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Intestinal adaptation during lactation in the mouse

I. ENHANCED INTESTINAL UPTAKE OF DIETARY PROTEIN ANTIGEN

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SUMMARY

Small quantities of dietary protein antigens cross the intestinal epithelium of the lactating mouse, enter the circulation, are transferred across the mammary gland into the milk and reach the suckling neonate. In this study, we sought to determine whether intestinal uptake of ovalbumin (OVA) was enhanced in lactating compared to control mice. OVA was administered by gavage under ether anaesthesia. Blood was obtained at 15, 30, 60 and 120 min and immunoreactive OVA (iOVA) measured by enzyme immunoassay. At 30 and 60 min, a three- to four-fold higher concentration of iOVA was detected in lactating compared to control mice. Because this increase in concentration of iOVA might be explained by changes in plasma volume, rate of clearance of OVA from the circulation or altered uptake from the intestine, plasma volume was measured by isotope dilution after i.v. injection of ^{125}I-bovine serum albumin (BSA) and clearance was assessed by measuring elimination of OVA from the circulation after i.v. injection of OVA. In comparison to controls, plasma volume of Day 7–10 lactating mice was increased two-fold and no difference in clearance rate was noted. Because the increase in concentration of iOVA in lactating mice is several-fold greater than in controls, we suggest that increased intestinal uptake of the protein occurs during lactation.

INTRODUCTION

Nursing infants may develop allergic reactions to substances in the maternal diet (Gerrard, 1979). Recent studies have demonstrated that in some infants, colic (Jakobsson & Lindberg, 1983), atopic dermatitis (Cant *et al.*, 1986) and colitis (Lake, Whitington & Hamilton, 1982) can be attributed to a specific protein in the mother's diet. Because maternal dietary antigens appear to be involved in the induction and persistence of certain diseases of infants and because of the potential importance of these antigens for the development of the neonate's normal immune response, we have examined the process of antigen transfer from lactating mother to infant (Harmatz *et al.*, 1986a,b). This transfer requires that ingested proteins penetrate the gastrointestinal barrier of the lactating mother, reach the systemic circulation, avoid clearance from the systemic circulation before reaching the mammary gland, and be transferred by the mammary gland from the circulation into the milk. In this study, we examined whether the initial step in the process, penetration of the gastrointestinal barrier, was altered during lactation.

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MATERIALS AND METHODS

Animals

Male and nulliparous female C57BL/6NTacfBr × DBA/2NTacfBr (BDF1), mice were purchased from Taconic Farms (Germantown, NY), mated in our colony, and maintained on autoclaved NIH-31 diet (Taconic, OVA-free) and tap water throughout the study. Lactating mice were selected for study 1–2 days (early lactation), 6–10 days (mid-lactation) and 18–19 days (late lactation) after delivering their first litter. Age-matched virgin females served as controls.

Antigen

Ovalbumin (OVA, grade V, Sigma Chemical, St Louis, MO) was dissolved in normal saline at 20 mg/ml; the concentration was verified by the method of Waddell & Hill (1956), and the solutions were stored at 4° for use within 24 hr. ^{125}I (NEN-033A, New England Nuclear, Boston, MA) was linked to OVA by the chloramine-T method (Ishizaka & Okudaira, 1973).

Antigen feeding

OVA [10 mg or 25 mg in 0·5 ml normal saline (NS)] was administered intragastrically (i.g.) via a silastic catheter (Dow-Corning, Midland, MI) during light ether anaesthesia. To maintain normal lactation, the mice were not fasted prior to antigen feeding.

Enzyme immunoassay (EIA) for OVA in serum

Blood was collected from the retro-orbital venous plexus at 15, 30, 60 and 120 min, was permitted to clot and was then centrifuged (12,000 *g*, 10 min). The EIA for detection of iOVA is described in detail elsewhere (Harmatz *et al.*, 1986b). Briefly, sera were diluted with whole normal mouse serum (NMS), followed by a final dilution of 1:3 in phosphate-buffered saline (PBS; 0·01 M, pH 7·3). Duplicate samples, and identically diluted OVA standards in NMS, were incubated in vinyl microtitre wells precoated with the IgG fraction of rabbit anti-OVA antiserum (Miles Scientific, Mapierville, IL), followed by washing and addition of the IgG fraction of rabbit anti-OVA antiserum conjugated with horseradish peroxidase (Miles Scientific). The assay was developed with orthophenylene-diamine substrate and examined in a Dynatech MR-600 ELISA reader. Concentrations of iOVA were estimated by fitting absorbance readings to the standard curve in the linear range of the assay. The sensitivity of the assay varied from 1 to 10 ng/ml.

