routes to produce lactogenic antibody responses.

Groups/immunogen	Days								
	1	7	14	21	28	35	42	49	56
	Prebleed 1° immuniz.[c]	Bleed	Bleed	Bleed 2° immuniz.	Bleed	Bleed and Breed	Bleed and 3° immuniz.	Bleed	Expect littering
1. BRV$_K$[a]	O/50 μg/d H$_2$O	Bleed	Bleed	O/50 μg/d H$_2$O	Bleed	Bleed and Breed	O/50 μg/d H$_2$O	Bleed	Expect littering
2. BRV$_K$	O/50 μg/d H$_2$O	Bleed	Bleed	IM/50 μg/FIA	Bleed	Bleed and Breed	IM/50 μg/FIA	Bleed	Expect littering
3. BRV$_K$	IM/50 μg/FCA	Bleed	Bleed	IM/50 μg/FIA	Bleed	Bleed and Breed	IM/50 μg/FIA	Bleed	Expect littering
4. BRV$_K$[b]	IMam/50 μg/d H$_2$O	Bleed	Bleed	IMam/50 μg/d H$_2$O	Bleed	Bleed and Breed	IMam/50μg/d H$_2$O	Bleed	Expect littering
5. BRV$_L$[b]	O/50 μg/d H$_2$O	Bleed	Bleed	O/50 μg/d H$_2$O	Bleed	Bleed and Breed	O/50 μg/d H$_2$O	Bleed	Expect littering
6. BRV$_L$	O/50 μg/d H$_2$O	Bleed	Bleed	IM/50 μg/FIA	Bleed	Bleed and Breed	IM/50 μg/FIA	Bleed	Expect littering
7. BRV$_L$	IM/50 μg/FCA	Bleed	Bleed	IM/50 μg/FIA	Bleed	Bleed and Breed	IM/50 μg/FIA	Bleed	Expect littering
8. BRV$_L$	IMam/50 μg/d H$_2$O	Bleed	Bleed	IMam/50 μg/d H$_2$O	Bleed	Bleed and Breed	IMam/50 μg/d H$_2$O	Bleed	Expect littering
9. BRV$_L$	IMam/50 μg/d H$_2$O	Bleed	Bleed	IM/50 μg/FIA	Bleed	Bleed and Breed	IM/50 μg/FIA	Bleed	Expect littering
10. Negative control[d]	Saline	Bleed	Bleed	Saline	Bleed	Bleed and Breed	Saline	Bleed	Expect littering

[a] BRV$_K$ = bovine rotavirus killed.

[b] BRV$_L$ = bovine rotavirus live.

[c] The quantities given are per mouse (3 mice per group) and volume of antigen given = 100 μl; O = oral; IM = intramuscular; IMam = intramammary; FCA = Freund's complete adjuvant; FIA = Freund's incomplete adjuvant.

[d] Control received saline either with FCA or FIA in those groups where adjuvants were used.

blood samples were taken weekly during the course of immunization to monitor antibody responses as indicated in the immunization protocol (Table 1). The immunization intervals were chosen arbitrarily at three weeks, since in most instances, maximal immunity occurs three weeks post-vaccination.

The newborn mice were allowed to stay with their mother throughout the period of the experiment. In order to collect samples of colostrum and milk, three neonates from each group were sacrificed every second day post parturition starting on days 1 through 11. Milk from their stomach was collected and diluted twofold in MEM. Samples were spun in an Eppendorf centrifuge and supernatants were aliquotted into Eppendorf tubes and stored at −20°C.

Enzyme-linked immunosorbent assay (ELISA) for detection of anti-rotavirus antibodies

Antibody levels in serum and milk samples against BRV were determined by the method described previously (Bidwell et al., 1977). Briefly, 96-well microtiter plates (Immunolon 2: Dynatech Laboratories Inc., Alexandria, VA) were incubated at 4°C overnight with 1 μg of BRV (isolate C-486) antigen diluted in 1 × carbonate-bicarbonate (pH 9.6). The volume of BRV per well was 100 μl.

After incubation of plates with antigen, unadsorbed protein was removed by extensive washing in distilled water. The antigen was then overlaid with 75 μl of mouse antiserum per well in an undiluted form or diluted in 0.01 M phosphate-buffered saline containing 0.5% fetal bovine serum (FBS).

