Vitamin A Status Affects the Development of Diabetes and Insulitis in BB Rats

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BB/Wor rats develop autoimmune diabetes mellitus with many features in common with human insulin-dependent diabetes mellitus. Since retinoids are known to have effects on insulin secretion and immune function, these studies were designed to investigate the effects of retinoid deficiency on diabetes in BB/Wor rats and to identify a role for retinoid status in the pathogenesis of autoimmune diabetes mellitus. Litters of diabetes-prone (DP) and diabetes-resistant (DR) BB/Wor rats were divided at weaning and fed a diet either (1) devoid of retinoids and leading to clinical deficiency at approximately 60 days of age (A-def diet)—following 10 days of clinical deficiency, rats on the A-def diet were changed to a diet containing 2 μg/g retinoic acid (A-def/RA diet); (2) containing 2 μg/g retinoic acid but deficient in retinol (RA diet); or (3) replete in retinol with 4 μg/g retinyl palmitate (RP diet). Rats receiving RP or RA diets were pair-fed to rats on the A-def/RA diet. Diabetes by 120 days of age was greatly reduced ($P < .01$) in DP rats that received the A-def/RA diet (four of 27) or RA diet (four of 29) versus the RP diet (13 of 31). Insulitis progressed with age in nondiabetic DP rats receiving the RP diet ($P < .02$) or RA diet ($P < .05$), but not the A-def/RA diet ($P > .22$). Insulin secretion was measured in perfused pancreas of nondiabetic rats after age 120 days and correlated negatively with insulitis ($P < .05$). DP rats receiving the RP diet had reduced insulin secretion as compared with other DP and DR rats ($P < .05$). In DR rats, retinoid status had no effects on insulitis through 120 days of age or on insulin secretion after 120 days of age. In conclusion, retinol deficiency reduces diabetes and insulitis in DP BB/Wor rats, and retinoic acid can at least partly substitute for retinol in the development of insulitis.

THE BB/Wor rat is an animal model of human insulin-dependent diabetes mellitus.[1] The majority of rats in diabetes-prone (DP) lines demonstrate autoimmune destruction of pancreatic β cells associated with lymphocytic infiltration of the islets between approximately 60 and 120 days of age.[2] DP rats are lymphopenic and deficient in circulating lymphocytes expressing RT6.1 and CD8 surface antigens. Immunosuppressive treatments or transfer of RT6.1-positive ($RT6.1^+$) lymphocytes to DP rats can delay or prevent diabetes.[1,3] Intensive treatment with insulin at an early age also reduces the incidence of diabetes, presumably by altering self-recognition of β cells by the immune system.[4] The diabetes-resistant (DR) lines do not develop diabetes spontaneously, but it can be induced by manipulation of the immune system, for example, depletion of $RT6.1^+$ cells. Lymphocyte populations in DR rats are similar to those of normal rats.[1,5]

Diet has also been shown to influence the development of diabetes in the BB rat. Protein sources in the diet, especially in semisynthetic diets, can alter the incidence of diabetes.[6-8] Diets deficient in essential fatty acids also decrease the incidence of diabetes in BB rats, possibly by affecting macrophages.[9]

Vitamin A (retinol), an essential dietary nutrient, is required for normal growth, reproduction, and vision. Retinol is provided in the diet in the form of the precursor carotene or is "preformed" as retinyl esters, such as retinyl palmitate.[10] In the intestine, carotene and retinyl palmitate are converted to retinol, which is reesterified in the enterocyte and incorporated into chylomicrons, which are transported to the liver. In the liver, retinyl esters are taken up, processed, and metabolized, and retinol is stored in its ester form. The liver releases retinol bound to retinol-binding protein, which transports retinol to its target cells. Intracellularly, retinol is converted irreversibly to the active metabolite, all-*trans*-retinoic acid, which has marked effects on cell growth and differentiation.[11] This formation of retinoic acid from retinol is the major source of all-*trans*-retinoic acid. However, when added to the diet, retinoic acid can substitute for retinol in growth and differentiation of epithelial cells and at high doses in reproduction, but not in functions attributed specifically to retinol or retinaldehyde, such as vision.

