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Impaired Biliary Secretion of Immunoglobulin A in Vitamin A-Deficient Rats (42362)

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Abstract. The effect of vitamin A deficiency on biliary secretion of IgA was investigated. Rats used in this study were rendered vitamin A deficient following withdrawal of retinoic acid from the diet of retinoate-cycled animals. This procedure allows a precise control of both the onset of deficiency and dietary protein–energy input. Defective synthesis and transport of IgA antibodies into the bile was evident when vitamin A-deficient rats (A^-) were immunized by injections of either *Brucella abortus* or sheep red blood cells directly into the Peyer's patches. Antibody titers in the bile of A^- animals were significantly lower than those of A^+ controls ($P < 0.01$). These A^- rats also had significantly lower levels of total IgA in the bile compared with A^+ controls ($P < 0.05$). Moreover, the transport of labeled rat IgA injected intravenously was adversely affected in these animals. These results, together with our previous report on the impaired intestinal antibody in A^- rats, clearly indicate that vitamin A deficiency interferes with the transport of IgA antibodies into the bile of these animals.

Different lines of evidence suggest that vitamin A deficiency is associated with defective natural and acquired host defense mechanisms (1, 2). Such an association may contribute to the increased incidence and severity of infections, particularly those involving mucosal surfaces. Marked depletion of lymphocytes from the thymus and spleen has been noted (3, 4). Lymphopenia has also been observed, but such a defect is readily reversible following appropriate repletion with either vitamin A or its analogue (5–7). Moreover, the functions of these lymphocytes were also found to be impaired.

The mucosal immune system appears to be compromised in vitamin A deficiency (8). Several years earlier we noted that the secretory IgA (SIgA) levels in nasal washings of children with protein–calorie malnutrition, particularly in those complicated by vitamin A deficiency, were lower than in well-nourished controls (9). It was recently reported that the intestinal immune response of rats rendered vitamin A deficient by a cyclical retinoic acid supplementation–deprivation regime was impaired (10). In this study, the effect of vitamin A status on biliary secretion of IgA was investigated. The data obtained suggest that vitamin A deficiency also impairs biliary transport of IgA.

Materials and Methods. *Animals and diets.* Male Wistar rats were obtained from the Animal Center, Faculty of Science, Mahidol University. These animals were kept in individual stainless steel cages at approximately 28°C with light provided 12 hr/day. They were rendered vitamin A deficient by the withdrawal of retinoic acid from the diet as previously described (11, 12). Briefly, weanling rats were fed a vitamin A-free diet *ad libitum* for 3 weeks until the early weight plateau. Thereafter, the animals were fed a diet supplemented with and later lacking in 5 μg retinoic acid (Eastman, Rochester, N.Y.) per gram of diet in at least four repeating 18- to 10-day cycles. These animals were then given a maintenance dose of 2 μg retinoic acid per gram of diet for 8–10 days or until they were to be used in the experiment when the retinoic acid would be ultimately withdrawn from the diet (T_0). Those animals selected to be vitamin A-sufficient (A^+) received a total of 1000 μg retinyl palmitate (K and K Laboratories, Plainview, N.Y.) (in safflower oil) in 500 μg split doses 2 days (T_{-2}) and 1 day (T_{-1}) before retinoic acid withdrawal. Those selected to be vitamin A-deficient (A^-) received only the safflower oil. Thereafter, both groups of animals were tube-fed twice daily with stock 18% casein vitamin A-free diet (ICN, Nutritional Biochemicals, Cleveland, Ohio), with sucrose in place of starch until the end of the experiment. With this protocol, the animals receiving retinyl palmitate (A^+) would have sufficient vitamin A reserve to last for several weeks, thus representing our control group, while those

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receiving only the oil (A^-) would become vitamin A deficient within 3–4 days following retinoic acid withdrawal (11).