Determination of the rate of clearance of unlabelled OVA from the circulation after i.v. injection

Monomeric OVA was prepared for i.v. injection by Sephadex G-100 (Pharmacia, Piscataway, NJ) gel filtration (2·5 × 100 cm, buffer: PBS) of OVA and pooling of the fractions in the major peak. Lactating (Day 6–10) or control mice were injected with this preparation of monomeric OVA (100 μg) in 200 μl of normal saline. Blood was obtained from the retro-orbital venous plexus at 1 min and 15 min in one group of mice and at 1 min and 60 min in another group. The concentration of iOVA in serum was measured by EIA and the percentage of the 1 min concentration remaining in the circulation at 15 min and 60 min was determined.

Estimation of the ^{125}I-BSA distribution space (plasma volume) in lactating and control mice

Lactating (Day 6–10) or control mice were injected intravenously with 1 mg of bovine serum albumin (BSA fraction V; Sigma) in 100 μl normal saline followed 30 min later by a tracer dose of ^{125}I-BSA in 100 μl normal saline. One minute after the second injection, blood was obtained from the retro-orbital venous plexus into pre-weighed vials; the haematocrit was also determined. Radioactivity was measured by gamma scintillation spectroscopy. The plasma volume was calculated by the isotope dilution technique (Crispell, Porter & Nieset, 1950).

Characterization of immunoreactive OVA in the serum of lactating or control mice given OVA by gavage

After feeding 25 mg of monomeric OVA by gavage to 6–10 day lactating mice or controls, blood was obtained at 60 min from three animals in each group. The sera from each group were combined and applied to a Sephadex G-100 gel permeation column (90 × 1·5 cm). The column was eluted with PBS containing 0·02% sodium azide; fractions were collected and the iOVA content was measured. Serum protein was estimated in each fraction by determining absorbance at 280 nm in a Gilford Response II Spectrophotometer. The area under each peak was determined using a computer-assisted digitizer (Sigma Scan, Jandel Scientific, Corte Madera, CA).

RESULTS

Concentration of OVA in the serum of lactating and control mice after feeding OVA

Ten micrograms of OVA were administered by gavage to lactating and control mice and the serum concentration of iOVA determined at intervals (Fig. 1). On Days 1–2 of lactation, there was no significant difference in concentration of iOVA at 15, 30, 60 and 120 min (Fig. 1a). On Days 6–10 postpartum, there was a significant difference in iOVA at 30 and 60, but not at 15 and 120 min (Fig. 1b). On Days 18–19, lactating females continued to show a significantly greater serum concentration of iOVA at 30 and 60 min (Fig. 1c); the difference was not as great as that seen on Days 6–10. We next sought to determine whether the increase in OVA concentration during lactation was attributable to a decreased rate of clearance of OVA from the circulation or to decrease in plasma volume in lactating mice.

Clearance of OVA from the circulation of lactating and control mice after i.v. injection of OVA

After i.v. injection, the clearance of iOVA in Day 6–10 lactating mice ($n=3$) did not differ from that of controls ($n=3$) (Fig. 2).

Measurement of the ^{125}I-BSA distribution space (plasma volume) in lactating and control mice

The plasma volume of Day 6–10 lactating mice ($n=4$) estimated by isotope dilution technique was 3 ml, that of controls ($n=4$) was 1·5 ml.