Retinoids have been shown to be required for normal insulin secretion from islets[12] and rat insulinoma cells.[13] Retinoic acid is thought to be the active metabolite of retinol for many vitamin A actions.[11] However, retinol is also metabolized to other derivatives that have specific functions, such as 9-*cis*-retinoic acid, the ligand for retinoid X receptors,[11] and *retro*-retinoids, which have a distinct configuration of double bonds and are formed from retinol and not retinoic acid.[14] Retinoids are known to have profound effects on the immune system.[15] Vitamin A deficiency is associated with infections in animals and humans.[16,17] Immunosuppressive effects of retinoid deficiency on T and B lymphocytes in vivo and in vitro have been described.[18-20] Recent reports have identified *retro*-retinoids as necessary for normal function of B and T lymphocytes and other cells.[14,19,21] However, other studies

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have suggested that retinoic acid, acting through its nuclear receptors, is also active in regulating lymphocyte functions.[22-24]

To explore the role of retinoid status in diabetes and to open a potential area for intervention in the steps leading to autoimmune diabetes mellitus, we examined the effects of retinoid deficiency on the incidence of diabetes and insulitis and insulin secretion in BB/Wor rats. Significant reductions in both insulitis and the development of clinical diabetes occurred in rats that were made deficient in retinoids.

MATERIALS AND METHODS

Experimental Design

Studies were designed to determine the effects of retinoids on diabetes in BB rats. Since rats cannot remain completely vitamin A–deficient for the entire 60 days of peak susceptibility to diabetes, study groups were planned to reflect either a deficiency of both retinol and retinoic acid, a deficiency of retinol but with retinoic acid supplementation, or no deficiency whatsoever. Disease activity was measured both by occurrence of diabetes by 120 days of age and by the degree of insulitis if diabetes had not developed. To control for potential effects of the semisynthetic nature of the diet, all animals received the same basic diet, with addition of the test retinoid the only difference. Pair-feeding was performed to control for any differences in food consumption. To determine whether vitamin A status or insulitis could alter islet function or morphology without clinically apparent diabetes, pancreas perfusions with measurement of glucose-induced insulin secretion were performed on rats that had not developed diabetes by 120 days of age, and pancreases were examined histologically for insulitis at various ages.

Animals and Diets

Male and female BB/Wor DP and DR rats for breeding were obtained from the National Institutes of Health central breeding colony at the University of Massachusetts Medical School (Worcester, MA) and maintained in isolation from other animals and humans (viral antibody–free conditions after the central breeding colony became viral antibody–free; specific pathogen–free conditions previously).[1] Vitamin A deficiency in pups was induced by standard methods previously described in detail.[12] In brief, breeding pairs were maintained on chow (Purina Rodent Laboratory Chow #5001; Ralston-Purina, St Louis, MO) until midgestation. Pregnant females were then changed to a vitamin A–deficient powdered diet supplemented with vitamins E, K, and D (A-def diet) prepared as described by Suda et al,[25(p 1050)] except the fat-soluble vitamin mix was modified to provide 750 μg DL-α-tocopherol, 90 μg menadione (ICN Biomedicals, Costa Mesa, CA), and 4.5 μg cholecalciferol (Sigma, St Louis, MO) three times weekly and soybean oil replaced cottonseed oil. The diet contained glucose monohydrate 642 g/kg diet (Koehl, Reading, OH), casein 180 g/kg diet (ICN Biomedicals), soybean oil 100 g/kg diet (Hunt-Wesson, Fullerton, CA), nonnutritive fiber 30 g/kg diet (Teklad Premier, Madison, WI), and the salts and soluble vitamins described previously.[25]

At weaning, individual pups from each litter were distributed to different treatment groups so they either (1) continued on the A-def diet (maximal retinoid deficiency); (2) received the A-def diet made replete with enough added retinoic acid (2 μg/g) for purposes of growth and health, except for the processes of vision and reproduction (RA diet, retinol deficiency)[26,27]; (3) received the A-def diet made replete in vitamin A with 4 μg/g retinyl palmitate (RP diet, retinol-replete controls); or (4) were killed for histologic examination of the pancreas. Male and female pups within each litter were distributed as evenly as possible among the treatment groups to avoid any spurious results due to differences in diabetic incidence among individual litters or between genders, as seen in non-obese diabetic mice. With weaning, rat pups were moved to individual stainless steel cages and fed a specific test diet. Rat pups receiving the A-def diet developed clinical vitamin A deficiency, evidenced by a weight plateau at approximately 60 days of age. These rats were continued on the A-def diet for 10 days after clinical vitamin A deficiency developed, and then their diet was changed to the RA diet (A-def/RA diet treatment in the maximal deficiency group). Besides cessation of growth, clinically deficient rats showed other signs of deficiency including conjunctival irritation and poor grooming; all these signs reversed with retinoic acid supplementation of the diet. Some rats from each treatment group were randomly selected and killed during the weight plateau period of rats on the A-def diet for histologic examination of the pancreas. To control for any differences in food intake during the period of weight plateau, rats continuously on RA and RP diets (described earlier as treatments 2 and 3) were pair-fed to rats of the same sex and line that were on the A-def diet. Pair-feeding to rats that had been clinically deficient was continued following the change to the RA diet until all rats were finally killed. All rats were weighed twice weekly through age 120 days, and diabetes was diagnosed by weight loss and random blood glucose of at least 11.1 mmol/L (200 mg/dL). Groups of DP pups were treated with all three diets, while DR pups were started on only A-def or RP diets because preliminary experiments demonstrated no diabetes in either DR group.

