ORIGIN OF IgA-SECRETING PLASMA CELLS IN THE MAMMARY GLAND*

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Secretory IgA (sIgA),[1] the predominant antibody in milk of most species, is not absorbed after ingestion by the nursing infant (1, 2), but acts locally in the intestinal tract to protect against infections. To perform such a role, the antibodies must have immunological specificity for the antigens of intestinal microorganisms. Indeed, several observations (3–8) support the idea that the various specificities of milk sIgA reflect prior antigenic encounters in the intestinal tract. For example, neutralizing antibodies against the enterotoxins of *Escherichia coli* and *Vibrio cholerae* have been found in human milk (3, 4). Furthermore, the results of colonization experiments in pregnant women suggest that lymphoid cells in the gut-associated lymphoid tissue (GALT) home to the mammary gland where they produce IgA antibodies to intestinal microorganisms (5). A similar suggestion has been made to account for the appearance of specific sIgA in milk after oral immunization of rabbits (7).

In mice, as in humans and rabbits, the predominant Ig in milk is IgA (2, 9, 10). Its probable source is the local plasma cells which lie adjacent to the glandular epithelium of the breast and which dramatically increase in number late in pregnancy and during lactation (11). Because IgA plasma cells in the intestinal tract appear to be derived from precursors which emigrate from mesenteric lymph nodes (MN) (12, 13) and because IgA plasma cells in mammary gland have been suggested to originate from precursors in the GALT, we decided to test directly whether MN could seed the mammary gland with IgA plasma cells. We found that MN lymphoblasts do home to the mammary gland, but only in the latter stages of pregnancy and during lactation. Moreover, they give rise to IgA plasma cells at sites where these are normally found. Our results thus provide an explanation for the observation (3-6) that IgA antibodies in milk are specific for antigens of the intestinal microflora.

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[1] *Abbreviations used in this paper:* AR, autoradiography; CRL, complement receptor lymphocytes; GALT, gut-associated lymphoid tissue; IF, immunofluorescence; ^{125}IUDR, [^{125}I]iododeoxyuridine; MN, mesenteric lymph node; PI, preference index; PN, peripheral lymph nodes; PP, Peyer's patches; sIgA, secretory immunoglobulin A.

Materials and Methods

Mice. CAF_1/J mice of both sexes, obtained from The Jackson Laboratory (Bar Harbor, Maine) at 7–9 wk of age, were bred in the animal facilities of New York University Medical Center. The stage of pregnancy was determined according to methods described in (14).

Cell Transfers. The procedure used is fully described in (15). Suspensions of cells from virgin young adult females were prepared from MN or peripheral lymph nodes (PN) in RPMI 1640 medium with HEPES buffer (Grand Island Biological Co., Grand Island, N. Y.) at 0°C. They were incubated for 90 min at 37°C in medium containing 10% fetal calf serum (Grand Island Biological Co.) and 2 μCi/ml of ^{125}I-iododeoxyuridine (^{125}IUDR; Amersham/Searle Corp., Arlington Heights, Ill.) to label cells synthesizing DNA. Occasionally, the whole population was labeled by incorporating 20 μCi/ml of ^{51}Cr (as sodium chromate; Amersham/Searle Corp.) into the labeling medium for the last 30 min of incubation. Alternatively, 10 μCi/ml of [^{3}H]uridine (New England Nuclear, Boston, Mass.) alone was used. The cells were then washed three times in ice cold RPMI 1640 medium and filtered twice through nylon wool to prepare suspensions of single viable cells free of unbound label. Approximately 10^7 cells/recipient were transferred intravenously. In some experiments selectively-depleted populations were transferred. Populations depleted of cells bearing surface Ig were prepared by exposing labeled MN cells to rabbit antiserum specific for mouse IgA, IgG, or IgM, plus guinea pig complement, for 45 min at 37°C in medium 199 (Grand Island Biological Co.). Populations depleted of complement-receptor lymphocytes (CRL) were prepared by removing rosette-forming cells on a discontinuous bovine serum albumin gradient (16). Suspensions to be injected were washed and filtered as above. In all cases the viability of the transferred cells was greater than 90% as assessed by trypan blue dye exclusion. Three or four recipients were injected with each cell population.

