Oral Exposure to Diabetes-Promoting Food or Immunomodulators in Neonates Alters Gut Cytokines and Diabetes

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Disease development in diabetes-prone BB rats is modified by the type of diet fed after weaning. The aim of this investigation was to determine whether exposure during the first week of life to antigens from a known diabetes-promoting diet (NIH-07) could modify diabetes incidence and, if so, to what extent this occurs via alterations in systemic T-cell reactivity, gut cytokines, or islet infiltration. Diabetes-prone BB (BBdp) rats were hand-fed twice daily between age 4 and 7 days with vehicle, a hydrolyzed casein (HC)–based infant formula, Pregestimil (PG), PG + cereal-based NIH-07 diet, PG + lipopolysaccharides (LPS) or PG + LPS + silica. After weaning, they were fed either an NIH-07 diet or a semipurified HC (diabetes-retardant) diet until 150 days. In separate studies, 5-day-old BBdp rat pups were administered the aforementioned treatments, and expression of intestinal mRNA for γ-interferon (IFN-γ) or transforming growth factor-β (TGF-β) was quantified using reverse transcriptase–polymerase chain reaction. The effect of early oral treatment with NIH-07 or PG on systemic T-cell reactivity was evaluated using footpad swelling delayed-type hypersensitivity (DTH) and the popliteal lymph node assay. Oral exposure of neonates to a complex mixture of antigens from the diabetes-promoting diet delayed onset of diabetes (79 vs. 88 days) and prevented disease in approximately one-third of animals. A similar protective effect was seen for neonatal exposure to wheat gluten in animals subsequently weaned onto a semipurified wheat gluten diet. By contrast, LPS-treated neonates displayed more severe insulitis and developed diabetes at an increased rate, which was significantly suppressed by co-administration of silica particles. The protective effect of early exposure to diabetogenic diets was not associated with significant reduction of islet infiltration, and there was no impact on the DTH response to food antigens. However, whereas diabetes-resistant BBc rats developed systemic tolerance to NIH-07 antigens fed chronically, BBdp rats did not. The lack of effect of the early oral antigen regimen on the DTH reaction in the footpad, a classic Th1-mediated reaction, suggests little effect on systemic T-cell reactivity. However, local effects were observed in the small intestine. Oral exposure to diabetes-promoting food antigens or LPS downregulated the Th1 cytokine IFN-γ and decreased the IFN-γ/TGF-β ratio. Thus, oral exposure to diabetes-promoting food antigens and immune modulators in neonates can modify diabetes expression in association with changes in local cytokine balance in the gut. *Diabetes* 51:73–78, 2002

Type 1 diabetes occurs in individuals with certain gene expression patterns that increase their risk of developing diabetes when they are exposed to common factors in the environment, such as infectious or dietary agents (1,2). Because diabetes is modified by diet, it has been suggested that handling of antigens by the gut of susceptible individuals may be abnormal (3–7).

The immune response of the gut of the neonate is less well-developed than in adults, and this relative immune deficiency makes it easier to induce tolerance to orally administered antigens (8,9). Indeed, neonates normally display a Th2 cytokine bias in the gut (9,10). This is the opposite in the adult BB rat (11); moreover, the gut exhibits sporadic inflammation and damage (12; F.W.S., A.M. Mowat, W.J. Malaisse, P. Courtois, S. Graham, H. Kolb, and H. Wang, unpublished data). These findings plus the fact that diet is a major factor in BB rat diabetes are consistent with the suggestion that the gastrointestinal tract must play a role in diabetes pathogenesis in the BB rat (2,3,6).

