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Ontogeny of Mink IgG, IgA, and IgM (40039)

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Aleutian disease (AD) in adult mink is characterized by marked hyperglobulinemia, plasmacytosis and immune complex arteritis and glomerulonephritis (1). The hypergammaglobulinemia and immune complex disease in young mink infected in utero with AD virus (ADV) was found to be less than that found in infected adult mink, presumably because of decreased immunological responsiveness in mink kits (2). Furthermore, mink kits infected neonatally with ADV frequently developed AD with atypical features rarely found in mink infected as adults (W. J. Hadlow, personal communication). Because immune responsiveness is required for development of AD (3), knowledge about the ontogeny of immunity in mink may help in elucidating the pathophysiology of AD in neonatal mink. Neonatal humoral immunity in mammals is a summation of passively acquired antibody (via placental and milk transmission) and actively synthesized Ig by the host. The relative amount of maternal Ig transferred before and after parturition varies widely among mammals as does the particular Ig which is transmitted (for review see Ref. 4). The present report contains information about the transmission to and synthesis of IgG, IgA and IgM in young mink.

Materials and methods. Animals. Sapphire mink were obtained from a single closed herd free of AD and New Zealand white rabbits were obtained from a local breeder. Serum or plasma was obtained from normal mink by cutting a toenail or by cardiac puncture. Blood was obtained by decapitating pre- and postnatal mink kits.

Antisera. Antisera specific for mink IgG, IgA and IgM were made in rabbits as previously described (5), except that the antigen was absorbed by coupling it to Sepharose 4B (Pharmacia, Uppsala, Sweden) with cyanogen bromide (6).

Quantitation of Ig. Concentrations of mink Ig were determined by ring diffusion assay in which five lambda of serum were delivered into a 14-gauge needle hole made in antibody-agar covered glass plates. Appropriate concentrations of specific antibody and test serum were used to obtain suitable ring diameters. Concentration of protein in an unknown sample was calculated by comparing ring diameter (squared) with the dilution curve of the standard control serum. Isolated Ig of known protein concentration (Kjeldahl method) was used to determine concentrations of IgG, IgM and IgA in standard control serum (7). The molecular size of IgA in milk and young mink serum was not determined; for quantitation purposes this IgA was assumed to be similar in size to the adult serum IgA standard which was 12S (5).

[^{14}C] Tissue culture. Mink tissues [axillary, prescapular, and mesenteric lymph nodes, spleen, thymus, liver, ileum, and kidney] obtained at necropsy were promptly minced into 1 mm pieces that were incubated in culture tubes containing [^{14}C] isoleucine and [^{14}C] lycine (ICN, Irvine, CA) tissue culture medium and placed on a roller drum for 12 hr at 37° (8). After the cultures were frozen, thawed and centrifuged, the supernatant fluid was dialyzed extensively and then lyophilized. Presence of radioactivity in a particular Ig was examined by autoradiography in which the reconstituted [^{14}C] tissue culture supernatant fluid and normal mink serum carrier were added to a well of a 2% agarose covered slide and specific antiserum added to an adjacent well. Concentration of antiserum and carrier were adjusted so that a sharp precipitin line developed after 24 hr. The slides were then washed extensively for 4 days with

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PBS, dried, stained, and applied to Kodak contrast film for 7 days. Under the area of the superimposed agarose precipitin line [^{14}C]-labeled Ig was seen as a dark line on the developed film.

Results. Ontogeny of serum Ig. Serum levels of IgG, IgA and IgM were determined in blood obtained from normal sapphire kits exsanguinated at various intervals after birth (Fig. 1). The normal adult Ig values in Fig. 1 were obtained by examination of serum from eight 6-month-old male sapphires. Serum from a single kit was obtained on most days, although sera from two animals were examined on postnatal days 10, 20, 24, 31, and 50. IgM and IgG were detected within 24–48 hr after birth; concentration of IgM gradually increased over 128 days, whereas that of IgG rapidly attained adult levels (7–10 mg/ml) 8 days after birth. IgG levels then decreased to 1–2 mg/ml during the fifth week of life and before the kits were weaned on day 56. Serum IgA was not detectable until 39 days after birth and remained at low levels (0.01 mg/ml) until after day 128 (Fig. 1).