In Situ Pancreas Perfusion

Glucose-induced insulin secretion from the in situ–perfused pancreas of BB rats that did not develop diabetes by age 120 days was determined as described previously.[28-30] In brief, rats were anesthetized with pentobarbital 50 mg/kg. Following sedation, the abdomen was opened, and vessels connecting pancreatic and colonic circulations were ligated. Then the small and large intestines were secured and resected, leaving about 4 cm of the duodenum, descending colon, and rectum in place. Renal and adrenal arteries and veins, splenic vessels, esophagus and left gastric vessels, the vena cava below the kidney, and the aorta near the ileolumbar vessels were ligated. A catheter was inserted into the aorta below the origin of the renal arteries, advanced cephalad to the level of the celiac trunk, and secured with ligatures and clamps on the aorta located just above the catheter insertion point and just below the diaphragm. Plasma-free pancreas perfusion medium[29] was then used to supply oxygen and circulation to the pancreas with an incoming flow rate of 1.8 mL/min. The rat was then killed while still anesthetized. Finally, the portal vein was catheterized near its entry into the liver, and perfusate from this aortic-pancreatic-portal circulation was collected. Following the initial equilibration steps, 2-minute portal venous samples were collected over consecutive 20-minute periods with glucose concentrations of 5.6, 16.7, 5.6, and 16.7 mmol/L. Insulin level was measured by radioimmunoassay[31] compared with rat insulin (Eli Lilly, Indianapolis, IN) standards, and secretion over the entire 80 minutes was compared among treatment groups.

Histology

Pancreas histology was examined in BB rats either at weaning, at weight plateau of rats on the A-def diet and simultaneously in the rats pair-fed to them (about 60 days old), or after the usual period of diabetes development ($\geq$120 days old). Pancreases from rats

that developed clinical diabetes were not included in these analyses. Pancreases were fixed in Bouin's solution and Formalin and stained with hematoxylin and eosin. All islets in one section were graded for insulitis by one observer unaware of treatment status, and a mean score was calculated for each rat. Insulitis was graded using a semiquantitative scale from 0 to 4 based on the report by Mathieu et al[32]: 0, no mononuclear cells in or around islet; 1, periductular and periislet infiltration without capsular penetration; 2, periislet infiltration with capsular penetration; 3, intraislet infiltration occupying less than 50% of the islet; and 4, intraislet infiltration occupying more than 50% of the islet.

Statistical Analyses

Analysis of the onset of diabetes was performed using a computerized statistical program (life-table analysis) and the log-rank test (SAS Institute, Cary, NC). All other analyses used another statistical package (SPSS, Chicago, IL). Incidence of diabetes by 120 days of age was analyzed by the chi-square test. Male or female rat weights for any age were compared using one-way ANOVA and the Student-Neuman-Keuls procedure for multiple comparisons. For analysis, insulin secretion values were log-transformed to ensure homogeneity of variance. Values were then compared by ANOVA and the Student-Neuman-Keuls procedure. Histology scores were compared by one-way ANOVA with the least-significant difference procedure for multiple comparisons and by linear regression against age or insulin secretion.

RESULTS

Incidence of Diabetes

Diabetes occurred at a greatly reduced rate in DP rats that were started on the A-def diet and became clinically deficient (four of 27, 14.8%) and in DP rats fed the RA diet continuously from weaning (four of 29, 13.8%) as compared with the rate (13 of 31, 41.9%) in DP rats fed the RP diet continuously from weaning ($P < .02$). Comparison of individual treatments showed the protective effects of A-def/RA and RA diets to be equivalent; each of these resulted in less diabetes than the RP diet ($P < .05$). Life-table analysis (Fig 1) confirmed the differences among treatments ($P < .01$). The age of onset of diabetes in 13 rats on the RP diet that developed diabetes was 99.4 ± 3.4 days (mean ± SE), somewhat older than the population mean of 91 days reported by others before establishment of viral antibody–free conditions at the central breeding colony.[1] None of 19 DR rats made clinically vitamin A–deficient or 24 DR controls fed the RP diet developed diabetes by 120 days of age.