Recovery of Label. Organs were weighed as they were removed and gamma activity was measured in a Nuclear Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.). ^{3}H activity was extracted with hot trichloroacetic acid and measured in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) (15). Radioactivity recovered in recipients is expressed as a percentage of that injected.

Antisera. The preparation and specificity of these reagents are fully described in (17). Rabbit antisera to mouse IgA, IgG, and IgM were raised against the respective heavy chains isolated from partially reduced and alkylated proteins. These antisera and normal rabbit serum were absorbed with mouse thymic leukemia cells (L1210) before use. All antisera, with the exception of anti-IgA, were cytotoxic for MN cells in the standard dye exclusion assay (17). Anti-IgA antiserum was not cytotoxic by this criterion since only a small percentage of the entire MN cell population bears IgA, and death of these cells, if it occurred, could not be distinguished from background. However, the anti-IgA antiserum was specifically cytotoxic to the population of MN blast cells in its effects on homing (see Results). The specificity of the anti-IgA antiserum was checked by absorption with the IgA myeloma protein TEPC-15 coupled to APB-cellulose (18). Absorption removed all anti-IgA activity detectable by both Ouchterlony analysis and effects on the homing cells.

Immunofluorescence and Autoradiography. Indirect immunofluorescence (IF) was used. Combined immunofluorescence and autoradiography (AR) was performed as previously described (13).

Results

Distribution of Radioactivity in Recipients of ^{125}IUDR-Labeled Cells. The percentages of injected radioactivity recovered from recipient mammary glands (and small intestines) 16–24 h after transfer of ^{125}IUDR-labeled MN and PN cells to virgin, pregnant, lactating, and postlactating mice are presented in Table I. Near term and during lactation, recovery of label in mammary glands is greater in recipients of MN cells than of PN cells. This preferential seeking of the mammary gland by MN lymphoblasts is conveniently expressed by the preference index (PI), defined as the ratio of percentage recovery of injected radioactivity per gram of mammary gland in recipients of MN cells versus recipients of PN cells. A PI above 1 indicates a greater tendency of MN cells

TABLE I

*Percent Injected Label Recovered after Transfer of ^{125}IUDR-Labeled MN and PN Cells to Virgin, Pregnant, Lactating, and Postlactating Mice**

