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Transmission of maternal antibody prenatally and from milk into serum of neonatal rabbits

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SUMMARY

Rabbit dams fed 0·1% BSA for various periods before and during lactation produced anti-BSA of low avidity and IgG isotype in serum and milk. Milk anti-BSA and IgG concentrations were one-third to one-half of those in the serum. At birth, kits had IgG and anti-BSA serum concentrations approximately equal to their dams. Both fell rapidly for the first 10–20 days, levelling off at about 1 mg IgG/ml. Kits born to unimmunized dams and suckled by dams with anti-BSA in the milk showed increasing anti-BSA in serum for the first 12–16 days, falling by 20 days. Foster-suckling on immunized dams beginning at various times after birth showed antibody uptake from birth through 12 days of age. Thus immunoglobulins are among factors absorbed from milk that have potential for regulating the immune responses of rabbit neonates.

INTRODUCTION

Transmission of immunoglobulins between rabbit dam and kit is said by some to been entirely *in utero* through the yolk sac splanchnopleur (Brambell *et al.*, 1949; Kleinman *et al.*, 1983). It is generally thought that the rabbit neonate, unlike the rodent (Kraehenbuhl & Campiche, 1969; Culbertson, 1939) does not absorb milk immunoglobulins through the gastrointestinal tract (Sonoda & Schlamowitz, 1972), but Adler & Adler (1982) demonstrated considerable uptake of foreign Ig allotype from milk in rabbits. In the course of foster-suckling experiments on immunized dams, we detected anti-BSA in the serum of rabbit kits born to unimmunized dams. This suggested that the suppression of immune response to ingested BSA in kits nursed by an orally immunized dam (Peri & Rothberg, 1981) might relate to specific antibody or other factors absorbed from the milk. The present study confirms maternal IgG anti-BSA transmission through milk into the circulation of nursing kits.

MATERIALS AND METHODS

Animals

Rabbits derived from a cross between New Zealand White (NZW) and Flemish strains were kindly supplied by Dr Kay Knight, University of Illinois Medical Center, Chicago, from a closed colony maintained for allotype uniformity. They were bred and maintained as a closed colony in our animal facility.

Immunization

Female rabbits were given tap water containing 0·1% bovine serum albumin (BSA) (Fraction V from bovine plasma, Armour Pharmaceutical Co., Kankakee, IL) *ad libitum* for various periods prior to or during nursing. Females ingested 300–350 mg BSA/day, increasing to as much as 1000 mg/day when fed during lactation. Dams A and B were fed as young adults for two 10-day periods, and kits were born 3 months after the second feed. Dam C was similarly fed and then given 1 day of BSA ingestion 18 days before delivery. Dam D was fed for three 10-day periods before breeding, and again for 3 days at 3 weeks and 2 weeks before delivery. Females E and F were fed at the age of 20–30 days, then injected i.m. with 5 mg BSA in incomplete Freund's adjuvant at 49 days of age. They were bred when 8 months old, and fed for 3 days at 3 weeks and 2 weeks before delivery. Dam G is Dam E in a subsequent pregnancy, fed from 1 week prior to 2 weeks after delivery. Dam H was fed for 21 days prior to breeding and from 1 week prior to 2 weeks after delivery. Milk and serum samples were obtained and treated as previously described (Peri & Rothberg 1981). Kits were bled by cardiac puncture under ether anaesthesia for the first 16 days, and from the marginal ear vein thereafter. At 4 or more days of age, kits nearly always recovered from cardiac puncture and individuals could be bled repeatedly.

Antibody

Anti-BSA was measured in individual samples by a modified Farr test (Minden & Farr, 1978). Radioloabelled BSA antigen was used at a final concentration of 20 ng or 2 ng BSA-N/ml, and antibody-binding calculated as the ABC-33. Antibody avidity in samples was compared using the effect of dilution (ED), calculated by the formula:

$$ED = \frac{\text{ABD-33 at 2 ng N}}{\text{ABD-33 at 20 ng N}} \times 100.$$

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The concentration of IgG was measured in radial immunodiffusion plates (Miles Laboratories, Elkhart, IN). The isotype of anti-BSA was determined by fractionation on an Ultrogel ACA-34 column (Peri *et al.* 1982), followed by precipitin ring tests with specific anti-rabbit IgG or IgA (Miles Laboratories). Samples were also tested by absorbtion on a Protein A-Sepharose column (Pharmacia Inc., Piscataway, NJ) and elution in 3 M sodium thiocyanate, followed by measurement of antibody in the fractions. Antibody specificity was confirmed by total inhibition of binding of ^{125}I-BSA with small amounts of unlabelled BSA.