Incubation of antigen was carried out for 1 h at room temperature, after which time unbound antibody was removed by washing in distilled water. A 1:2000 dilution of rabbit antimouse horseradish peroxidase conjugate (Boehringer Mannheim Biochemical, Calgary, Alberta) was then added per well, and incubation of the conjugate proceeded at room temperature for an additional hour.

After extensive washing to remove excess conjugate, the bound conjugate was reacted with 75 μl of chromagen and enzyme substrate (recrystallized 5-amino salicylic acid, 1 mg/ml in 0.01 phosphate buffer, pH 5.95 ± 0.05, Aldrich Chemical Co., Milwaukee, WN) per well, to which 0.005 % hydrogen peroxide was added immediately before use. The reaction was allowed to proceed for 30 min at room temperature before the absorbance (450 nm) of each well was determined with a micro-ELISA reader (Dynatech Instruments, Inc., CA) and titers were expressed as the reciprocal of the highest dilution with an OD of >5 SD over mean background levels.

For the quantitation of different classes of antibody in mouse milk, mouse monoclonal subtyping kit 55051-K was used following the procedure recommended by the manufacturer (Hyclone Laboratories, Logan, UT).

Micro-neutralization test

Neutralization of BRV C-486 by milk samples taken from hyperimmunized dams was determined by a micro-neutralization test using 96-well plates containing confluent monolayers of BSC-1 cells grown in Eagle's minimal essential media (MEM) with 10% FBS (Gibco Laboratories, Grand Island, NY). Milk samples were se-

rially diluted and incubated for 30 min at 56°C. Virus dilutions representing 200 $TCID_{50}$ were added 1:1 with various dilutions of milk and the virus-milk mixture was transferred to the plates containing confluent monolayers of BSC-1 cells in each well in duplicate. The plates containing virus-milk mixture were incubated for 1 h at 37°C. The cell monolayers were washed with MEM and replaced with 100 µl of MEM. The plates were incubated at 37°C for two days. The dilution sample giving 50% cytopathic effect (CPE), as observed under the microscope, was taken as end point.

Results

Since passive protection is important for preventing rotavirus infection in neonates, it is important that neonates receive sufficient quantities of neutralizing antibodies in milk from their mothers. In this study we were interested in determining the best regimen of immunizations against BRV which would result in the highest neutralizing antibody levels in milk. To achieve this goal, mice were immunized with either live or killed BRV via different routes and the levels of serum antibody against rotavirus were monitored throughout the immunization schedule up to parturition. After parturition, anti-rotavirus antibodies were determined in lacteal secretions for 11 days, using an ELISA and virus neutralization test. Antibody titers in serum, colostrum and milk are shown in Figs. 1–4. Milk samples

TABLE 2

The ELISA Ig subtypes in mammary secretions of mice immunized with killed bovine rotavirus.

Immune regime	Isotype	Days after parturition[a]					
		1	3	5	7	9	11
O × 3	IgM	24.0	29.0	29.0	26.0	27.0	27.0
	IgG	75.0	68.0	66.0	66.0	63.0	61.0
	IgA	8.0	7.0	8.0	11.0	12.0	10.0
O × IM × 2	IgM	30.0	27.0	25.0	26.0	26.0	29.0
	IgG	68.0	65.0	64.0	64.0	67.5	59.0
	IgA	8.0	10.0	10.0	10.0	12.0	10.0
IM × 3	IgM	33.0	26.0	24.0	27.0	29.0	25.0
	IgG	61.0	67.0	73.0	67.0	66.0	66.0
	IgA	10.0	6.0	6.0	7.0	12.0	9.0
IMam × 3	IgM	33.0	32.0	31.0	31.0	28.0	26.0
	IgG	56.0	60.0	62.0	62.0	62.0	61.0
	IgA	11.0	8.0	8.0	9.0	13.0	12.0

[a]Milk samples obtained from the stomachs of neonate mice at various ages were suspended in MEM and clarified to obtain fat-free samples by centrifugation in an Eppendorf centrifuge. They were subjected to subtypic analysis by ELISA using sub-subtyping kit following manufacturer's procedure. The values indicate the means of triplicate determinations of Ig subtypes on each of the three samples.