There have been attempts to induce oral tolerance in nonobese diabetic mice (13) and diabetes-prone BB (BBdp) rats with insulin (14) to delay or prevent diabetes, as well as clinical trials in humans (15). However, immune reaction after administration of antigens by the oral route can be immunostimulatory, even resulting in autoimmunity (16), or induce tolerance (17) depending on age, amount of antigen, and the presence of other compounds. Thus, attempts to prevent autoimmune diseases by inducing oral tolerance to selected individual autoantigens have met with mixed success in animals and little success in

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BLU, Boehringer light units; DTH, delayed-type hypersensitivity; HC, hydrolyzed casein diet; IFN-γ, γ-interferon; IL, interleukin; LPS, lipopolysaccharide; NIH, NIH-07 diet; PG, Pregestimil diet; PLN, popliteal lymph node; RT-PCR, reverse transcriptase–polymerase chain reaction; TGF-β, transforming growth factor-β; WG, wheat gluten diet.

humans (18). One reason for these disparities may be the use of single autoantigens or peptides when in fact diabetes-promoting conditions are favored by several diabetes-promoting antigens. The present studies was carried out to determine whether oral exposure to antigens from a complex diabetes-promoting diet in the first week of life has an impact on later disease development. A known diabetes-promoting mixture of dietary antigens and/or immune modulators, LPS and silica, was fed to BBdp rat neonates, and animals were weaned onto diets of widely differing diabetes-inducing potential. The effects on diabetes incidence and islet inflammation, systemic T-cell–mediated immunity as measured by delayed-type hypersensitivity (DTH), and local gut immune balance were measured.

RESEARCH DESIGN AND METHODS

Animals. Male and female BBdp and control BB rats (BBc) were obtained from the Animal Resources Division of Health Canada, where they are maintained under specific pathogen-free conditions. BBc rats derive from an early subline of animals in this colony that does not spontaneously develop diabetes. Animals were antibody-free with respect to Sendai virus, pneumonia virus of mice, rat corona virus/sialodacryoadenitis virus, Kilham rat virus, Toolan's H-1 virus, reovirus type 3, and mycoplasma pulmonis. Animals were weaned at 23 days of age, caged in banks of 30 wire-bottom cages, and given free access to food and water. The principles of laboratory animal care as described by the Canadian Council on Animal Care were followed.

The animals were weighed at weekly intervals between the ages of 30 and 100 days. Animals were tested twice weekly for glucose in the urine after 60 days of age (Testape; Lily, Toronto, Ontario, Canada). Animals with a value of 2+ or greater were fasted overnight, and blood glucose was measured the next morning in tail blood using glucose test strips and a glucometer. Diabetes was diagnosed in an animal when fasting blood glucose was >11.1 mmol/l (200 mg/dl). Diabetic animals were killed within 24 h of diagnosis by exsanguination while under 3% halothane in oxygen anesthesia.

Diets

NIH-07. Diets were fed in powdered or meal form in open bowls. A standard rodent diet, NIH-07 (NIH), was purchased in meal form from Ziegler Brothers (Gardners, PA). NIH-07 is an open-formula, nonpurified diet that provides dietary amino acids mainly from plants. The NIH-07 diet is composed of 5% dried skim milk, 10% fish meal, 12% soybean meal, 4% alfalfa meal, 3% corn gluten meal, 24.5% ground yellow shelled corn, 23% ground hard winter wheat, 10% wheat middlings, 2% brewer's dried yeast, 1.5% molasses, 2.5% soybean oil, 0.5% sodium chloride, 1.25% dicalcium phosphate, 0.5% ground limestone, and 0.25% premixes (19).

Wheat gluten diet. Wheat gluten (WG) semipurified diets were made up of 22.5% wheat gluten (ICN Biochemicals, Cleveland, OH), 50.2% corn starch, 12.0% sucrose, 5.0% corn oil, 5% alphacel, 3.5% AIN-76 mineral mix (ICN), 1.0% AIN-76A vitamin mix (ICN), 0.2% choline bitartrate, 0.02% DL-methionine, 0.5% L-lysine, and 0.08% L-threonine.