Experimental design

Kits were exchanged at birth between unimmunized dams and orally immunized dams with various levels of anti-BSA. Samples of milk and serum from dams and serum from kits were usually taken at weekly intervals. The timing of immunoglobulin absorption from milk was studied in a second group of kits. Two animals from each litter were exchanged between three immunized and two unimmunized dams on Days 0, 4, 8 and 12 after birth. Kits were bled and dams bled and milked at birth and at 4-day intervals thereafter. Kits were individually weighed at intervals to monitor growth.

Statistics

Mean ± SEM were calculated from individual samples, and significance between groups was determined by Student's *t*-test.

RESULTS

Growth of kits

The NZW–Flemish rabbit is somewhat larger than the NZW, and these kits grew faster (mean gain of 20 g/day) than the average gain of 9·3 g/day for NZW rabbit kits from birth to 2 weeks (Rao *et al.*, 1977). The size of the litter born or suckled made a considerable difference in the weights, but there was no significant difference between foster-suckled kits and their littermates suckled by their own dam.

Concentration of IgG

Since rabbits fed BSA produce primarily IgG anti-BSA in serum and colostrum or milk (Peri *et al.*, 1982), total IgG was measured. Kit serum IgG at birth varied within a litter, but the mean level was approximately equal to that of their dam. A rapid decrease in IgG occurred for the first 10 or more days, depending on the initial concentration, levelling off at about 1–2 mg/ml (Fig. 1).

At parturition, dam serum IgG levels varied from 2 to 12 mg/ml and this usually increased during lactation. Milk IgG concentrations averaged from one-quarter to one-half that of the corresponding serum, ranging from 1·3 to 4 mg/ml. Colostrum collected immediately after delivery had IgG levels equal to or slightly lower than that in milk collected later in lactation.

Isotype of anti-BSA

Anti-BSA in various milk samples and in serum from dams and kits was found to be almost entirely IgG. A few milk samples had anti-BSA in the IgA-containing fractions, ranging from trace amounts to 3% of the total anti-BSA.

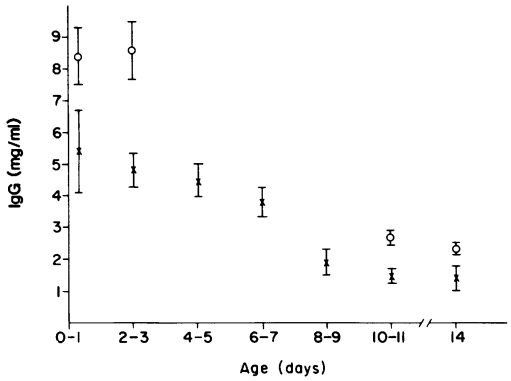

Figure 1. Rabbit kit serum IgG concentration. Mean ± SEM of four to eight kits from six dams with higher serum IgG levels (○), and from six to 11 kits from six litters from older dams with lower serum IgG(×).

Anti-BSA concentration in dams

Anti-BSA levels in milk and serum of selected, individual orally immunized dams are compared in Table 1. As with IgG, milk levels of antibody were one-quarter to one-half of those in serum. The ratio of milk to serum antibody and the amount of anti-BSA in most individuals remained relatively constant or decreased somewhat during the first 14 days in dams fed BSA only prior to pregnancy (Dams A–C).

In the group of hyperimmunized dams fed for several periods during pregnancy (Dams D–F) or prior to and during lactation (G–H), both milk and serum antibody increased, but again the ratio remained fairly constant during lactation.