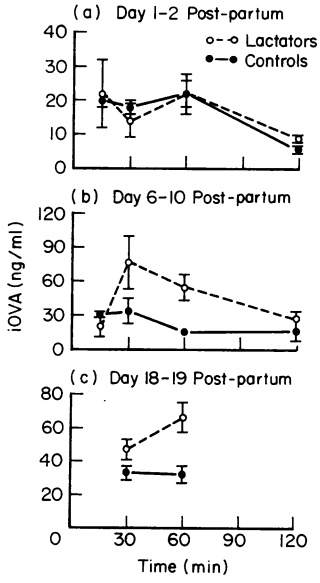

Figure 1. Concentration of iOVA in the circulation of lactating and control mice fed 10 mg OVA by gavage. Mean ± SEM are shown. (a) There was no significant difference between lactators versus controls; (b) the difference between lactators and controls was significant, $P \leq 0{\cdot}002$ as determined by ANOVA; (c) the difference between lactators and controls was significant, $P \leq 0{\cdot}02$ as determined by ANOVA with repeated measures.

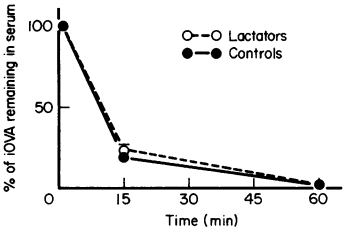

Figure 2. Clearance of OVA from the circulation of lactating and control mice after i.v. injection of 100 μg OVA. Fifteen and 60 min points each represent the mean of three animals. No significant difference in clearance was determined by ANOVA.

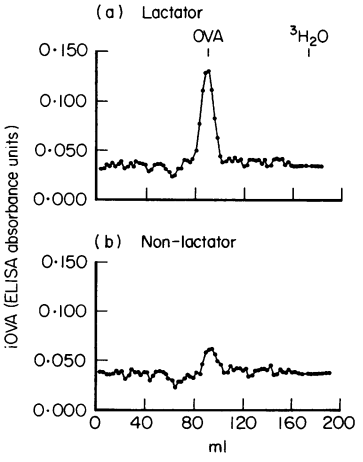

Figure 3. Sephadex G-100 gel permeation (80 × 1·5 cm; buffer PBS) of serum from 6–10 day lactating or control mice given 25 mg OVA by gavage. 2·8-ml fractions were collected; the iOVA content was measured by EIA. iOVA eluted in the same fractions of serum from lactating and non-lactating mice.

Characterization of OVA in the serum of lactating and control mice after feeding OVA

Immunoreactive OVA in serum from lactating and control mice eluted from a Sephadex G-100 gel permeation column (Fig. 3) in the same fractions as native OVA (not shown in Fig. 3). Although a minimal elevation of the baseline was noted after the major iOVA peak was eluted, no clearly defined peaks representing fragments were identified in serum from lactators or controls. Despite loading equal volumes of serum from each group, an increased amount of iOVA was detected in the eluted fractions obtained from the pooled sera of lactating mice. By determining the area under the major peak generated with pooled control or lactator serum, we confirmed the three- to four-fold increase in the iOVA concentration found earlier on testing of whole sera by EIA.

DISCUSSION

In the present study, we examined the effect of lactation on the intestinal uptake of a dietary protein antigen in mice. In contrast to the controls, a three- to four-fold increase in serum concentration of iOVA was noted at mid-lactation (6–10 days). This difference in iOVA concentration persisted through late lactation (Day 18), although the difference was not as great as at mid-lactation. No significant difference was noted early in lactation (Day 1), suggesting that the physiological changes of lactation and not those of pregnancy, contributed to the observed differences in concentration of serum iOVA.

The concentration of serum iOVA after feeding a selected dose of OVA is related to (i) the amount of OVA absorbed from the intestine; (ii) the plasma volume; and (iii) the rate of clearance of OVA from the circulation. Based on measurement of (ii) and (iii), we can estimate the difference in OVA uptake from the intestine of lactating and control mice. In mid-lactation, the plasma volume, determined by the isotope dilution technique, was increased two-fold. This finding is in agreement with that of Jepson & Lowenstein (1966) who also noted a two-fold increase in the plasma volume of mice at Day 15 of lactation. This increase in the plasma volume of lactating mice leads to an under-estimation of intestinal uptake based on differences in serum concentration. In Day 6–10 lactating mice, the difference in estimated gut uptake in our studies would therefore be six- to eight-fold (assuming clearance of OVA from the circulation does not differ between lactating and control mice).