DP rats tolerated clinical vitamin A deficiency less well than normal rats in previous studies,[12,27,33] with a mortality of 12 of 39 (30.8%) at the time of clinical deficiency without either hyperglycemia or clinical evidence of infection such as nasal discharge, pulmonary congestion, or hepatosplenomegaly. In comparison, DR rats had a mortality of one of 20 (5.0%, $P = .05$ *v* DP rats). No rats fed RA or RP diets died during this period. DP and DR rats that died while acutely vitamin A–deficient during the period of weight plateau were excluded from analyses of diabetes incidence and insulitis.

Despite pair-feeding, rats that were fed the RP diet tended to maintain a greater weight than the other groups, commencing at the time when rats on the A-def diet were clinically deficient. DP rats on the RA diet and on the A-def/RA diet had similar weight profiles except for slight differences during the period of clinical vitamin A deficiency (Fig 2).

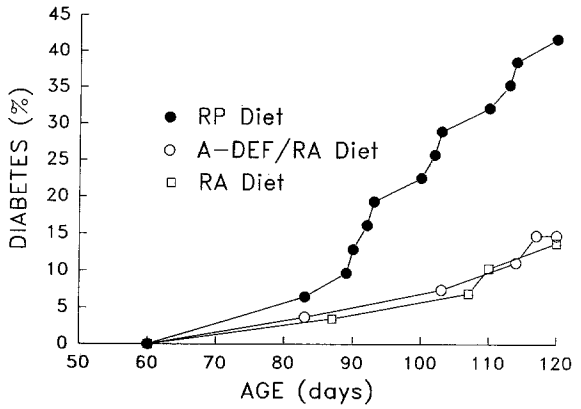

Fig 1. Cumulative incidence of diabetes in BB/Wor DP rats receiving diets of differing retinoid content. Each point showing an increase in diabetes from the previous point represents development of diabetes in 1 to 2 rats. Rats receiving retinoic acid at 2 μg/g diet with (A-DEF/RA Diet, n = 27) or without (RA Diet, n = 29) a 10-day period of clinical deficiency around 60 days of age had an improved diabetes-free survival as compared with rats on a retinol-replete diet (RP diet, n = 31) with 4 μg/g retinyl palmitate ($P < .01$, log-rank test).

Insulin Secretion

Glucose-induced insulin secretion was measured in perfused pancreases of DP and DR rats from all diet groups that did not develop diabetes by 120 days of age (Fig 3). DP rats fed the RP diet (the treatment with the highest incidence of diabetes) had significantly less insulin secre-

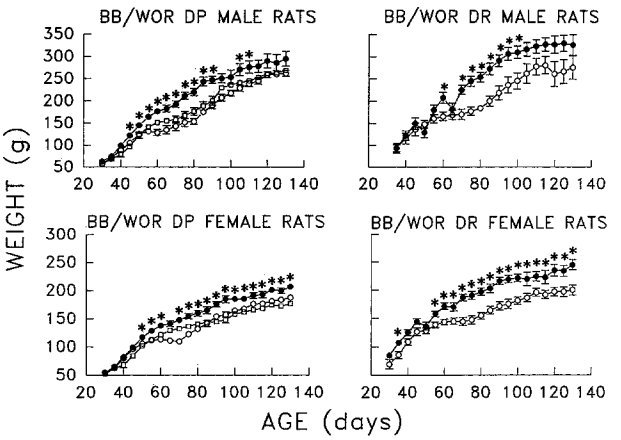

Fig 2. Growth (weight, mean ± SE) of BB/Wor rats receiving diets of differing retinoid content. (○) A-def/RA diet; (□) RA diet; (●) RP diet. DP rats: *$P < .05$ for both A-def/RA *v* RP and RA *v* RP; differences between RP diet and only one other diet and differences between A-def/RA diet and RA diet have been omitted for clarity. DR rats: *$P < .05$ for A-def/RA *v* RP. The median number of animals represented by the points of each growth curve is 11 and 14 for DP males and females on the A-def/RA diet, 10 and 14 for DP males and females on the RA diet, 12 and 13 for DP males and females on the RP diet, 7 and 12 for DR males and females on the A-def/RA diet, and 14 and 9 for DR males and females on the RP diet.