TABLE 3

The ELISA Ig subtypes in mammary secretions of mice immunized with live bovine rotavirus.

| Immune regime	Isotype	Days after parturition[a]					
		1	3	5	7	9	11
O × 3	IgM	35.0	23.0	25.0	26.0	30.0	29.0
	IgG	57.0	74.0	78.0	64.0	61.0	61.0
	IgA	11.0	11.0	9.0	12.0	13.0	12.0
O × IM × 2	IgM	28.0	20.0	23.0	21.0	28.0	23.0
	IgG	62.5	73.0	66.0	71.0	60.0	65.0
	IgA	13.0	9.0	10.0	11.0	13.0	12.0
IM × 3	IgM	28.0	24.0	24.0	24.0	22.0	20.0
	IgG	69.0	70.0	72.0	66.0	65.0	71.0
	IgA	10.0	8.0	8.0	9.0	13.0	9.0
IMam × 3	IgM	28.0	29.0	25.0	26.0	22.0	27.0
	IgG	62.0	71.0	64.0	66.0	71.0	62.0
	IgA	11.0	8.0	8.0	8.0	11.0	8.0
IMam × IM × 2	IgM	29.0	29.0	29.0	27.0	26.0	25.0
	IgG	63.0	65.0	65.0	63.0	62.0	63.0
	IgA	10.0	9.0	8.0	10.0	12.0	12.0

[a]The values indicate the means of triplicate determinations of Ig subtypes on each of the three samples.
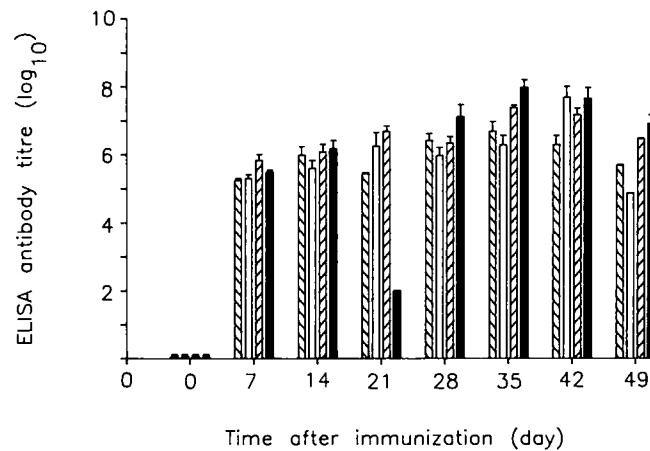
Fig. 1. Serum anti-rotavirus antibody response in mice immunized with killed bovine rotavirus. Animals were immunized with killed bovine rotavirus via different routes and blood samples were collected at different time intervals (Table 1). The titer of anti-rotavirus antibody was determined by ELISA assay as described in the text. O × 3 = ▨▨▨; O × IM × 2 = ===; IM × 3 = ▨▨▨; IMam × 3 = ▬▬. The results are expressed as the means ± SD of triplicate determinations.
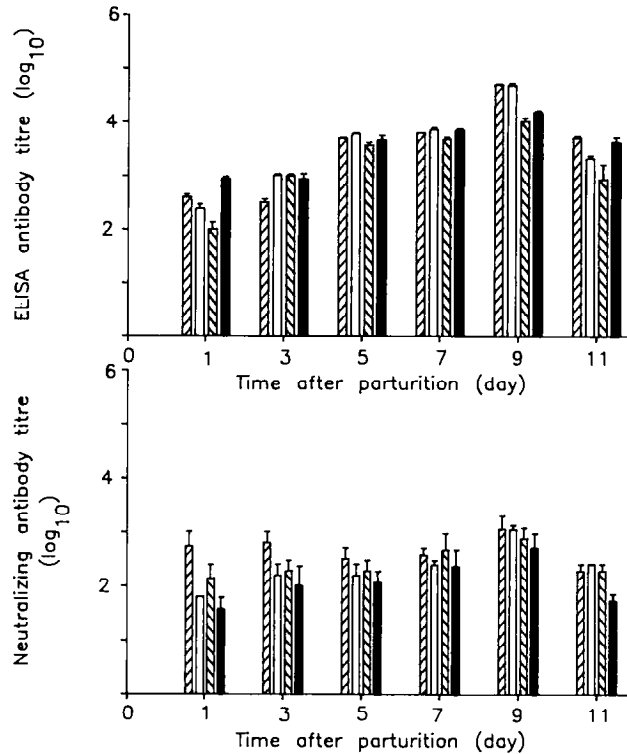
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Fig. 2. Lactogenic anti-rotavirus antibody response in mice immunized with killed bovine rotavirus. Milk samples obtained from the stomach of the neonates were processed as described in the text. The titer of anti-rotavirus antibody was determined by ELISA assay. The neutralizing ability of the anti-rotavirus antibodies present in the milk samples was determined by virus neutralization test as mentioned in the text. O × 3 = ▨; O × IM × 2 = ☰; IM × 3 = ▨; IMam × 3 = ■. The results are expressed as the means of ± SD of triplicate determinations.