Anti-BSA concentration in kits

As with IgG, kits born to an immunized dam had anti-BSA serum concentrations at birth varying within a litter, some greater and some less than the serum concentration of their dam. Figure 2 shows two representative litters from a total of eight litters followed from birth. As shown, kits in some litters had serum antibody concentrations greater than their dam's. The decline in antibody for the first 20–30 days (Fig. 2) was similar to the decline in IgG (Fig. 1). As might be expected from the relatively constant milk/serum relationship of anti-BSA in the dam, the amount of antibody in the kit's serum was closely related to the milk as well as the serum antibody concentration of their dams (Fig. 3). Immunization schedules of the dams did not detectably affect the ratio of milk to kit serum antibody.

In order to investigate the amounts of anti-BSA from milk passing into kit's serum, kits born to unimmunized dams were foster-suckled by immunized dams from birth. Figure 4 presents the data from two representative litters showing increasing antibody levels until 16 days of age, with an abrupt drop thereafter. Similar transfer of antibody was observed in other litters regardless of the dam's immunization schedule. Two additional litters were exchanged at birth between dams with different milk anti-BSA titres. Table 2 compares antibody in individual kits suckled by their own dam with that in littermates

Table 1. Anti-BSA concentration in rabbit milk and serum during lactation

	Days postpartum								
	0			7			14		
Dam*	Milk	Serum	Ratio	Milk	Serum	Ratio	Milk	Serum	Ratio
A	0·9†	2·7†	0·33	1·3	2·4	0·54	0·5	1·9	0·26
B	0·8	2·2	0·36	0·7	1·9	0·37	0·5	1·3	0·38
C	3·3	6·5	0·51	2·1	4·2	0·50	1·0	3·7	0·27
D	1·1	4·8	0·23	1·9	4·7	0·40	1·5	4·4	0·34
E	5·5	19·2	0·29	10·0	33·3	0·30	11·5	36·5	0·32
F	6·9	16·5	0·42	7·5	19·5	0·38	6·7	22·1	0·30
G	15·8	52·2	0·30		ND‡		25·7	93·2	0·28
H	3·0	6·1	0·49		ND		9·5	24·5	0·39

* Dams A–C fed 0·1% BSA prior to pregnancy: Dams D–F immunized previously, then fed BSA on Days 5–7 and 11–12 of pregnancy; Dams G–H fed continuously prior to and during lactation.

† ABC-33 in μg N bound/ml at 20 ng BSA N/ml.

‡ ND, not determined.

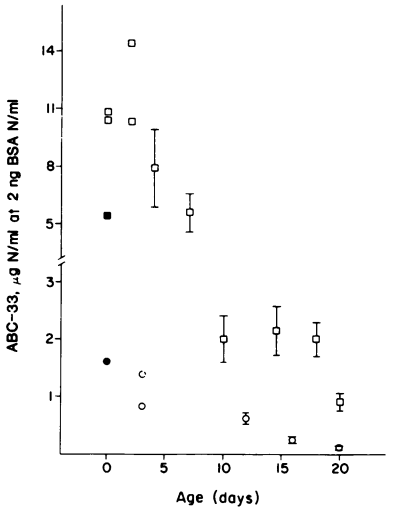

Figure 2. Decline in kit serum anti-BSA when suckled by their own immunized dam. Points represent individual kits on Day 0 and 3, and mean ± SEM of five to seven kits thereafter; (●) Dam D, (■) Dam E serum concentration of anti-BSA.

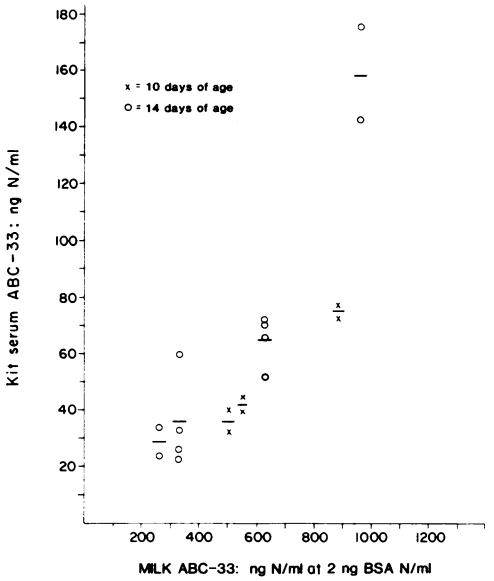

Figure 3. Ratio of kit serum anti-BSA to milk antibody titre of their immunized dams A, B, C and E. Bar is mean of each group.

on a foster dam. Although starting with similar amounts of antibody, by 14 days of age, kits ingesting milk with more anti-BSA had two to three times as much serum anti-BSA as their littermates.