Hydrolyzed casein-based diet. The semipurified hydrolyzed casein (HC) diet consisted of 53.0% corn starch, 12.0% sucrose, 20.0% casein hydrolysate (Champlain Industries, Mississauga, Ontario, Canada [presently RedStar Bioproducts, Tara, Ontario, Canada]), 5% corn oil, 5% alphacel, 3.5% AIN-76 mineral mix, 1.0% AIN-76A vitamin mix, 0.2% choline bitartrate, and 0.3% DL-methionine (19).

Oral feeding regimen. To test the possibility that early oral exposure to a diet known to be diabetes-promoting might alter diabetes outcome, we removed BBdp rat pups from their dams and hand-fed them twice daily from 4 to 7 days of age a mixture of Pregestimil (PG) (Mead Johnson, Belleville, Ontario, Canada), a hypoallergenic infant formula diet based on hydrolyzed casein (HC) plus ground NIH-07 diet or plus wheat gluten or PG alone. Pregestimil consists of 13% hydrolyzed casein, 62% carbohydrates (corn starch and corn syrup solids), and modified coconut oil, supplemented with vitamins A, B_1, B_2, B_3, B_6, B_{12}, C, D, E, K; biotin; folic acid; α-pantothenic acid; the amino acids L-tryptophan, L-cystine, and L-tyrosine; and mineral supplements. Because of the labor-intensive nature of these studies, which limited the size of individual trials, three separate experiments were carried out with early NIH-07 feeding and the data were pooled. The total number of animals treated was as follows (neonatal treatment/weaning diet): PG/HC, 37; PG/NIH, 39; and NIH/NIH, 42. In a separate study, two groups of animals were treated as follows: PG/WG, 15; and WG/WG and then weaned at day 23 onto a WG diet, 14. A control group, PG/HC, contained 15 rats.

The pups were fed through Technicon-B rubber tubing attached to a 23-G needle and a 1-ml syringe. The diet mixture was kept at body temperature during feeding. After feeding, the pups were returned to their dams. Feces- and urine-soaked bedding were applied to the pups to reduce maternal cannibalism. The amount of protein in the NIH-07 slurry fed to the pups was equivalent to 1 mg/g body wt, which at the weight of the pups at the time of feeding (~15 g) meant a dose of 67 mg NIH per rat per feeding. WG was given at a dose of ~20 mg/0.45 ml to deliver 1 mg protein/g body wt. This was delivered in 0.45 ml of fluid. After the 4 days of hand-feeding, the pups remained with the dams until weaning. The animals were weaned onto one of three diets: two modified, semipurified diets in which HC or WG was the sole source of amino acids or the standard rodent diet, NIH-07. Lipopolysaccharides (LPS) (*Escherichia coli* serotype 0127:B8; Sigma) was administered to neonates as above at a concentration of 0.75 mg/0.4 ml, and silica (Sigma) was administered concurrently at a concentration of 1.2 mg/0.4 ml.

Footpad swelling and popliteal lymph node assays. BBc and BBdp animals that were treated in the first week of life with either oral PG or NIH were fed a diabetes-promoting NIH-07 diet or protective HC diet from weaning. At 60 days of age, the animals were lightly anesthetized and received an injection of autoclaved NIH-07 diet in PG (0.5 mg/ml, equivalent to ~3 μg of protein from NIH-07 diet in 25 μl) in the left footpad and, as a control, with PG in the right footpad. Footpads were cleaned with an iodine solution. Footpad thickness was measured before the injection for a zero time measurement. The time was noted, and subsequent measures were performed at approximately the same time of day. Footpad thickness was measured with a micrometer (Mitutoyo, Montreal, Canada) after 24 h. All procedures took place between 8:00 A.M. and 12:00 P.M.. After 7 days, the animals were killed, popliteal lymph nodes (PLNs) were excised, the fat was trimmed, and each lymph node was weighed.