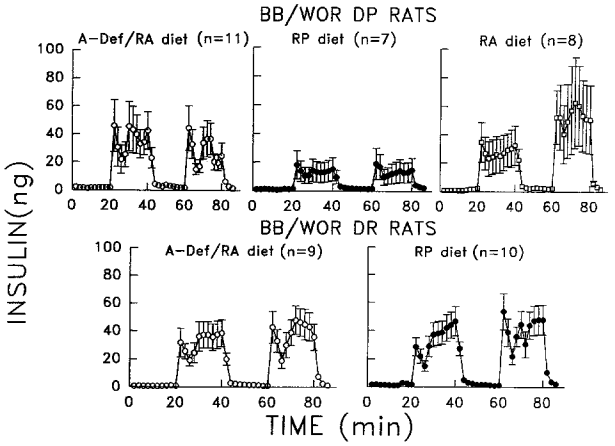

Fig 3. Insulin secretion by nondiabetic BB/Wor rats ≥120 days old receiving diets of differing retinoid content. Each point represents the mean ±SE insulin secretion from perfused pancreas during the preceding 2 minutes for each treatment group. (○) A-def/RA diet; (□) RA diet; (●) RP diet. Perfusate glucose concentration was 5.6 mmol/L during minutes 0 to 20, 40 to 60, and >80. Glucose concentration was 16.7 mmol/L during minutes 20 to 40 and 60 to 80. Cumulative insulin secretion (mean ± SE) over two low-glucose and two high-glucose 20-minute periods was as follows: DP rats on RP diet, 308 ± 153 ng (n = 7); DP rats on A-def/RA diet, 708 ± 205 ng (n = 11); DP rats on RA diet, 868 ± 370 ng (n = 8); DR rats on A-def/RA diet, 747 ± 174 ng (n = 9); and DR rats on RP diet, 832 ± 167 ng (n = 10). Total insulin secretion over minutes 0 to 80 was less for DP rats on the RP diet than for any other group ($P < .05$ for each comparison).

tion than any other diet treatment group ($P < .05$ for each comparison).

Insulitis

Histology scores grouped according to type of rat, diet treatment, and age revealed significant differences among the groups ($P < .001$). The results are detailed in Table 1 and can be summarized by noting that (1) DP rats receiving the A-def diet had low levels of insulitis, similar to all DR rats, (2) DP rats continuously on RA or RP diets through at least 120 days of age showed the most insulitis, and (3) although insulitis increased by 120 days of age in DP rats on the RA diet, it was low at 60 days of age.

Table 1. Histology Scores (mean ± SD) for Severity of Insulitis in BB/Wor Rats

Age and Diet Treatment	DP Rats	DR Rats
Weanling	1.08 ± 0.36 (13)	1.06 ± 0.33 (9)
A-def, ~60 days	0.99 ± 0.59 (6)	1.30 ± 0.50 (6)
A-def/RA, ≥120 days	1.36 ± 0.77(17)*	1.07 ± 0.61(18)
RA, ~60 days	1.12 ± 0.96 (7)	
RA, ≥120 days	1.96 ± 1.12(17)†	
RP, ~60 days	1.42 ± 0.98 (5)	1.02 ± 0.63 (6)
RP, ≥120 days	1.80 ± 0.96(14)‡	0.80 ± 0.32(20)

NOTE. DP rats on the RA diet or the RP diet at ≥120 days had the highest insulitis scores, and DR rats, young DP rats, and DP rats on the A-def/RA diet tended to have lower scores. Number of rats is shown in parentheses.

*Greater than DR rats on the RP diet at ≥120 days ($P < .05$).

†Greater than DP or DR weanlings, DP rats on the A-def diet at ~60 days (weight plateau), DP or DR rats on the A-def/RA diet at ≥120 days, DP rats on the RA diet at ~60 days, DR rats on the RP diet at ~60 days, and DR rats on the RP diet at ≥120 days ($P < .05$ for each comparison).

‡Greater than DP or DR weanlings, DP rats on the A-def diet at ~60 days (weight plateau), DR rats on the A-def/RA diet at ≥120 days, DP rats on the RA diet at ~60 days, DR rats on the RP diet at ~60 days, and DR rats on the RP diet at ≥120 days ($P < .05$ for each comparison).