Recipient mice	Exp.	Donor cells	Mammary gland		PI‡	Small intestine
			%/g	%/organ		%/organ
Virgin	1	MN	2.7 ± 2.1	1.6 ± 1.3	1.2	5.7 ± 0.3
		PN	2.2 ± 0.7	0.9 ± 0.2		4.3 ± 0.2
	2	MN	0.4 ± 0.2	0.4 ± 0.2	1.0	4.6 ± 0.8
		PN	0.4 ± 0.1	0.4 ± 0.1		2.6 ± 0.5
	3	MN	0.5 ± 0.2	0.5 ± 0.3	1.2	8.0 ± 1.4
		PN	0.4 ± 0.1	0.6 ± 0.3		1.2 ± 0.2
9 days antepartum	1	MN	0.3 ± 0.3	0.2 ± 0.1	1.5	4.6 ± 1.2
		PN	0.2 ± 0.1	0.1 ± 0.1		0.6 ± 0.1
	2	MN	1.3 ± 0.8	1.0 ± 0.6	1.4	3.1 ± 0.6
		PN	0.9 ± 1.3	0.9 ± 1.2		1.8 ± 1.1
1 day antepartum	1	MN	0.8 ± 0.4	1.1 ± 0.4	4.0	3.1 ± 0.5
		PN	0.2 ± 0.1	0.4 ± 0.2		0.8 ± 0.1
	2	MN	3.1 ± 2.0	4.2 ± 2.9	10.3	4.9 ± 0.5
		PN	0.3 ± 0.0	0.3 ± 0.1		1.1 ± 0.2
4 days postpartum (lactating)	1	MN	2.2 ± 0.4	3.3 ± 0.9	2.8	3.4 ± 0.4
		PN	0.8 ± 0.4	1.1 ± 0.5		1.4 ± 0.2
	2	MN	0.6 ± 0.1	1.0 ± 0.2	3.0	5.8 ± 2.2
		PN	0.2 ± 0.1	0.2 ± 0.1		1.4 ± 0.3
	3	MN	1.6 ± 0.2	4.0 ± 0.4	3.2	4.1 ± 0.6
		PN	0.5 ± 0.1	1.3 ± 0.4		1.4 ± 0.2
10 days postpartum (lactating)	1	MN	0.8 ± 0.0	1.5 ± 0.2	1.0	3.9 ± 0.4
		PN	0.8 ± 0.3	1.4 ± 0.5		1.2 ± 0.4
	2	MN	2.3 ± 1.1	3.5 ± 0.9	3.3	5.1 ± 1.8
		PN	0.7 ± 0.0	1.3 ± 0.2		1.5 ± 0.1
	3	MN	1.1 ± 0.3	2.3 ± 0.6	3.7	5.8 ± 0.7
		PN	0.3 ± 0.2	0.6 ± 0.4		2.0 ± 0.2
20 days postpartum (lactating)	1	MN	2.1 ± 1.0	3.6 ± 2.2	5.2	3.4 ± 1.0
		PN	0.4 ± 0.2	0.7 ± 0.4		1.4 ± 0.1
15 days postlactating	1	MN	0.02 ± 0.0	0.03 ± 0.0	1.0	3.7 ± 0.3
		PN	0.02 ± 0.0	0.03 ± 0.0		1.3 ± 0.3
	2	MN	0.2 ± 0.1	0.3 ± 0.2	0.7	4.2 ± 0.3
		PN	0.3 ± 0.1	0.3 ± 0.1		1.3 ± 0.2

* Figures are the means ± 1 SD of three animals.

‡ PI = $\frac{\text{\% injected radioactivity/gram mammary gland in MN recipients}}{\text{\% injected radioactivity/gram mammary gland in PN recipients}}$

than PN cells to migrate to the mammary glands, i.e., homing. An index near 1 indicates no difference in the behavior of donor cells from the two sources. Indices between 0.7 and 1.5 were observed in virgin mice, at 9 days antepartum (mid-pregnancy), and at the postlactating stage. In contrast, at 1 day antepartum and throughout the normal period of lactation (20 days), higher indices were observed. The results indicate a marked increase in the ability of mammary gland tissue during late pregnancy and lactation to attract and/or retain MN lymphoblasts. This homing tendency by MN blasts is evident whether the index is calculated on a per gram of tissue basis, as in Table I, or on a per organ basis, a potentially important consideration since the glands increase in size somewhat variably during pregnancy and lactation.

TABLE II

Combined Recovery of Radioactivity in Mammary Glands and Small Intestine of Lactating vs. Nonlactating Mice Injected with the Same Population of MN Blasts

Exp.	Recipient mice	Mammary glands	Small intestine	Mammary glands plus small intestine
		%/organ	*%/organ*	
1	4 days postpartum (lactating)	4.0 ± 0.4	4.1 ± 0.6	8.1
	Virgin	0.4 ± 0.2	4.6 ± 0.8	5.0
2	10 days postpartum (lactating)	9.4 ± 1.7	6.2 ± 1.6	15.6
	Virgin	0.4 ± 0.2	5.7 ± 1.2	6.1
3	20 days postpartum (lactating)	3.6 ± 2.2	3.4 ± 1.0	7.0
	Postlactating	0.3 ± 0.2	4.2 ± 0.3	4.5

The percentage of label recovered from the small intestine is also given in Table I. In agreement with previously reported data (12, 15), MN blasts evidenced a greater tendency than PN blasts to go to this site. Homing occurred to the small intestine at all times.

When ^{51}Cr or [^{3}H]uridine was used to label the entire population of injected cells, the distinction between MN and PN cell populations was rarely statistically significant with respect to either lactating mammary gland or small intestine.

