

**TRANSPORT OF IMMUNOGLOBULINS
FROM SERUM INTO COLOSTRUM***

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The origin of immunoglobulins in mammalian exocrine secretions has been a subject of considerable recent interest. The secretory IgA that is found in high concentrations in intestinal secretions, saliva, tears, milk, etc. has been assumed to be derived directly from local synthesis rather than from other secretory tissues via the serum. Support for this hypothesis is provided by adoptive transfer experiments that have shown a preferential migration of thoracic duct lymphoblasts or mesenteric lymph node lymphoblasts to mucosal lymphoid tissue, particularly the gut (1, 2). In addition, Roux et al. (3) and Lamm et al. (4) have observed that the mesenteric lymph node lymphoblasts can selectively lodge in the mammary gland of the lactating mouse. Further, these same studies indicate that the migrating cells from the mesenteric lymph node bear surface IgA and are already committed to IgA synthesis. These findings provide a rational explanation for the observations that antigenic stimulation in the gut may result in specific IgA antibodies in the milk. Nevertheless, a quantitative evaluation of the amount of immunoglobulin in a secretion provided by local cells has not been made (5). Further, as noted by Hall et al. (6), the salivary and lacrimal glands contain only trivial numbers of lymphoid cells and thus their secretory immunoglobulins must be provided by specific and/or nonspecific transudation of serum proteins. In fact, there are a number of recent observations suggesting that some of the IgA in intestinal secretions is derived from serum (7, 8). Jackson et al. (9) have shown that dimeric IgA in serum is rapidly secreted into the intestinal lumen via the bile duct. They also observed high levels of secretory IgA in rats with bile duct obstruction (10) and their data suggested that ~90% of the IgA in intestinal washings reached this secretion from the serum (8). Finally, it should be noted that the finding of low serum levels of IgA is not a relevant observation in assessing the quantitative significance of transport from serum to secretions (11). To assess the ability of the breast to export serum IgA we have injected labeled IgA dimer into the lactating mouse and have measured its transport into colostrum. To evaluate the selectivity of this process, we have compared this transport with the passage of several different classes and subclasses of immunoglobulins by this same route.

Materials and Methods

Animals. Postpartum BALB/c females were used in all experiments. Except where specifically noted, they were injected intravenously or subcutaneously within 24 h of delivery of a litter. In most of the experiments reported here the mothers were injected with labeled proteins subcutaneously in the back. The drinking water contained 0.01% KI.

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Protein Preparation. Ascites fluid collected from mice with TEPC-15 myeloma was mixed with an equal volume of 100% saturated ammonium sulfate. The precipitate was washed in the cold (4°C) and then dissolved and dialyzed against borate-buffered saline (0.05 M borate, 0.15 M NaCl, pH 8.0). This protein was chromatographed on a phosphorylcholine-Sepharose (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) column and the bound TEPC-15 was eluted with 10^{-3} M phosphorylcholine. The dialyzed TEPC-15 was fractionated on a calibrated Bio-Gel A5M (Bio-Rad Laboratories, Richmond, Calif.) column to separate dimeric TEPC-15 from the monomer. MOPC-300 (IgG1) was prepared from ascites by ammonium sulfate precipitation, DE-52 (Whatman, Inc., Clifton, N. J.) column chromatography, and affinity chromatography on a staphylococcal protein A Sepharose column. The MOPC-11 protein (IgG2b) was purified from ascites by Dr. Barbara Birshtein, Albert Einstein College of Medicine, Bronx, N. Y. The inulin-binding myeloma protein J606 (IgG3), was obtained from ascites by ammonium sulfate precipitation, immunoabsorption on an inulin-cellulose column, and elution with 1 M LiCl. The mouse IgM protein was purified from a hybridoma (TEPC-15 idiotype) by affinity chromatography on a phosphorylcholine-cellulose column. Human IgG was prepared by DEAE-cellulose chromatography of commercial human gamma globulin, Cohn fraction II (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.). A human IgM (kappa chain) was prepared from patient serum by euglobulin precipitation and Bio-Gel A5M chromatography. A commercial preparation of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was used without further purification. The myeloma proteins were judged to be pure by immunoelectrophoresis and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. As has been found by others (12), isoelectric focusing indicated some charge heterogeneity for all of these proteins.

Radioiodination of Proteins. The proteins were iodinated with carrier-free Na^{125}I (Amersham Corp., Arlington Heights, Ill.) using the iodine monochloride (13) or the chloramine-T method (14), and separated from unbound iodine by dialysis and/or chromatography on Sephadex G-25 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.). Specific activities were $\sim 0.1 \mu\text{Ci}/\mu\text{g}$. The iodinated material eluted with the native protein when examined by gel filtration on Sephadex G-200 columns, and furthermore, the label was found to be $>90\%$ precipitable by 8% trichloroacetic acid, 20% polyethylene glycol, and the appropriate specific antisera. In addition, the labeled proteins comigrated with native material when examined by SDS-polyacrylamide gel electrophoresis and by isoelectric focusing.