The rate of clearance of OVA from the circulation was examined in lactating and control mice after i.v. injection of small (microgram) amounts of OVA. Clearance of iOVA was not different in the two groups of mice over a 1-hr study period. After intravenous injection of monomeric OVA, we assessed the clearance of OVA administered as a single bolus. OVA transferred to the systemic circulation after feeding might be subject to different rates of clearance because it might consist of several molecular species produced by digestion. Examination of the molecular size of iOVA appearing in the circulation after feeding (Fig. 3) did not show intermediate size fragments of OVA different from that of intact OVA. Based on the studies of plasma volume and clearance of OVA from the circulation, we conclude that enhanced intestinal uptake of OVA in the lactating mouse is likely to be responsible for the increased iOVA found in the circulation.

The observed change in intestinal uptake of OVA may be explained by changes in intestinal structure or function seen during lactation (Campbell & Fell, 1964; Fell, Smith & Campbell, 1973; Craft, 1970; Cripps & Williams, 1975; Burdett & Reek, 1979; Palmer & Rolls, 1980). Increases in total intestinal length, weight, nitrogen-content, villus height and crypt depth have been described in the rat (Campbell & Fell, 1964; Fell *et al.*, 1973; Craft, 1970; Cripps & Williams, 1975). Absorption of the nutrients, leucine and glucose, from the entire intestine of lactating rats was increased, although nutrient absorption per unit length or per intestinal weight was increased to a lesser extent or actually decreased (Cripps & Williams, 1975). Although an increase in the small intestinal and pancreatic enzymes responsible for protein digestion has been reported (Rolls, 1975; Rolls, Henschell & Palmer, 1979), changes in protein digestion during lactation have not been specifically examined.

The Peyer's patch has been suggested as a site for increased uptake of macromolecules within the intestine (Keljo & Hamilton, 1983); its contribution in relation to the remaining epithelium has not been determined. Fell *et al.* (1973) failed to find a correlation between the number of Peyer's patches in the intestine and intestinal hypertrophy in the lactating rat; these findings suggest that the Peyer's patch is unlikely to be responsible for the changes we observed in antigen uptake.

Increased intestinal permeability might contribute to the increased uptake of OVA we observed. Falth-Magnusson *et al.* (1985) examined intestinal permeability during lactation in the human. They fed polyethylene glycol (PEG) 400 and 1000 to lactating and control women and measured the 6-hr urinary recovery of these two species of PEG. They determined maximum recovery (an estimate of surface area), and recovery ratio (an estimate of the ability of the mucosa to exclude larger molecules). They found no difference between lactating women and controls for either of these two determinations. Even if differences in intestinal permeability to 'small' molecules had been found, recent studies suggest that tests conducted with such probes need not reflect enhanced permeability to macromolecules. In comparisons of macromolecular uptake (BSA and beta-lactoglobulin) with the uptake of small water soluble 'sugar' molecules, no direct correlation was observed (Weaver & Coombs, 1988; Turner *et al.*, 1988). It was concluded that there was no simple correlation between intestinal permeability to small markers and uptake of macromolecular proteins.

The enhanced uptake of dietary protein observed after a single feeding of the test antigen is presumably amplified by the 250–350% increase in dietary intake reported for the lactating rat (Campbell & Fell, 1964; Cripps & Williams, 1975). Increased dietary intake, in combination with enhanced intestinal uptake, may lead to the transfer of antigenic proteins to the nursing infant in immunologically significant quantities. The immune response in the neonate of several animal species can be influenced by feeding a specific antigen to the mother either prior to mating or during pregnancy or lactation (Troncone & Ferguson, 1988; Nicklin & Miller, 1987; Peri & Rothberg, 1981; Wold *et al.*, 1987).

In summary, our findings suggest that increased uptake of OVA from the gastrointestinal tract during lactation is largely responsible for the increased circulating iOVA found in these mice. Possible mechanisms for this change in intestinal processing of dietary protein antigen will be examined in subsequent studies.

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