To determine whether there is competition between mammary gland and gut for the same population of cells, we compared the distribution of labeled MN blasts in lactating and nonlactating individuals (Table II). In each of three experiments in which lactating and nonlactating mice were injected with the same population of donor cells, the combined recovery of radioactivity in the mammary glands and small intestine was greater in lactating mice. The possible significance of this finding is discussed later.

Occurrence of IgA in Homing Cells in Recipients. Sections of mammary gland taken from mice injected with labeled lymphoblasts late in pregnancy and during lactation were studied by combined AR and IF to determine what proportion of the homing lymphoblasts contained IgA. Table III and Fig. 1 show the results. Although only 5–10% of the labeled MN cells showed IgA in the cytoplasm before transfer, the overwhelming majority (84–93%) of the labeled cells found in the mammary glands 16–20 h after transfer of MN blasts contained IgA. On the other hand, labeled cells of PN origin were less frequent in the mammary glands, and although PN blasts could give rise to IgA-positive plasmablasts, they did so much less often than MN blasts.

Effect of Depletion of Ig-Bearing Cells or CRL from the Donor Population. The previous data show that MN blasts are potentially able to seed the mammary gland with precursors of IgA plasma cells. Subsequent experiments were designed to determine whether the MN blasts which home to the mammary gland are precommitted to IgA synthesis, i.e., already produce IgA while still in the MN rather than only after emigration. Antisera to IgA, IgG, or IgM,

TABLE III
Combined IF and AR of Sections of Mammary Glands of Pregnant and Postpartum (Lactating) Mice Injected with ^{125}IUDR-Labeled Cells

Stage of recipient	Source of donor cells	Number of*		IgA-positive donor cells
		Donor cells (AR)	Donor cells with IgA (IF + AR)	
				%
1 day antepartum	MN	172	150	87
	PN	81	24	30
	MN	42	39	93
	PN	27	8	30
1 day postpartum	MN	100	90	90
	PN	45	11	24
	MN	63	54	86
	PN	32	13	41
4 days postpartum	MN	156	143	92
	PN	60	21	35
	MN	91	80	88
	PN	45	20	44
10 days postpartum	MN	93	84	90
	PN	33	15	45
	MN	101	85	84
	PN	43	21	49

* Averages per section of the mammary glands of three recipients.

plus complement, were used to treat labeled cell populations before transfer. Results of such experiments are shown in Table IV. Anti-IgA antiserum markedly diminished homing to the mammary gland and small intestine, though it consistently gave a cytotoxic index close to zero. This probably reflects the fact that cells bearing IgA occur in limited numbers in the population of MN cells taken as a whole, though they represent the bulk of the homing cells. Anti-IgG and anti-IgM antisera, on the other hand, were demonstrably cytotoxic but had no effect on homing. Rinsing the cells at 37°C (experiment 2) to promote elution of passively adsorbed IgA (13, 19, 20) before treatment with anti-IgA antiserum did not alter its effect on homing. Absorption of the antiserum with insoluble IgA (experiment 6) did remove its activity.

The effect of anti-IgA antiserum plus complement on the subsequent homing of labeled MN blasts was also assessed by combined IF and AR of recipient mammary glands. This treatment reduced both the number of donor cells and the percentage containing IgA (Table V).

Labeled MN cells were also depleted of CRL to determine whether cells homing to the mammary gland lack complement receptors, as do cells homing to the gut (15). In two experiments, removal of more than 95% of the CRL had no effect on homing to either the mammary gland or gut. Evidently, B