Antisera. A rabbit antiserum to the TEPC-15 protein was raised by challenging two rabbits with the purified TEPC-15 monomer suspended in complete Freund's adjuvant followed by several boosts using incomplete adjuvant. To make α -chain-specific antisera, these sera were absorbed with a mouse IgG-Sepharose conjugate. A rabbit anti-mouse IgG serum was obtained from rabbits immunized with DEAE-cellulose-purified pooled mouse IgG.

Experimental Procedures. For the 23-h studies of uptake, 5 μg of labeled protein was injected and then at several time points, the live neonates were counted (whole body counts) in a Packard 5240 gamma scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.). The neonates were immediately returned to their mother to nurse. The animals were killed at 1 d at which time blood samples were taken and the gut tissue (stomach to large intestine) was removed. The total blood volume in the neonate was estimated to be 6% of the body weight.

In some cases, 15–25 μg were injected into the mother at 6–10 d post-partum to collect sufficient labeled material for analysis. The milk was extracted manually into a capillary tube after injection of 0.2 ml oxytocin (10 USP U/ml) and 0.3 ml of 0.5% phenobarbital. The whey was prepared by addition of 0.25 M sodium acetate, pH 5.5, and two centrifugations at 15,000 rpm for 20 min (Sorvall SS-34 rotor; DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.).

Labeled proteins before injection and those recovered in whey were evaluated on a Sephadex G-200 column (0.9 \times 20 cm) equilibrated in Tris-buffered saline, pH 7.4. The column was standardized with dextran blue and dilute $\text{Na}_2\text{Cr}_2\text{O}_7$ with a sample application vol of 0.3 ml in all cases.

Results

After the injection of 5 μg of labeled protein into postpartum BALB/c mice, transport from serum to colostrum was measured by following isotope uptake into the

TABLE I
Transport of Labeled Proteins into Whey and Uptake by Neonates

Protein	Uptake of protein by neonates in 23 h (percent of total injected)*	Analysis of labeled material in whey	
		Percent macromolecular on Sephadex G-200 column	Percent precipitated by trichloroacetic acid
IgA dimer (TEPC-15)	31 ± 2	85	89
IgM (mouse hybridoma)	24 ± 2	87	86
IgG1 (MOPC-300)	10 ± 3	96	97
IgG3 (J-606)	11 ± 2	80	88
IgG2b (MOPC-11)	13 ± 1	98	95
Human IgG	10 ± 1	ND†	96
Human IgM	27 ± 1	ND	74
Bovine serum albumin	26 ± 1	84	83

* Mean ± SEM. Each value from two to five litters of four to eight neonates per litter.

† ND, not done.

nursing offspring. As can be seen in Table I, all the labeled proteins that were injected into the lactating females were transported to some extent into the colostrum. Of the mouse immunoglobulins studied, the IgA dimer and IgM were transported at a faster rate than the three subclasses of IgG. By 24 h, the amount of TEPC-15 dimer had reached levels about three times that found for IgG. Similar results were obtained at higher doses (50 µg). Several heterologous serum proteins were also examined. Here, also, the polymeric, J-chain-containing immunoglobulin (human IgM) was transported faster than IgG. Kinetic profiles (data not shown), where uptake by the neonates was measured at various time points, demonstrate that the uptake of IgA dimer and IgM is faster throughout the 24-h period after injection.

The data in Table I indicate that the labeled immunoglobulin obtained by the nursing neonates is still largely intact. Thus, 80–98% of the isotope in the whey is still macromolecular as indicated by elution on Sephadex G-200 columns and 74–98% of the isotope in the whey was precipitated in 8% trichloroacetic acid. In addition, for some of the proteins studied, the antigenic integrity of the material transported into whey was examined with specific antisera. When labeled IgA dimer, MOPC-300 protein, and human IgG were injected and the whey was analyzed, it was found that 82, 93, and 92% of the isotope was precipitated with the appropriate antisera.

The body distribution of isotope in the nursing neonates 23 h after injection into their mother was examined. For the immunoglobulins studied, the gut, which included the ingested milk, contained 42–57% of the isotope. Most of these counts were found to be on intact proteins. The isotope in the blood contained significant quantities of low molecular weight material, but in these samples quantitation was difficult because of the small samples available.