Linear regression analysis further substantiated the impact of dietary retinoids on insulitis by demonstrating significant correlations between insulitis score and rat age for DP rats on either the RA diet ($r = .33, P < .05$) or the RP diet ($r = .42, P < .02$) continuously. These correlations indicate an increase in insulitis over time despite the lack of progression to clinical diabetes in these individual rats. No such correlation of insulitis with age was found for DP rats that started on the A-def diet and became clinically deficient ($P > .22$), suggesting a greater suppression of insulitis by the more complete deficiency of vitamin A. In addition, DR rats showed no correlation of insulitis with age either for those that had been clinically vitamin A–deficient ($P > .98$) or for those continuously on the RP diet ($P > .38$).

Histology scores correlated negatively with insulin secretion ($r = -.35, P < .05$), so rats with less insulitis had better insulin secretion regardless of previous retinoid deficiency.

DISCUSSION

These studies demonstrate a reduction in the incidence of diabetes in BB/Wor DP rats given a diet either deficient in all retinoids (A-def diet) or deficient in retinol but containing enough retinoic acid to permit growth (RA diet). The dose of retinoic acid chosen, 2 μg/g diet, was previously shown to be sufficient to permit growth.[26,27] However, this restriction was sufficient to disrupt the sequence of events that would otherwise culminate in the autoimmune destruction of β cells and diabetes in DP rats. Our finding that DP rats that continuously received the RA diet developed more insulitis than DP rats that had received the A-def/RA diet and had been clinically deficient suggests that retinoic acid substitutes at least partly for retinol in the processes leading toward diabetes. However, another possible explanation for this finding is that retinol or a specific metabolite of retinol is required for diabetogenesis in BB/Wor rats. This explanation is supported by studies showing effects of 14-hydroxy-*retro*-retinol on lymphocyte function.[14,19,21]

The unexpectedly high mortality from clinical vitamin A deficiency initially raised the question of whether rats that were destined to develop diabetes could be more sensitive to hypovitaminosis A. In that case, the reduction in incidence could be explained by deletion of susceptible rats rather than by genuine protection. However, the reduction in diabetes incidence in rats on the RA diet, which did not have any increased mortality, disproves that hypothesis and is consistent with a partial deficiency of retinoids resulting from retinoic acid at 2 μg/g diet. Histology scores of pancreases at approximately 60 days of age do not indicate that rats destined to develop diabetes in the A-def or RA diet groups were killed for these studies. Although not statistically different, the scores are in fact trending lower in

the protected groups than in the higher-incidence RP diet group.

Growth curves of male and female DP rats on the RP diet are identical to previous growth curves of DP rats on semisynthetic diets,[6] and greater weight gain in male and female DR rats than in DP rats has been observed previously.[34] Another interesting observation was that despite pair-feeding, rats fed RA or A-def/RA diets remained at a slightly lower weight than comparable rats fed the RP diet, in agreement with Wiedermann et al.[33] Decreased absorption or utilization of nutrients in rats receiving the RA diet may explain the weight differences, especially since vitamin A deficiency is known to have effects on intestinal mucosa.[33,35] However, this process could not account for our findings, because it has previously been shown that caloric restriction alone does not alter the incidence of diabetes in DP rats.[9] Any effects of restriction of retinoids to pregnant dams[36] on the development of diabetes in the offspring are not clarified in these studies, since all diet groupings were made at weaning.

The overall incidence of diabetes in DP rats fed the RP diet is lower than the current breeding colony incidence of approximately 80%.[37] Two factors may contribute to the difference. First, the majority of data on incidence were collected before establishment of the high-incidence viral antibody–free breeding colony, when the incidence was 60% to 70% by 120 days.[1] Second, a reduction in diabetes with semisynthetic diets has been reported without vitamin A deficiency.[6-8] Notwithstanding these differences in incidence, in our study the only difference among diets was retinoid supplementation, so different incidences of diabetes among treatment groups must be due to retinoid content and not to variations in protein, fat, or carbohydrate sources. In addition, the RP diet did not contain any excess of retinol that could lead to pharmacologic or toxic effects. Retinoid content of the RP diet is 4 μg retinyl palmitate (7.3 IU vitamin A activity)/g diet. This content is less than the content of 15.0 IU/g for chow (Purina Rodent Laboratory Chow #5001; Ralston-Purina) and more than the 4.0 IU/g provided by the AIN-76 diet.[38] The minimum dietary requirement to normalize sensitive indicators of retinol effects in deficient rats has been estimated at 1.1 to 2.6 IU/g diet.[26]

Insulin secretion was decreased in DP rats fed the RP diet that did not develop clinical diabetes by 120 days of age. The higher levels of insulitis in this group and the correlation of insulitis with reduced insulin secretion suggest that the immune attack on β cells permitted by retinoid sufficiency resulted in impaired insulin secretion even without frank diabetes.