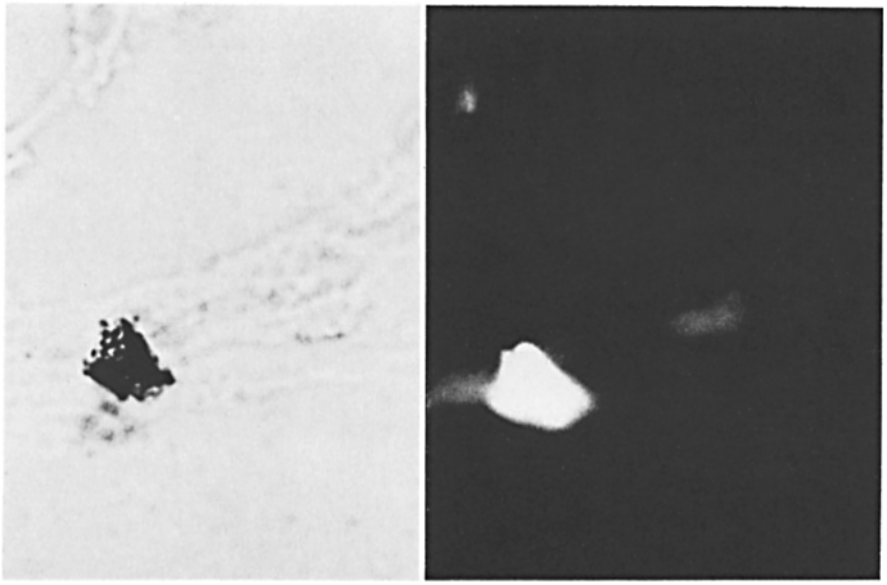

FIG. 1. Combined AR (left) and IF (right) of recipient mammary gland showing a donor cell producing IgA.

TABLE IV

Effects of Various Antisera, Plus Complement, on Homing of ^{125}IUDR-Labeled MN Cells to Mammary Glands of Lactating Mice 6 Days Postpartum

Exp.	Antiserum	Cytotoxic index	Percent injected label recovered in	
			Mammary glands	Small intestine
1	NRS*	–	1.3 ± 0.3	5.0 ± 0.4
	anti-IgA	0	0.7 ± 0.0	2.4 ± 0.6
2‡	NRS	–	4.3 ± 1.1	5.4 ± 0.6
	anti-IgA	0	1.4 ± 0.9	3.0 ± 0.4
3	NRS	–	7.0 ± 1.6	7.1 ± 0.3
	anti-IgA	1	3.5 ± 0.5	4.5 ± 0.7
	anti-IgM	22	6.0 ± 1.2	6.7 ± 0.4
4	NRS	–	3.0 ± 1.2	5.7 ± 0.8
	anti-IgA	0	1.7 ± 1.5	2.8 ± 1.3
	anti-IgG	20	2.7 ± 0.5	4.6 ± 1.0
5	NRS	–	2.2 ± 0.1	4.7 ± 0.3
	anti-IgG	20	3.1 ± 1.6	3.8 ± 0.6
	anti-IgM	38	2.3 ± 0.1	4.6 ± 0.4
6	NRS	–	7.7 ± 2.9	4.9 ± 0.7
	anti-IgA	0	2.1 ± 0.2	4.0 ± 0.1
	anti-IgA absorbed with insoluble IgA	0	8.0 ± 1.1	5.0 ± 0.1

* Normal rabbit serum.

‡ In experiment 2 the cells were rinsed three times at 37°C, instead of at 0°C as in the other experiments, before treatment.

TABLE V
Combined IF and AR of Sections of Mammary Glands of Lactating Mice Injected with ^{125}IUDR-Labeled MN Cells 6 Days Postpartum

Treatment of donor cells	Number of donor cells (AR)*	Number of donor cells with IgA (AR + IF)*	IgA-positive donor cells
			%
NRS	27	20	74
Anti-IgA	12	3	25

* Averages per section of the mammary glands of three recipients.

lymphoblasts homing to the mammary gland and small intestine are alike in bearing surface IgA and lacking complement receptors.

Discussion

Secretory IgA antibodies specific for antigens of gastrointestinal microorganisms can be found in milk after oral immunization (3–7). In the GALT, B cells are selectively committed to IgA synthesis (9, 10, 12, 13, 21–30). These observations led to the suggestion that intestinal exposure to microorganisms stimulates gut-associated lymphoid cells which migrate to the mammary glands and contribute to the antibodies in milk (5, 7). Until now, direct proof that GALT cells can migrate to the mammary gland has been lacking. In the present work, we have shown that MN lymphoblasts already committed to IgA synthesis can home to the mouse mammary gland late in pregnancy and throughout lactation, but not to the resting mammary gland.