were also examined for determination of different immunoglobulin (Ig) classes. Data showing ratios of various subtypes in milk collected from dams immunized with either live or killed BRV are summarized in Tables 2 and 3. The distribution of different subtypes in milk samples revealed IgG to be the most predominant antibody followed by IgM and IgA.

It is interesting to note that regardless of the route of immunization, a gradual increase in the antibody titer in the lacteal secretions was observed up to 9 days post-partum, with the greatest increase on day 9, followed by a decrease in titer on day 11; a unique observation compared with other species.

Immunization with killed bovine rotavirus

To determine whether killed BRV can produce lactogenic anti-rotavirus antibody response, experiments were performed using this antigen orally and in combinations of other routes. Animals were immunized by the following route using killed BRV: O × 3, O × IM × 2, IM × 3 and IMam × 3. Freund's complete
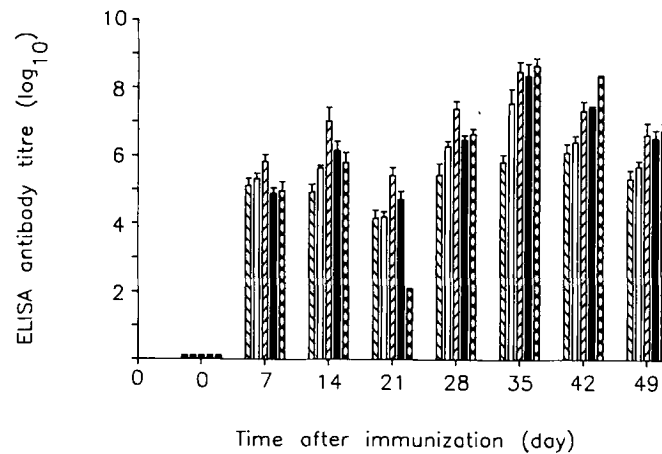
Fig. 3. Serum anti-rotavirus antibody response in mice immunized with live bovine rotavirus. Animals were immunized with live BRV via different routes and blood samples were collected at different time intervals (Table 1). The titer of anti-rotavirus antibody was determined by ELISA assay as described in the text. O × 3 = ▨▨; O × IM × 2 = ≡≡; IM × 3 = ▨▨; IMam × 3 = ▬▬; IMam × IM × 2 = ▨▨▨. The results are expressed as the means ± SD of triplicate determinations.

adjuvant (FCA) and Freund's incomplete adjuvant (FIA) were used only with the IM route of immunization (Table 1). Immunization with killed BRV significantly increased anti-rotavirus antibodies in serum in all groups regardless of the route of immunization (Fig. 1). The rapid immune response observed a week after the first immunization as compared to an almost negligible titer on day 0, does not reflect that animals were primed before immunization because mice used in this study were pre-tested for anti-rotavirus antibodies and all were found to be seronegative and control mice remained seronegative throughout the study. However, it does show that rotavirus is highly immunogenic.

Among all the groups immunized with killed BRV, the group immunized 3 times via the IMam route showed the best anti-rotavirus serum antibody response. An interesting but unexplainable decrease in antibody occured 21 days post-immunization in animals immunized via the IMam route (Fig. 1). However, a second immunization at 21 days increased the antibody titer above that of any other group. Although the second immunization boosted the antibody titers in all groups, the third immunization at 42 days did not show any effect on the level of antibody production in any of the groups under study (Fig. 1).