The young kits in Fig. 1 had been maintained until exsanguination with their mothers and presumably started suckling shortly after birth. One litter was examined which had not suckled; the parturient mother was maintained in a cage with a course screen bottom (1 × 2 in. mesh) and nest box without floor so that directly after delivery the kits fell through the screen out of the mothers' reach. Sera obtained from these four kits revealed no detectable IgA or IgM but an average IgG level of 0.56 mg/ml (0.35, 0.47, 0.67 and 0.73 mg IgG/ml). A serum pool also was obtained from one litter of fetal mink (1–2 weeks prepartum) and contained 0.1 mg IgG/ml without detectable IgA or IgM.

The high level of serum IgG in neonatal mink during the nursing period suggested its maternal origin. Twelve samples of colostrum and milk obtained 1–50 days after parturition were assayed for IgG, IgA and IgM (Table I). IgG was present in greatest quantity (mean 2.9 mg/ml), although small amounts (mean 0.17 mg/ml) of IgA were also detected. IgM was not present in any of the samples. Sera were obtained from

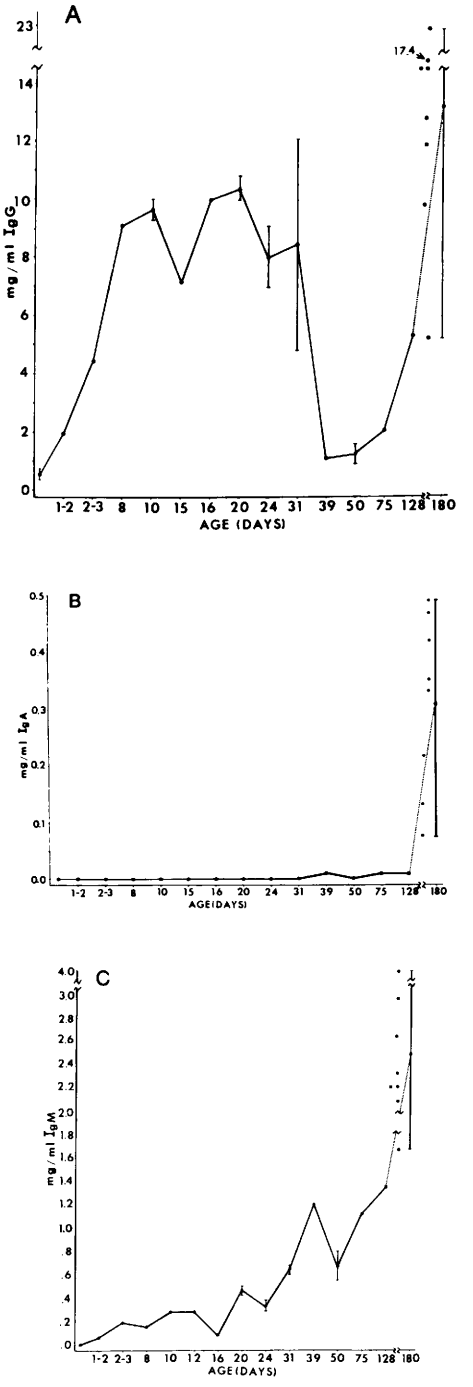

FIG. 1. Ontogeny of serum Ig in mink; mg/ml of IgG (A), IgA (B) and IgM (C) shown at various intervals after birth (day 0). Solid line connects mean values. Range is shown by brackets. Adult individual values (dots) and means were obtained from sera of eight normal male sapphire mink at 180 days of age.

TABLE I. Ig Levels in Milk and Serum of Lactating Female Mink.

	IgG[a]	IgA[a]	IgM[a]
Milk[b]	2.9 (1.0–6.3)	0.17 (0.04–0.5)	0
Maternal serum[c]	23.4 (11.6–37.5)	0.51 (0.1–1.8)	3.5 (2.1–7.5)
Milk Ig × 100[d]/serum Ig	14.5% (4.4–25.6)	45.6% (27.7–84.0)	0

[a] Average mean value in mg/ml and (range).
[b] Twelve samples, days 1–50.
[c] Eight samples, days 1–50.
[d] Eight individually paired samples, days 1–50.

eight lactating females concurrently with the milk sample and the amount of serum Ig was determined. Calculations of milk/serum Ig ratios for individual mink showed that, on an average, relative IgA secretion was threefold greater than IgG secretion; that is, mean IgA level in milk was 45.6% of serum level, whereas mean IgG concentration in milk was 14.5% that of serum (Table I). No significant trend of change in Ig levels of milk and serum with time were seen, although the highest levels (mg/ml) of IgA in milk (0.5 and 0.34) were on days 39 and 50 and the lowest on days 1 and 2 (0.04 and 0.08). The highest IgG (5.0 and 6.3 mg/ml) levels in milk were from mothers that had the highest IgG (32.2 and 37.5 mg/ml respectively) levels in serum.