Tissue collection. After exsanguination under halothane anesthesia or decapitation (pups only), pancreata were dissected free, trimmed of fat, and placed in Bouin's solution for fixation. A 12-cm piece of small intestine beginning at the stomach was removed and flushed with ice-cold phosphate-buffered saline, and the distal 2 cm was frozen immediately in liquid nitrogen and stored at −80°C for reverse transcriptase–polymerase chain reaction (RT-PCR) analyses.

Evaluation of islet infiltration. Hematoxylin and eosin–stained sections (5 μm) were read for insulitis and degree of islet damage using a light microscope (20). Briefly, the overall extent of mononuclear cell infiltration of the islets (insulitis) and damage in a section was graded according to a scale of 1 to 5 as follows: 1, normal section, good number of islets (~32), no infiltration; 2, mild infiltration (up to 30% of islets infiltrated), still a good number of islets observed (~30); 3, several islets were present (~28), but most (~90%) were infiltrated; 4, few islets remained (~14), most were infiltrated (~78%) or very small; and 5, end-stage disease, displaying very few small islets (<5), with little inflammatory infiltrate remaining.

Gut cytokine gene expression measured using RT-PCR. In two separate experiments, gut cytokines were measured in pups fed on day 5 as above, then killed the following morning. Total RNA was isolated from the duodenal section of the small intestine using Trizol Reagent (Life Technologies, Karlsruhe, Germany) following the manufacturer's instructions. RT-PCR was performed as described elsewhere (21). Primer sequences (β-actin, γ-interferon [IFN-γ], and transforming growth factor-β [TGF-β]) were obtained from Clontech (Palo Alto, CA). PCR conditions were within the semiquantitative range as verified by initially performing serial dilutions of cDNA and varying the number of cycles used for PCR. The resulting cycle numbers were 22 for β-actin and 34 for IFN-γ and TGF-β. In all amplifications, template-negative controls confirmed the absence of contaminating cDNA. Amplified products were visualized in a 1.5% agarose gel containing ethidium bromide. For quantification, the fluorescence intensity of each band was determined as Boehringer light units (BLU) using a Lumi-Imager (Boehringer Mannheim, Mannheim, Germany) and the specific analysis software (LumiAnalyst 3.0). A curve was generated by plotting the amount of cDNA versus the corresponding BLU. Linear regression was performed with this curve, and the mRNA amount was set as the BLU value for 1 μl of cDNA. The correlation between different amounts of cDNA and the corresponding fluorescence intensity ranged between 0.90 and 0.99. The relative amounts of cytokine mRNA were expressed as arbitrary units as the ratio of cytokine to the respective β-actin mRNA.

RESULTS

Impact of neonatal exposure to a diabetes-promoting diet on disease development. Survival curves for BBdp