Discussion

The data in Table I showing faster transport of IgA dimer and both of the IgM proteins indicate that an efficient mechanism exists in the mammary gland to remove these polymeric immunoglobulins from the circulation. It is of particular interest to note that the immunoglobulins selectively transported are those with the potential to bind secretory component (15, 16). The possibility that secretory component facilitates this selective transport process by functioning as a receptor on epithelial cells in secretory tissue is supported by several recent reports (17, 18). One would predict

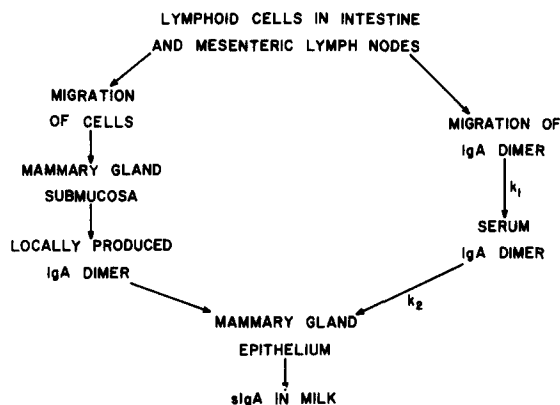


FIG. 1. Two mechanisms for secretory IgA to reach mammary gland secretion. Here k_1 is the rate constant for transfer of IgA dimers from lymph to serum and k_2 is rate constant for removal from the circulation by membrane-bound secretory component in mammary gland epithelial cells.

from these observations that an IgA monomer (lacking J chain) would not be selectively transported because the secretory component interaction would not be possible. It is interesting to note in this regard that the studies of Jackson et al. (19) on the clearance of an unfractionated MOPC-315 IgA preparation demonstrated a biphasic clearance curve. The initial rapid phase might reflect the transport of dimeric IgA, whereas the much slower clearance may characterize the transport of monomeric IgA from serum. The observation of a rapid transport of serum albumin into mouse colostrum is consistent with what is known about the rat (20) in which it appears that all of the albumin in whey is derived from blood plasma.

The cellular origin of the immunoglobulins in mammalian secretions, particularly milk and colostrum, has not been clearly defined (5). For a number of secretions, local synthesis and direct secretion would appear to be of only minor quantitative significance (6). An alternative model (Fig. 1) is that each secretion contains immunoglobulins produced by both local and distant tissues of the mucosal immune system, as well as by the spleen. These antibodies may then be transported to the various secretory fluids via the circulation by mediation of secretory component in the membrane of the epithelial cells which operate to select the oligomeric, J-chain-containing immunoglobulins. The experiments reported here demonstrate that the mouse mammary gland can efficiently export IgA dimers and IgM from serum. Nevertheless, these findings do not provide us with the data to quantify the relative importance of the two mechanisms shown in Fig. 1: cell migration followed by local synthesis vs. synthesis and secretion at distant mucosal sites, especially the gut lamina propria, followed by passage in the circulation to other exocrine tissues.

Two observations are usually cited as evidence against a serum transfer mechanism. Measurements of the serum levels of IgA, particularly oligomeric IgA, indicate that relatively low levels are present. However, observations on total serum levels provide no information with respect to the relative importance of transport via serum to secretory fluids (6). As can be seen in Fig. 1, all that is required to keep the steady-state serum concentration low is for the rate of removal of oligomeric IgA to be larger than the rate of production and transfer into serum. If k_1 is the rate constant for transfer of IgA dimer into serum (compartment B) from the lymphoid tissue (com-

partment A) and k_2 is the rate constant for transfer to the milk (compartment C) then the steady-state level of serum IgA dimer is $[B] = (k_1/k_2)[A]$.¹ Thus, in such a circumstance, low serum levels will be observed even though large amounts of IgA reach the serum each day via the lymph from the mucosal lymphoid tissue.

There have been several reports dealing directly with the transport of IgA from serum to secretions and these have indicated that little IgA in the secretions comes via the serum. However, in the sow (21) and the cow (22) there is compelling evidence that IgA in the milk is derived from serum. Those that report no significant transport from the serum used either 7S IgA, secretory IgA, or undefined mixtures of IgA (23–25). Because one would predict that IgA monomer and IgA with bound secretory component would not be selectively transported, these studies with uncharacterized IgA populations cannot be readily interpreted.

Summary

Oligomeric, J-chain-containing immunoglobulins were observed to be transferred selectively from serum into colostrum. These studies suggest that, in the case of the mammary gland secretion, a significant role for extraglandular synthesis of IgA merits consideration. Thus, for example, colostrum may contain antibodies synthesized locally as well as antibodies synthesized in the much larger lymphoid tissues such as the gut lamina propria.

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¹ Because $\frac{d[B]}{dt} = 0 = k_1[A] - k_2[B]$ at steady-state.

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