The association of higher levels of insulitis with advancing age in DP rats fed the RA diet that did not develop diabetes again suggests that DP rats continuously fed the RA diet are intermediate in terms of vitamin A status between DP rats on the A-def/RA diet (which were protected from diabetes and insulitis) and DP rats on the RP diet (which developed clinical diabetes). DR rats showed no tendency to develop diabetes or insulitis through at least 120 days of age. These findings in DR rats suggest that the protective function of RT6.1$^+$ regulatory cells[1] is not impaired by hypovitaminosis A more than the function of the effector cells that are also present in DR rats in such a way that diabetes or insulitis develops.

In summary, vitamin A deficiency reduces diabetes and insulitis in DP rats. The more profound the deficiency is, the more complete the effect. Vitamin A status may therefore be relevant in the pathogenesis of human insulin-dependent diabetes mellitus, as well as in the recurrence of disease in transplanted islets or pancreas. Future studies need to address the mechanism by which vitamin A deficiency prevents diabetes on a more basic level, ie, the roles of retinoic acid versus retinol and their metabolites, which retinoid receptors mediate retinoid actions, and which target cells and gene products affect the autoimmune process. Consideration can be given to intervention with new classes of retinoids with precise and limited spectra of effects,[39] which can be devised to interrupt specifically the processes that lead to diabetes. Currently, studies examining the effects of vitamin A deficiency on lymphocytes in BB rats are under way to help define the mechanism by which retinoid deficiency leads to prevention of diabetes.

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REFERENCES

1. Crisá L, Mordes JP, Rossini AA: Autoimmune diabetes in the BB rat. Diabetes Metab Rev 8:9-37, 1992

2. Logothetopoulos J, Valiquette N, Madura E, et al: The onset and progression of pancreatic insulitis in the overt, spontaneously diabetic, young adult BB rat studied by pancreatic biopsy. Diabetes 33:33-36, 1984

3. Burstein D, Mordes JP, Greiner DL, et al: Prevention of diabetes in the BB/Wor rat by a single transfusion of spleen cells: Parameters that affect the degree of protection. Diabetes 38:24-30, 1989

4. Gotfredsen CF, Buschard K, Frandsen EK: Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. Diabetologia 28:933-935, 1985

5. Greiner DL, Mordes JP, Handler ES, et al: Depletion of RT6.1$^+$ T lymphocytes induces diabetes in resistant BioBreeding/Worcester (BB/W) rats. J Exp Med 166:461-475, 1987

6. Elliott RB, Martin JM: Dietary protein: A trigger of insulin-dependent diabetes in the BB rat? Diabetologia 26:297-299, 1984

7. Scott FW, Marliss EB: Conference Summary: Diet as an environmental factor in development of insulin-dependent diabetes mellitus. Can J Physiol Pharmacol 69:311-319, 1991

8. Hoorfar J, Scott FW, Cloutier HE: Dietary plant materials and development of diabetes in the BB rat. J Nutr 121:908-916, 1991

9. Lefkowith J, Schreiner G, Cormier J, et al: Prevention of diabetes in the BB rat by essential fatty acid deficiency: Relationship between physiological and biochemical changes. J Exp Med 171:729-743, 1990

10. Blaner WS, Olson JA: Cellular biology and biochemistry of

the retinoids, in Sporn MB, Roberts AB, Goodman DS (eds): The Retinoids: Biology, Chemistry, and Medicine (ed 2). New York, NY, Raven, 1994, pp 229-255

11. Giguère V: Retinoic acid receptors and cellular retinoid binding proteins: Complex interplay in retinoid signaling. Endocr Rev 15:61-79, 1994

12. Chertow BS, Blaner WS, Baranetsky NG, et al: Effects of vitamin A deficiency and repletion on rat insulin secretion in vivo and in vitro from isolated islets. J Clin Invest 79:163-169, 1987

13. Chertow BS, Moore MR, Blaner WS, et al: Cytoplasmic retinoid-binding proteins and retinoid effects on insulin release in RINm5F β-cells. Diabetes 38:1544-1548, 1989