Collection of bile and blood. Food was withheld for at least 12 hr before specimens were collected. Pentobarbital sodium (Abbott Laboratories, Chicago, Ill.) was injected intraperitoneally at a dose of 2.5–5.0 mg/100 g body weight depending on the vitamin A status of animals. The abdomen was then opened and the bile duct was cannulated. For most experiments bile was collected for approximately 2 hr, after which the rat was exsanguinated from the abdominal aorta. The paired samples of serum and bile were stored at −20°C until analyzed.

Administration of antigens. Abdomen of the animal was opened after deep ether anesthesia and injection was made into the Peyer's patches. For primary immunization, 10^8 sheep red blood cells (SRBC) or 10^9 killed *Brucella abortus* (Ba) in a total volume of 50 μl were injected into 3–5 patches. For study on secondary response, 10^5 SRBC or 10^6 Ba were used for both priming and boostered injections. Both 10^5 SRBC and 10^6 Ba when given without a booster were found to give undetectable or very low antibody titers (usually less than 1:4) in the bile or serum of pellet-fed rats.

Infusion of labeled IgA. Twelve days after the ultimate withdrawal of retinoic acid from the diet, both A^+ and A^- animals were injected intraperitoneally with pentobarbital sodium as indicated above. The femoral vein was cannulated and subsequently injected with 0.5 ml of ^{125}I-labeled IgA containing a total of 10 μCi. The abdomen was then opened and the bile duct was cannulated. Bile was collected at 30-min intervals during the next 2 hr and total radioactivity for each sample was counted directly in a gamma spectrometer.

Quantitation of immunoglobulins. The concentrations of biliary and serum IgA were determined by radial immunodiffusion (13) using specific rabbit antiserum to rat IgA prepared as described previously (10). Purified IgA used as primary standard was prepared from the IR-270 rat serum (kindly supplied by Dr. H. Bazin, University of Louvain, Belgium).

Antibody assay. Agglutinating antibodies to SRBC and to Ba were titrated in microtiter plates by standard methods using doubling dilutions in 25 μl of phosphate-buffered saline (PBS), pH 7.2. To prevent lysis of SRBC in some bile samples, bovine serum albumin (BSA) was added to all diluents to a final concentration of 2%.

Radiolabeling of immunoglobulin A. Rat IgA was purified from IR-270 serum by ion-exchange chromatography and gel filtration (10). The purified preparation was traced labeled with ^{125}I (Amersham, Buckinghamshire, England) by the chloramine-T method (14). Prior to being used, the labeled IgA was rechromatographed on a Sephadex G-200 column and concentrated with Sephadex G-25. The specific activity of the labeled IgA was 1.6 μCi/μg protein; about 90% of the radioactivity was TCA precipitable.

Other techniques. Total protein in the bile and serum was quantitated by the Folin method (15) using human serum albumin (Pentex, Kankakee, Ill.) as standard. The concentration of purified immunoglobulin was determined spectrophotometrically at 280 nm, using an $E^{1\%}_{280\,nm}$ value of 14 (16).

Statistical analysis. The significance of differences between the mean values of different groups of animals was determined by the Student's t test. A value of $P < 0.05$ was considered to be significant.

Results. *Effect of vitamin A status on the immune responses to SRBC and B. abortus antigens.* Immune responses to single or repeated injections of SRBC (T dependent) and *B. abortus* (T independent) in vitamin A-deficient animals were compared with those in vitamin A-sufficient controls. To compare primary immune responses in these animals, the antigens were adminstered on Day 7 following retinoic acid withdrawal (T_7) and the bile and blood samples were collected 7 days after the injection (T_{14}). Results diagrammed in Fig. 1 show that vitamin A deficiency markedly depressed biliary antibodies to both antigens ($P < 0.001$). Similarly, serum antibody to SRBC was significantly depressed in vitamin A-deficient animals (Fig. 1). On the other hand, the titers of serum antibody to Ba were the same for both groups of rats (Fig. 1). It should be noted that, for both antigens, the mean titer of antibody in the bile of A^+ controls was significantly higher than that in the serum ($P < 0.05$). In contrast, for the A^- group,