Table 3 compares anti-BSA in kits from unimmunized dams first transferred to an immunized foster dam at 4-day intervals after birth to determine when uptake was occurring. Antibody was present at 4 days of age in kits transferred at birth, while maximum serum concentrations occurred between 12 days and 16 days, and small amounts of antibody were taken up even when first foster suckled at 12 days of age.

Antibody avidity

When the effect of dilution (ED) (Minden & Farr, 1978) was calculated to determine antibody avidity, the anti-BSA in milk at parturition had an ED of 19–45, while that of serum ranged from 15 to 64. Antibody in milk and serum of a particular dam had similar avidity, however, and did not change appreciably throughout lactation. The ED of kit serum anti-BSA corresponded closely to that of their dam's antibody, whether it was derived prenatally, solely from milk, or both. It should be noted that this low avidity results in a two- to six-fold lower ABC when measured at 2 ng BSA N than would be obtained with 20 ng BSA N. The lower antigen concentration was used in Figs 2, 3 and 4 and in Table 3 to allow calculation of an ABC with small amounts of antibody, and therefore the ABC of dam's milk differs from that of the same dam in Table 1, measured at higher antigen concentration.

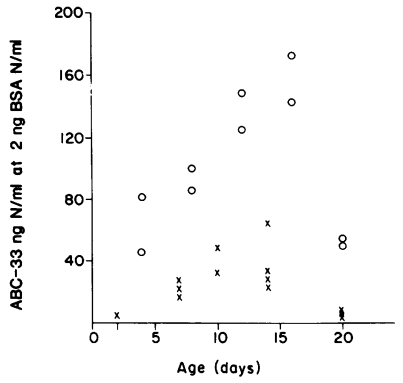

Figure 4. Anti-BSA uptake from milk by rabbit kits. Serum anti-BSA in randomly selected individual kits (×) from a litter born to an unimmunized dam and suckled by immunized Dam A from birth (milk ABC = 535 ng N/ml). Two kits (○) from an unimmunized dam suckled on hyperimmunized Dam E (milk ABC = 2629 ng N/ml at birth). Samples from same kits each time.

Table 2. Decline of passive anti-BSA in kit serum

	Milk antibody*	Kit antibody† at 14 days	Kit antibody at 20 days
Litter H	3·0–9·5	0·65	0·44
nursed on		0·80	0·82
Dam H		0·71	0·48
		0·63	0·46
Litter H	15·8–25·7	2·68	1·56
nursed on		2·43	1·24
Dam G		2·36	1·38
		1·41	1·15
Litter G	3·0–9·5	10·77	6·71
nursed on		9·16	6·05
Dam H		2·13	1·84
Litter G	15·8–25·7	20·13	15·07
nursed on		13·40	8·21
Dam G			

* Titre of milk ingested by kit at birth and at 20 days; dams fed BSA during the first 14 days. ABC-33 in μg N bound/ml at 20 ng BSA N/ml.

† ABC-33 in individual kit serum. Kits were suckled by their own immunized dam or a dam with a different milk anti-BSA content.

Table 3. Timing of anti-BSA uptake from milk

	Days after birth					
	0	4	8	12	16	20
Unimmunized	0*	47	85	125	143	55
litter on	0	81	100	150	176	53
Dam E		0	38	211	212	36
			0	78	46	35
				0	21	13
Dam's milk	2629†	2885	1268		959	
Unimmunized	0	0	0	2	0	0
litter on	0	0	11	4	ND‡	ND
Dam D		0	3	11	8	5
		0	ND	7	5	0
			0	4	4	3
			0	7	ND	ND
				0	2	2
Dam's milk	354	431	580		262	

* Serum ABC-33 in ng N/ml at 2 ng BSA N/ml in individual rabbit kits transferred from normal to immunized dam at the time indicated by initial 0.