We believe that the homing cells are members of the same population which regularly homes to the small intestine throughout adult life and gives rise to the large numbers of IgA plasma cells found normally in the lamina propria. The evidence is as follows:

(*a*) The ability to home to both mammary gland and small intestine is a property of MN blast cells, but not resting small lymphocytes.

(*b*) Pretreatment with antiserum to IgA, but not to IgG or IgM, in conjunction with complement, prevents homing of MN lymphoblasts to both mammary gland and gut. This treatment is effective after passively adsorbed surface Ig is eluted by thorough washing of the donor cells at 37°C. These results imply that only IgA-bearing cells home and that their surface IgA is endogenous.

(*c*) As in the case of cells homing to the gut (12, 13), a large proportion of lymphocytes homing to the mammary gland matures into IgA-containing plasmablasts within hours of transfer. In both organs the histological location of the homing donor cells is the same as that of host IgA plasma cells. After treatment with anti-IgA antiserum, relatively few of the transferred cells lodging in either organ contain IgA.

(*d*) The B blasts which home to the mammary gland, like those which home to the gut (15), lack receptors for complement.

(*e*) The spectrum of antibody specificities produced by IgA plasma cells in mammary gland and intestine is similar (3–6).

One piece of evidence seemingly contradicts the hypothesis that one cell type

homes to both mammary gland and intestine. On this hypothesis one might expect, given a limited number of cells capable of homing to either location, that they would be distributed between these organs in lactating recipients and would accumulate in the intestine of nonlactating recipients. In fact, however, a larger fraction of cell-associated radioactivity is recovered from the mammary glands and small intestine of lactating recipients than from the combined tissues of nonlactating recipients (Table II). This result could be taken to imply that separate populations of MN blasts are capable of homing to the gut versus the mammary gland. However, we prefer another explanation, i.e., that homing is never entirely efficient, and in the presence of increased trapping tissue more total homing can be observed.

The actual mechanism of homing to either site is not known. Homing to intestine is always observed; in contrast, homing to mammary gland occurs only during late pregnancy and lactation. This correlates with the period when, under natural conditions, the IgA plasma cell population of the gland is increasing most rapidly (11). Thus, homing to the mammary gland, in contrast to the gut, is probably an inducible phenomenon under hormonal control.[2]

The basic mechanism of homing to the gut must be antigen-independent since homing of MN blasts to antigen-free grafts of fetal gut has been described (12, 31, 32). The presence of antigen can, nevertheless, influence homing. For example, primed lymphoblasts making IgA specific for cholera toxoid localize preferentially at sites of antigen deposition in the intestine (33). Antigen, however, is unlikely to play a direct role in homing of GALT cells to the mammary gland since this organ appears not to contain enteric antigens absorbed from the gut (5, 7).

Recent results (34, 35) suggest that competition between inflammatory sites influences the migration of immunoblasts to the gut and, by analogy, might influence migration to the differentiating mammary gland. However, T blasts, not B blasts, account for the inflammation-induced increase of cells in the gut (34). The finding (35) that labeled thoracic duct lymphoblasts from parasite-infested rats accumulated in increased amounts in mammary glands of lactating syngeneic rats probably parallels our own. However, the association of label with particular populations of T or B lymphoblasts was not studied.

Our demonstration that B lymphoblasts committed to IgA synthesis have the potential to leave GALT and home to the mammary gland does not exclude the possibility that T cells derived from GALT can follow a similar pathway. This idea is supported by the finding that T lymphocytes in milk respond selectively to enteric antigens (36, 37). The possibility should be entertained that GALT-derived T cells play a role in development of the secretory IgA system in the mammary gland.

It now seems reasonable to incorporate the mammary gland into a model previously proposed by ourselves and others (9, 12, 27–30, 33, 38) for the circulation of IgA immunocytes to the gut. This model is shown in Fig. 2. Precursors of IgA plasma cells originally encounter antigen in Peyer's patches

[2] Weisz-Carrington, P., M. E. Roux, M. McWilliams, J. M. Phillips-Quagliata, and M. E. Lamm. Hormonal induction of the secretory immune system in the mammary gland. Manuscript submitted for publication.