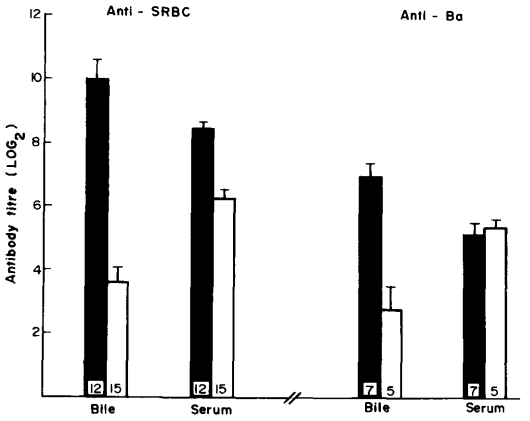

FIG. 1. The titers of antibody in the bile and serum of A^- (□) and A^+ control (■) rats that received a single injection of either sheep red blood cells (SRBC) or killed *B. abortus* (Ba) in the Peyer's patches. Bars and lines represent means ± SEM; the number at the base of each bar represents the number of specimens analyzed.

the mean antibody titer in the serum was significantly higher than in the bile ($P < 0.01$). These differences are more obvious when the paired bile–serum samples from individual animals are compared (Fig. 2).

In order to study the effect of vitamin A deficiency on immunological memory to previously exposed antigens, all animals were first primed with the antigen while they were on day 6 of the deprivation period of the last supplementation–deprivation cycle. At the end of the cycle (i.e., 4 days after priming) these animals were maintained on the minimal 2 μg retinoic acid/g diet for 8–10 days prior to being divided into A^+ and A^- groups as described under Materials and Methods. Both groups of animals were then boostered 8 days after the ultimate withdrawal of retinoic acid from the diet (T_8; 20–22 days after priming) and samples were collected 7 days later on Day 14. Results presented in Fig. 3 again show that vitamin A deficiency significantly depressed biliary secretion of antibody to both antigens ($P < 0.001$). However, unlike the primary responses, the titers of serum antibody to both antigens were not significantly affected by vitamin A status of the animals (Fig. 3). As in the primary immune responses, the titers of serum antibody to both antigens were always higher than the corresponding biliary antibody for all A^- rats (Fig. 4). However, when the paired bile–serum samples from individual A^+ animals were compared, the results seen with the boostered response were different from those of the primary response. Less than 50% of A^+ rats had higher biliary antibody titers following a boostered injection in comparison with almost 100% of A^+ animals in the primary response (Fig. 2 vs Fig. 4).

Effect of vitamin A status on biliary secretion of IgA. Because the predominant class of immunoglobulin and antibody in the bile of rats is IgA (17, 18), the effect of vitamin A deficiency on biliary secretion of this immunoglobulin isotype was investigated. The results presented in Table I and Figure 5 clearly

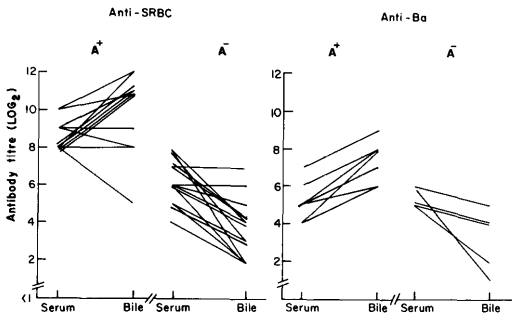

FIG. 2. The titers of antibody in the paired bile and serum samples from individual A^- and A^+ rats that received a single injection of either SRBC or Ba in the Peyer's patches. The <1 on the scale denotes sample that failed to give detectable reaction at a dilution of 1:2.