Antibody titers against rotavirus in lacteal secretions as determined by ELISA, varied from 2–3 $\log_{10}$ between groups one day post-parturition (Fig. 2). Animals immunized O × 3 had the highest titers whereas the IM × 3 had the lowest antibody titers. A gradual increase in antibody was observed in all groups with the maximum antibody titer in lacteal secretions being achieved 9 days after parturition. Unlike serum antibodies, the highest lacteal antibody titer occurred in the group immunized O × 3 and O × IM × 2.

The virus neutralization antibody titers in milk showed a similar pattern

throughout lactation as did the ELISA titers. However, anti-rotavirus antibody titers, as determined by ELISA, were consistently higher than virus neutralization titers, with the exception of the group immunized O × 3 which showed slightly higher neutralization titers on day 1 and 3 after parturition (Fig. 2).

Immunization with live bovine rotavirus

Since killed antigen produced an excellent response following oral immunization, attempts were made to do similar studies with live virus. Animals were immunized by the following routes using live BRV: O × 3, O × IM × 2, IM × 3, IMam × 3 and IMam × IM × 2 with or without adjuvant as indicated in the immunization schedule (Table 1).

Immunization with live BRV induced the production of high levels of anti-rotavirus antibodies in serum of dams in all groups. The pattern of immune response was rapid, similar to the groups immunized with killed BRV. One exception was the group immunized IMam × IM × 2, which revealed significantly higher anti-
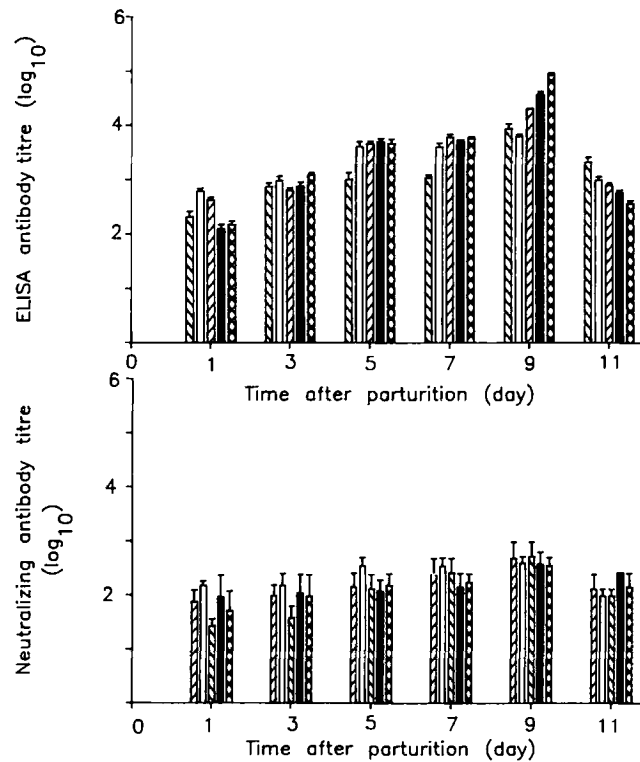
Fig. 4. Lactogenic anti-rotavirus antibody response in mice immunized with live bovine rotavirus. Milk samples obtained from the stomach of the neonates were processed as described in the text. The titer of anti-rotavirus antibody was determined by ELISA assay. The neutralizing ability of the anti-rotavirus antibodies present in the milk samples was determined by virus neutralization test as mentioned in the text. O × 3 = ▨; O × IM × 2 = ═; IM × 3 = ▨; IMam × 3 = ▬; IMam × IM × 2 = ▨. The results are expressed as the means ± SD of triplicate determinations.

rotavirus antibodies 35 days after primary immunization (2 wk after the second immunization). There was a rapid decrease in antibody titer in this particular group 21 days after the first immunization, but the second immunization at 21 days induced a higher level of antibody than in the rest of the groups. The third immunization at 42 days did not increase antibody production, and antibody levels in serum started declining towards the time of parturition (Fig. 3).

Titers of anti-rotavirus antibodies in lacteal secretions, as determined by ELISA, also revealed a similar pattern as seen in the groups immunized with killed BRV. The colostrum and milk samples collected up to a week after parturition showed a gradual increase in antibody titers as determined by ELISA. The highest anti-rotavirus antibody titer in all groups was observed on day 9 post-parturition. The maximum titer of antibody at this point was observed in the group immunized IMam × IM × 2. The titers started declining thereafter (Fig. 4).