[^{14}C] Synthesis of mink Ig. Lymph nodes (prescapular, axillary and mesenteric), spleen, thymus, liver, and intestine from the exsanguinated kits (Fig. 1) were assayed for Ig synthesis by [^{14}C] tissue culture. IgG and IgM synthesis were consistently detected in lymph node and spleen in all kits 1 to 128 days of age. Smaller, but yet definitive amounts of the IgG and IgM synthesis also were found in thymus and liver of kits up to 20 days of age. The first conclusive synthesis of IgA, however, was detected in spleen of a 75-day-old kit and then in spleen, lymph node, and particularly in intestine of mink 98 and 128 days old.

Discussion. Adult levels of IgG were found in sera of kits from the first to the fourth week of life, presumably because of maternal Ig transferred by milk (9, 10). Serum IgG concentration then fell precipitously during the fifth week of life and before weaning on day 56. Substantial amounts of IgG were present in milk throughout this interval, so the IgG decrease in kit serum occurred either because the capacity for its intestinal absorption decreased, as occurs in mice (11), or perhaps because suckling had essentially ended at this time. Sera of kits also contained autologous IgG during the neonatal period, as IgG synthesis was detected consistently from the first day of birth. The small amounts of IgG detected in fetal and neonatal mink prior to suckling could be of autologous and/or maternal (transplacental) origin. Kits also were synthesizing IgM at birth; the kinetics of increase during neonatal period and absence from milk indicate that kit serum IgM originates within the host.

In comparison to IgG, mink milk contained relatively small amounts of IgA, as also occurs in ruminants (12). However, when compared with milk-serum ratios of IgG (Table I), a threefold advantage of IgA secretion was found. The lack of detectable IgA in suckling kits may be explained by the small amount of IgA in milk. But mink kits, like suckling mice (13), may be unable to absorb milk IgA. Furthermore, little IgA of host origin was present, as IgA synthesis was not detected in young mink until they were more than 2 months old and significant serum levels were not achieved until after 4 months of age. Thus, ontogeny of mink IgA is similar to the slow maturation seen in other mammals (14). Also, the predominant synthesis of IgA by gut-associated tissues in young and adult normal mink (7) is similar to that in other animals (14).

Studies on the kinetics of serum Ig changes in adult mink after infection with ADV have shown that elevation of IgA levels was frequently preceded by increases in IgG levels (7), similar to serum levels in ontogeny. However, in sapphire mink with terminal AD, the serum Ig with greatest relative increase frequently was IgA, although AD resistant pastel mink showed minimal serum IgA increase (7). The question arises whether IgA-anti-ADV is an important component for development of AD pathology. Future experiments will determine if a minimal IgA-anti-ADV response

in sapphire kits infected with ADV can explain the observed mild disease in these young mink.

Summary. Serum levels and [^{14}C] tissue culture synthesis of IgG, IgA and IgM were determined in pre- and postnatal normal mink. Small amounts of IgG were found in serum from fetal and neonatal mink prior to suckling. This could represent maternal Ig (transplacental) or could be autologous Ig as IgG synthesis was found in day old kit spleen and lymph node tissues. The suckling kit achieved adult levels of IgG (7–10 mg/ml) 8 days after birth although IgG then decreased (before weaning) to 1–2 mg/ml during the fifth week of life. Generous amounts (mean 2.9 mg/ml) of IgG were found in mink milk throughout the nursing period. Small amounts of IgA (mean 0.17 mg/ml) were also found in mink milk, although IgA was not detectable in kit serum until 39 days of age and IgA synthesis in [^{14}C] tissue culture was not found in tissues from kits less than 75 days of age. IgM was not detected in milk, although [^{14}C] tissue culture synthesis of IgM was consistently found 1–2 days postpartum and serum levels gradually increased during neonatal development.

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