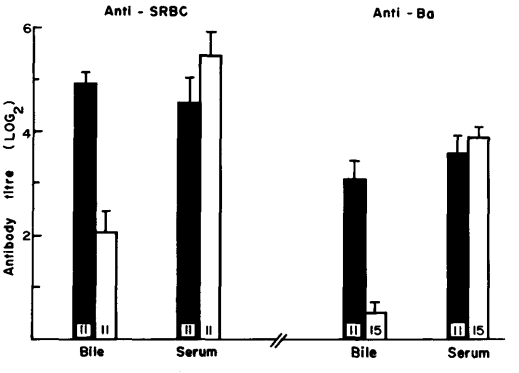

FIG. 3. The titers of antibody in the bile and serum of A^- (□) and A^+ control (■) rats that received a suboptimal dose-boostered injection of either SRBC or Ba in the Peyer's patches. Bars and lines represent means ± SEM; number at the base of each bar represents the number of specimens analyzed.

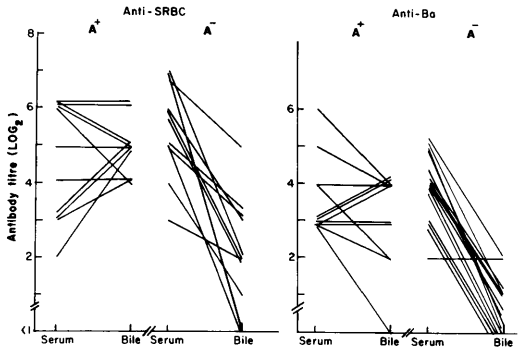

FIG. 4. The titers of antibody in the paired bile and serum samples from individual A^- and A^+ rats that received a boostered injection of suboptimal doses of either SRBC or Ba in the Peyer's patches. The <1 on the scale denotes sample that failed to give detectable reaction at a dilution of 1:2.

showed that vitamin A deficiency significantly depressed biliary secretion of IgA in the animals. There was a marked increase in IgA secretion into the bile following retinyl palmitate supplement, reaching a mean maximum level of 946 μg/ml (Table I) or 19% of total protein (Fig. 5) 12 days after supplementation. Both the absolute and the relative concentrations of biliary IgA measured on T_{12} in the A^+ animals were significantly higher than those measured on T_0. By contrast, there was no significant change in the concentrations of biliary IgA of the A^- group. When a slight increase in total protein concentration in the bile of A^- rats was taken into consideration, a gradual decline in the IgA secretion became obvious (Fig. 5). On T_{12}, the mean relative concentrations of biliary IgA in both groups of animals were significantly ($P < 0.05$) different from one another.

A depressive effect of vitamin A deficiency on biliary secretion of IgA on T_{12} was confirmed in an experiment using infused labeled rat IgA. The results presented in Fig. 6 showed that vitamin A-deficient animals had reduced capacity to transport IgA from blood circulation to bile, at least during the initial 2 hr of observation. During the 2-hr period, cumulative radioactivity in the bile of A^+ rats was significantly higher ($P < 0.01$) than that of A^- animals, which had their IgA transport reduced to approximately two-thirds of the controls. It should be mentioned at this point that the magnitude of such a reduction is similar to that noted for total biliary IgA in these animals (Table I).

Discussion. The results from the various experimental approaches used for this investigation clearly demonstrate that there is an impaired secretion of IgA antibodies into the bile of vitamin A-deficient rats. These observations complement our previous report demonstrating a defective intestinal immune response in these animals (10). These findings are not entirely unexpected as in rats, and to a lesser extent in other animals and man, bile contributes a substantial proportion of the IgA found in intestinal fluid (17, 18). Impaired biliary transport of IgA has also been observed previously in rats with protein–energy malnutrition (19). Considered together we should expect to find a local immune defect to be

TABLE I. EFFECT OF VITAMIN A DEFICIENCY ON BILIARY SECRETION OF IgA

Vitamin A status	No. of animals	Days after retinoic acid withdrawal	Bile	
			Total protein (mg/ml)	IgA (μg/ml)
Control (A^+)	4	0	5.99 ± 2.72[a]	453 ± 85
	4	4	5.42 ± 0.45	492 ± 89
	5	8	3.81 ± 0.41	501 ± 61
	7	12	4.93 ± 0.35	946 ± 121
Deficient (A^-)	4	0	4.62 ± 0.38	498 ± 18
	5	4	6.97 ± 1.41	435 ± 103
	7	8	7.76 ± 1.93	545 ± 114
	9	12	7.81 ± 1.07	627 ± 92

[a] Mean ± SEM.

more severe if these deficiencies occur simultaneously. In fact we previously demonstrated that the depressed secretory IgA levels in the nasal washings of children with protein–calorie malnutrition were reduced further in those complicated with vitamin A deficiency (9). Such a defect can and does result in an increased incidence and severity of infections that occur at the mucosal surfaces (1).