Virus neutralizing antibody titers in the lacteal secretions exhibited a similar pattern throughout lactation. The anti-rotavirus antibody titers, as determined by ELISA, were consistently higher than virus neutralizing antibody titers in all the groups (Fig. 4).

The distribution of immunoglobulin subtypes in mammary secretions of mice

The distribution of antibodies associated with various immunoglobulin (Ig) subtypes in mammary secretions are presented in Tables 2 and 3. Throughout the duration of the experiment, IgG antibodies predominated, followed by IgM and IgA in each group. Irrespective of the route of immunization and type of antigen used, there was a gradual reduction in the distribution of IgG and IgM antibodies until the end of the experiment. In contrast, IgA antibodies, after a gradual decrease up to day 7 post-parturition, showed an increase on day 9 before decreasing again.

Discussion

Since neonates are often exposed to enteric viruses before they have sufficient time to develop active immunity, they are highly susceptible to infection unless passively immunized before exposure to these pathogens. Lactogenic immunity has been shown to play a major role in providing protection to neonates from a variety of mammalian species against enteric infections (Hanson et al., 1983; Knight and Peaker, 1982; Offit and Clark, 1985; Opdebeeck, 1982). This further underlines the significance of passively required antibodies from colostrum and milk in the neonatal gut for preventing these infections (Hanson et al., 1983). Therefore, considerable efforts are underway in order to develop vaccines which will stimulate lactogenic immunity against enteric infections in order to improve the immune status of neonates and thereby, prevent early infection (Babiuk et al., 1985).

The role of maternal antibodies in the protection of neonates against diarrhea, caused by a number of viral agents, has been shown to depend on the continual presence of antibody in the intestinal lumen (Saif and Smith, 1983). In the case of rotavirus induced neonatal diarrhea in calves and lambs, it has been experimen-

tally shown that by feeding colostrum, milk, or serum containing suffcient anti-ro-
tavirus antibodies, infection of the intestine can be prevented (Snodgrass et al.,
1982). The degree of protection not only correlated with the quantity of anti-ro-
tavirus antibodies present but also with the subtype of the Ig involved (Snodgrass
et al., 1982). Local immunity against rotavirus can perform two functions. First,
the presence of antibody in the intestine should protect the neonate from disease.
Secondly, it should help in the reduction of rotavirus in feces and hence reduce
environmental contamination (Saif and Smith, 1983). Since the mouse model being
used in our protective-challenge experiments against rotavirus infection, utilizes
neonates at 7 days of age, the time when mouse enterocytes have the highest num-
ber of rotavirus specific receptors, thereby making them more susceptible to in-
fection (Riepenhoff-Talty et al., 1983). We studied lacteal antibody responses
spanning this time frame, plus a few days beyond. To achieve this, different routes
of immunization were evaluated.

Although administration of live or killed BRV at mucosal sites (intestine and
mammary regions) not only induced a significant elevation of IgA, IgM and IgG
antibodies in lacteal secretions there was also a marked increase in serum anti-ro-
tavirus antibodies as determined by ELISA. This might be due to rapid transmis-
sion of the viral antigen from mucosal sites to other body sites. Therefore, the sites
of inoculation of antigen might only serve as a "portal of entry". It could also be
due to spillover of mucosally produced Igs into the circulation (Chang et al., 1981).