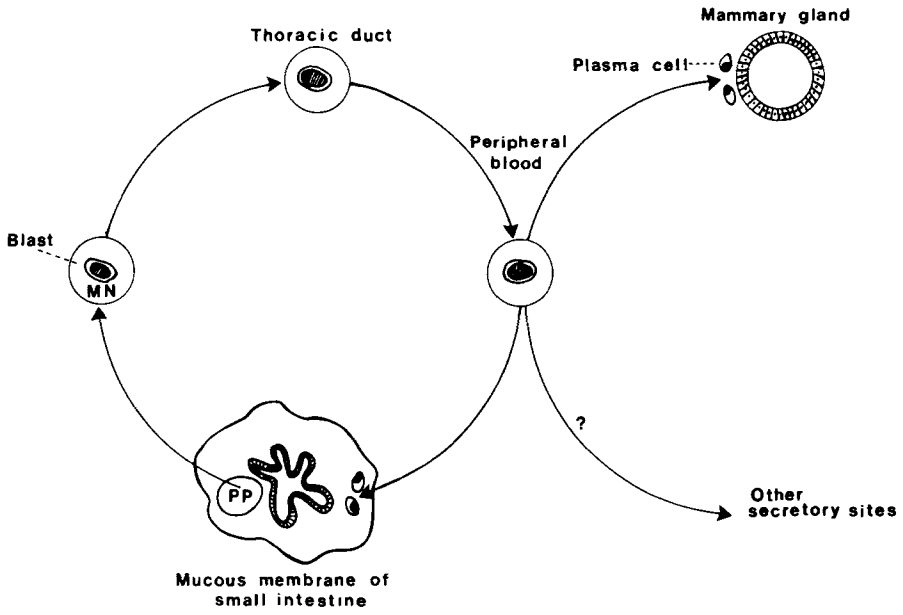

FIG. 2. Model for the circulation of IgA immunocytes to the gut and mammary gland. Lymphocytes originating in PP migrate to MN, where they divide and differentiate. They leave the MN via the thoracic duct and peripheral blood to lodge in the lamina propria of the gut or, during late pregnancy and lactation, the intralobular connective tissue of the mammary gland, where they assume the appearance of plasma cells.

(PP). They migrate to the MN, where they divide and differentiate. The precursors are clearly committed to IgA synthesis before leaving the MN, and probably before leaving the PP. Via the thoracic duct and peripheral blood they reach the lamina propria of the small intestine. During late pregnancy and lactation, these cells also lodge in the periglandular connective tissue of the mammary gland, which is postulated to contain the same receptor as the intestine. In these latter sites, maturation into fully developed IgA plasma cells occurs. Moreover, it is likely that GALT is capable under natural conditions of supplying B cells to other parts of the secretory immune system as well. Indeed, under experimental conditions, PP can repopulate the respiratory tract with IgA plasma cells, and cells from bronchial lymphoid tissue can serve in the same capacity to the gut (27). Under appropriate circumstances exposure to antigen at one secretory surface containing organized lymphoid tissue might thus confer adaptive immunity at other secretory surfaces. This has obvious implications for prophylaxis against disease.

Summary

Lymphoblasts from the mesenteric lymph nodes (MN) of mice home to the mammary glands of syngeneic recipients late in pregnancy and during lactation, and within hours of transfer most can be shown to contain IgA. Homing does not occur in virgins, in early pregnancy, or after weaning. Homing MN lymphoblasts are sensitive to antiserum to IgA plus complement, but not to other class-specific antisera. Thus, lymphoblasts in MN with the potential to

home to the mammary gland are already committed to IgA synthesis and bear surface IgA before reaching their destination. These results explain observations, made by others, of specific IgA antibodies and IgA plasma cells in milk and colostrum after oral immunization. Under natural conditions it is likely that IgA precursor cells, after stimulation in the gut-associated lymphoid tissue by intestinal antigens, migrate to the mammary gland where they secrete antibodies which constitute an important defense mechanism of the newborn. In the absence of lactation, these cells probably form part of the normal traffic to the lamina propria of the small intestine.

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