† Milk ABC-33 in ng N/ml at 2 ng BSA N/nl.

‡ ND, not determined.

DISCUSSION

Serum concentrations of IgG and anti-BSA in nursing rabbit dams remained fairly constant during the first 2 weeks of lactation at the same time that large amounts were being excreted in the milk. Thus, although serum IgG is lower during pregnancy in the rabbit (Peri *et al.*, 1982), after parturition there must be a rapid increase in IgG production, including specific anti-BSA, and probably increases in other immunoglobulins as well, to compensate for the loss into milk. These fluctuations would appear to be hormonally controlled. The serum anti-BSA produced during lactation was lower in avidity (ED = 15–64) than in non-pregnant rabbits (ED = 43–91) (Rieger, Kraft – Rothberg, 1980), suggesting another change in antibody production during pregnancy. There seemed to be no selection for higher or lower avidity antibody during transport into milk or uptake through kit mucosa, since antibody avidity in milk and kit serum corresponded to that in dam serum.

In newborn kits, both passively acquired IgG and anti-BSA were shown to fall rapidly after birth. At the same time, the cross-suckling experiments demonstrated that anti-BSA, and by inference, other IgG molecules, were being absorbed from the milk into serum. Thus, the IgG measured in kits represents rapidly decreasing passive immunoglobulin acquired *in utero* plus IgG derived from milk. The kit serum anti-BSA absorbed from milk, which was >98% IgG, reached a concentration of 10–15% of that in the milk ingested, and absorption seemed to have slowed down markedly or stopped by Day 20 (Fig. 4). This small amount of uptake superimposed on the rapid decline in the large amount of prenatally acquired immunoglobulin probably explains the belief that no uptake from milk occurs in rabbits. However, maternal IgG of a foreign allotype acquired solely by nursing has a concentration of 300–600 mg/ml in serum of 3-week-old rabbits, but was not measured at earlier ages (Adler & Adler, 1982). Assuming total IgG concentrations of 1–2 mg/ml in the serum of these kits (Fig. 1; Diechmiller & Dixon, 1960), between 15% and 60% of these kits' IgG would be derived from milk. At that age, our kits were well past the peak concentration of anti-BSA absorbed from milk (Fig. 4 and Table 3), suggesting a greater absorption of IgG at ages earlier than 3 weeks. However, comparison of allotypic measurements with specific antigen-binding capacity may be misleading, since the epitopes could be affected differently by digestive processes.

Cells producing IgG are present in considerable numbers in the mammary gland (Peri *et al.*, 1982), but their origin is unknown. Antigen stimulation may have occurred locally or they may have migrated from other lymphoid sites. Dams fed

during pregnancy or lactation are probably absorbing minimal amounts of BSA into the circulation, as do non-pregnant rabbits (Rothberg, Kraft & Farr, 1967). This BSA would complex with the large amounts of antibody present in their serum. Experiments are in progress to investigate the possible transfer of BSA into milk and into the kits.

In rabbits, IgG in milk has the potential to be a major factor affecting systemic immune responses of kits, as well as the protection of mucosal surfaces from environmental antigens, which is considered to be an important function of milk immunoglobulins (Walker *et al.*, 1975). Factors in milk, including IgG, suppress the IgE response in neonatal rats (Jarrett & Hall, 1979; Roberts & Turner, 1983) and the plaque-forming cell response in mice (Auerbach & Clark, 1975). The suppression in IgG responses we observe in rabbit kits differs from these, in that it follows oral, but not parenteral, immunization of the dam, and in the lack of correlation with anti-BSA concentrations in the dam (Peri & Rothberg, 1981). However, very small amounts of anti-BSA might be all that are required for regulation in kits, or regulatory factors other than IgG could be taken up from milk by neonates. Further experiments will explore this possibility.

In human infants, immunoglobulin absorption through the intestine may also occur (Iyengar & Selvaraj, 1972) and could play a role in the regulation of immune responses as well as in mucosal protection.

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