The most prominent feature of this study and other studies, employing the mu-
rine model (Halsey et al., 1982), is the increase of milk antibody production ap-
proximately 9 days following parturition. The increase in milk Igs on day 9 could
occur as a result of the production of antibodies by sensitized cells residing within
the mammary glands, which are turned on by hormones along with the influx of
antibodies from the serum (Halsey et al., 1982). It has been shown by Halsey et
al. (1982) that such a transfer does take place in the mouse with as much as 80%
of the milk IgA on the fourth day of lactation coming from serum as compared to
20% on the eighth day. The rest of the IgA is likely produced locally by sensitized
cells, in the mouse model. In contrast, there is no evidence for local production of
IgA in the mammary gland in early or mid-lactation in sheep (A.J. Husband et al.,
1986; 6th International Congress of Immunology, Toronto, Canada, 1.43.4). Han-
son et al. (1983) could not observe any transfer of dimeric IgA from the circula-
tory system to milk in rats. Bohl and Saif (1975) vaccinated pregnant cows via the
IMam route with live attenuated transmissible gastroenteritis (TGE) virus and
found that antibodies in milk from these animals were primarily of the IgG class.
In contrast, after natural infection with TGE virus via the oral route, they ob-
served that the antibodies in lacteal secretions are mainly of the IgA class (Bohl
et al., 1972). Thus, it suggests that the route of infection or vaccinations with virus
may influence the Ig class in secretions. Although in the present study the majority
of antibody in milk was of the IgG class, in natural infection with rotavirus it is
the secretory IgA which is closely related to protection in mice and humans be-
cause of its resistance to degradation by trypsin, chymotrypsins, and pepsin (Offit
and Clark, 1985).

The reason for relatively higher IgM titers than IgA in lacteal secretions is not clear. It could, however, reflect the primary nature of the immune response, as rotavirus-free mice were used in this study. Also in the case of IMam route of immunization, antigen was administered intradermally in the mammary regions.

Even though the IgM and IgG antibodies were predominant in lacteal secretions, their effectiveness in preventing enteric infection in mice has been questioned (Newby, 1984). Since IgA is not absorbed from the intestine, it will remain locally and therefore should be more effective in preventing infection as compared to IgG and IgM which are absorbed (Hanson et al., 1983).

In rats and mice, milk antibody is probably locally produced since a marked number of Ig-containing cells are present in the mammary gland from late pregnancy through parturition and lactation to involution (Lee et al., 1979). Thus, inoculation of antigen(s) into the mammary gland of the rat during pregnancy results in an increase of specific Ig-containing cells of IgA and IgG subtype (Lee et al., 1979). In contrast, the majority of milk Igs in ruminants, are essentially serum-derived, and the lactating gland contains few Ig-containing cells (Saif and Smith, 1983). Thus the physiology of the mammary gland of the specific species being immunized must be taken into consideration when designing immunization protocols for controlling enteric infections in neonates.

The results of our study do not correlate with the work of Offit and Clark (1985) particularly regarding the antibody titer in serum and milk. Following oral immunization they reported that anti-rotavirus antibody response in serum and milk, even with homotypic virus, was approximately 15-fold and 80-fold lower, than that found after parenteral inoculation. Whereas in our study, different routes of immunization did not seem to make any difference as far as antibody titer in serum and milk were concerned. The plaque reduction neutralization (PRN) titer of milk in particular was much lower (3 $\log_{10}$) after oral immunization compared to parenteral immunization (Offit and Clark, 1985). Even though one would expect high antibody titer in milk when immunizations are carried out using the mucosal versus the parenteral route. No explanation for this obvious difference was given by the authors. Whether the differences in our study and their work is related to different quantities and quality of antigen remains to be determined.

Further studies are required to explore possible factors influencing the regulation of lactogenic antibody response in mice and other animals against rotavirus if effective control measures are to be implemented. These studies must address the effect of hormones and neuropeptides on the development of immunity and secretion of Igs (Diamond, 1982; Halsey et al., 1982; Stanisz et al., 1986; Weisz-Carrington, 1978). Recent studies clearly indicate that neuropeptides can modulate in vitro immune response either in a positive or a negative manner. Furthermore, different antibody subtypes may be altered more than others (Stanisz et al., 1986; 6th International Congress of Immunology, Toronto, Ontario, Canada, 3.63.44). These studies clearly indicate the potential of regulating specific lactogenic immune responses by neuroimmunoregulation. The role of neuropeptides and hormones in the regulation of anti-rotavirus lactogenic immunity is presently under investigation. Finally, selective production of IgA by an antisuppressor (AS)

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mechanism may selectively enhance IgA production and thereby contribute to intestinal and lactogenic immune regulation (Ernst et al. 1986; 6th International Congress of Immunology, Toronto, Canada, 3.27.16). An understanding of these mechanisms and appropriate exploitation should greatly aid in the enhancement of rotavirus immunity in the neonate and newborn and thus have a tremendous impact on the morbidity and mortality due to rotavirus infections in animals and humans.

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