14. Eppinger TM, Buck J, Hämmerling U: Growth control or terminal differentiation: Endogenous production and differential activities of vitamin A metabolites in HL-60 cells. J Exp Med 178:1995-2005, 1993

15. Semba RD: Vitamin A, immunity, and infection. Clin Infect Dis 19:489-499, 1994

16. West KP, Howard GR, Sommer A: Vitamin A and infection: Public health implications. Annu Rev Nutr 9:63-86, 1989

17. Semba RD, Graham NMH, Caiaffa WT, et al: Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. Arch Intern Med 153:2149-2154, 1993

18. Carman JA, Smith SM, Hayes CE: Characterization of a helper T lymphocyte defect in vitamin A–deficient mice. J Immunol 142:388-393, 1989

19. Garbe A, Buck J, Hämmerling U: Retinoids are important cofactors in T cell activation. J Exp Med 176:109-117, 1992

20. Buck J, Ritter G, Dannecker L, et al: Retinol is essential for growth of activated human B cells. J Exp Med 171:1613-1624, 1990

21. Buck J, Derguini F, Levi E, et al: Intracellular signaling by 14-hydroxy-4,14-*retro*-retinol. Science 254:1654-1656, 1993

22. Chun TY, Carman JA, Hayes CE: Retinoid repletion of vitamin A–deficient mice restores IgG responses. J Nutr 122:1062-1069, 1992

23. Friedman A, Halevy O, Schrift M, et al: Retinoic acid promotes proliferation and induces expression of retinoic acid receptor-α gene in murine T lymphocytes. Cell Immunol 152:240-248, 1993

24. Cantorna MT, Nashold FE, Hayes CE: In vitamin A deficiency multiple mechanisms establish a regulatory T helper cell imbalance with excess Th1 and insufficient Th2 function. J Immunol 152:1515-1522, 1994

25. Suda T, DeLuca HF, Tanaka Y: Biological activity of 25-hydroxyergocalciferol in rats. J Nutr 100:1049-1052, 1970

26. National Research Council: Nutrient Requirements of Laboratory Animals, vol 10 (ed 3 rev). Washington, DC, National Academy of Sciences, 1978

27. Lamb AJ, Apiwatanaporn P, Olson JA: Induction of rapid, synchronous vitamin A deficiency in the rat. J Nutr 104:1140-1148, 1974

28. Weir GC, Knowlton SD, Martin DB: Glucagon secretion from the perfused rat pancreas: Studies with glucose and catecholamines. J Clin Invest 54:1403-1412, 1974

29. Driscoll HK, Gottlieb PA, Mordes JP, et al: Plasma from BB/Wor rats increases insulin secretion by perfused rat pancreas. Endocrinology 126:1241-1249, 1990

30. Chertow BS, Driscoll HK, Blaner WS, et al: Effects of vitamin A deficiency and repletion on rat glucagon secretion. Pancreas 9:475-484, 1994

31. Herbert V, Lau K-S, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab 25:1375-1384, 1965

32. Mathieu C, Laureys J, Sobis H, et al: 1,25-Dihydroxyvitamin D_3 prevents insulitis in NOD mice. Diabetes 41:1491-1495, 1992

33. Wiedermann U, Hanson LÅ, Kahu H, et al: Aberrant T-cell function in vitro and impaired T-cell dependent antibody response in vivo in vitamin A–deficient rats. Immunology 80:581-586, 1993

34. Markholst H, Eastman S, Wilson D, et al: Decreased weight gain in BB rats before the clinical onset of insulin-dependent diabetes. Diabetes Res Clin Pract 21:31-38, 1993

35. Gmoshinskii IV, Khvylya SI, Ya KI: Effect of vitamin A deficiency on permeability of the small intestinal mucosa for macromolecules in adult rats. Bull Exp Biol Med 103:179-182, 1987

36. Gardner EM, Ross AC: Dietary vitamin A restriction produces marginal vitamin A status in young rats. J Nutr 123:1435-1443, 1993

37. Like AA, Guberski DL, Butler L: Influence of environmental viral agents on frequency and tempo of diabetes mellitus in BB/Wor rats. Diabetes 40:249-262, 1991

38. Bieri JG, Stoewsand GS, Briggs GM, et al: Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 107:1340-1348, 1977

39. Fanjul A, Dawson MI, Hobbs PD, et al: A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. Nature 372:107-111, 1994