The exact mechanism(s) of action of vitamin A in facilitating the transport of IgA from blood or glandular tissues to external secretions including bile remain to be determined. From its well-recognized action on somatic cell differentiation and synthesis of glycoproteins (20), it is possible that vitamin A deficiency interferes with the synthesis of secretory component required for such a transport. This glycoprotein is synthesized and found on the membrane of epithelial cells and hepatocytes (21). We previously noted that the immunofluorescent staining for secretory component in the intestinal columnar cells of A^- rats was less intense than that of the A^+ controls, particularly during the later stage of deficiency (10).

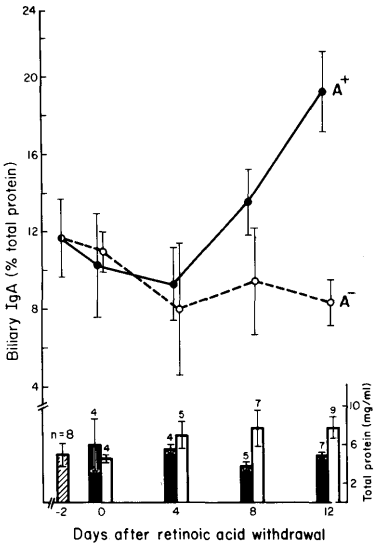

FIG. 5. Biliary IgA levels (means ± SEM) in vitamin A-deficient (A^-, ○) and control (A^+, ●) rats, expressed as percentage of total biliary protein (A^-, □; A^+, ■; means ± SEM). At T_{-2}, A^+ controls were given retinyl palmitate, while A^- animals received oil carrier alone. Retinoate control (▨); see Materials and Methods for explanation. n = Number of animals in each group.

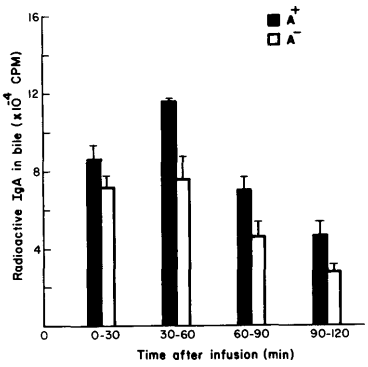

FIG. 6. Effect of vitamin A deficiency on the transport of intravenously injected ^{125}I-labeled rat IgA. The experiment was performed 12 days after the ultimate withdrawal of retinoic acid from the diet (T_{12}) of vitamin A-deficient (A^-, □) and control (A^+, ■) rats. The results are expressed as means and SEM of four animals.

In addition to a possible impairment of the transport of circulating IgA, evidence has been presented previously that vitamin A deficiency may affect recirculation and homing of lymphocytes to the mucosal tissues (22). It is possible to attribute these defects to an altered nature or distribution of glycoproteins on the lymphocyte membrane (23); the latter is known to be associated with lymphoid cell traffic (24). Changes in the membrane glycoproteins of vitamin A-deficient rats can also alter lymphocyte-trapping mechanism(s) (25). And lastly, the possible adverse effect of such a deficiency on the antigen-presenting cells should not be overlooked. We have recently demonstrated that the reticuloendothelial cell function of these animals is defective, judging from their inefficient ability to clear bacteria from the circulation (12). It is not unrealistic therefore to predict that other macrophage functions, including antigen processing and handling, are also affected. An alteration of major histocompatibility antigens, which are also glycoprotein in nature, could also interfere with cell cooperation. These various possibilities are of considerable interest and should be further investigated.

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