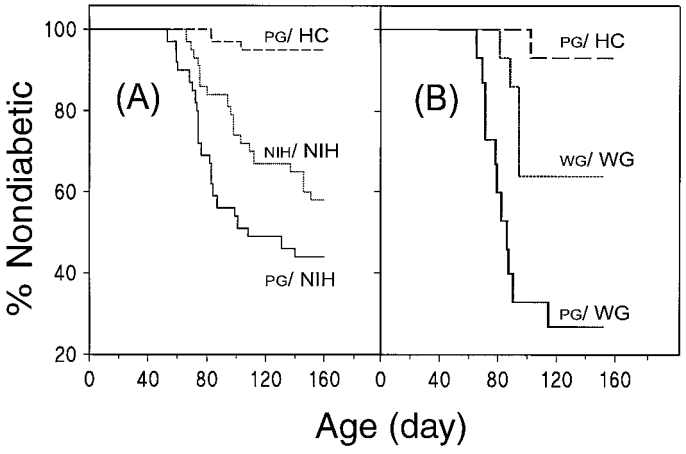

FIG. 1. Early oral exposure to a diabetes-promoting diet inhibits development of diabetes in neonates. BBdp rats were hand-fed twice daily between ages 4 and 7 days. Early antigen treatment is denoted by a smaller font, and the weaning diet is shown in standard upper-case font. NIH/NIH versus PG/NIH ($P = 0.02$); WG/WG versus PG/WG, $P = 0.008$ (Kaplan-Meier, log rank statistic). (*A*): Pooled data from three separate experiments; total number of animals: PG/HC, 2/37; NIH/NIH, 16/42; PG/NIH, 22/39. *B*: Data from an individual experiment in which the numbers per group were as follows: PG/HC, 15; WG/WG, 14; PG/WG, 15 (PG/WG versus WG/WG, $P = 0.05$).

rats fed PG between days 4 and 7 and weaned at 23 days onto the semipurified HC diet (PG/HC group) or weaned onto cereal-based NIH-07 (PG/NIH) showed a major and significant difference in diabetes rate by 150 days of age, confirming the important modulatory role of diet after weaning (2) (Fig. 1*A*). In addition, when the diabetes-inducing diet, NIH-07, was fed in the first week of life, the onset of diabetes was delayed (79 ± 6 vs. 88 ± 7 days) and approximately one-third fewer diabetes cases were observed (16 out of 42 vs. 22 out of 39 [38 vs. 56%]; $P = 0.02$, Kaplan-Meier, log rank test) (Fig. 1*A*). There was no effect of early antigen treatment on islet infiltration as measured by the number of islets infiltrated or on overall insulitis rating (Table 1). Animals fed PG in the first week of life and weaned onto an HC diet had low diabetes frequency (2 of 37 [5%]). Fewer islets were infiltrated, two to three times more were normal, and unaffected islets and the overall insulitis rating were lower than in animals weaned onto NIH-07 (Table 1).

TABLE 1
Early dietary antigens and inflammation in the pancreas

Early oral antigen fed to neonates	HC weaning diet PG	NIH weaning diet PG	NIH weaning diet NIH
n	34	36	42
Islets unaffected	44 ± 29†‡	19 ± 17†	22 ± 21‡
Islets infiltrated*	4 ± 6	6 ± 11	8 ± 9
Islets infiltrated (%)	12 ± 18†‡	30 ± 3†	33 ± 30‡
Mean overall insulitis rating per rat	1.9 ± 1.1†‡	2.7 ± 1.5†	2.8 ± 1.3‡

Data are means ± SD. *Islet infiltration defined as ≥5 mononuclear cells/islet; † and ‡, in each row, numbers with the same symbol are significantly different at $P \leq 0.02$ by analyses of variance with least significant difference post-hoc comparisons.

Because NIH-07 is a complex mixture of cereal and other proteins, the experiment was repeated with a semipurified diet containing WG as the sole source of protein and being isocaloric and isonitrogenous with the HC diet. As shown in Fig. 1*B*, this diet was as diabetes-promoting as NIH-07 when fed from weaning. Onset of diabetes was again delayed (82 vs. 91 days), and fewer diabetes cases were observed (PG/WG versus WG/WG, 73 vs. 35%; $P = 0.008$, Kaplan-Meier log rank test) after neonatal exposure to the diabetes-promoting wheat antigens. Of 15 rats, 1 developed diabetes in the PG/HC group.

Footpad swelling and popliteal lymph node assays of DTH and cellular immunity. Because of the apparent priming effect of early contact with a cereal-based diet, we searched for signs of altered systemic cellular immunity to NIH-07 antigens. We examined the ability of NIH-07 antigens to induce a DTH reaction, which is a classic measure of systemic Th1 cell recruitment and activation. As shown in Fig. 2*A*, subcutaneous injection of NIH-07 induced substantial footpad swelling within 24 h. Animals with previous neonatal exposure to NIH-07 showed slightly suppressed DTH responses (Fig. 2*A*), but this was not statistically significant.

Next we analyzed for the popliteal lymph node response

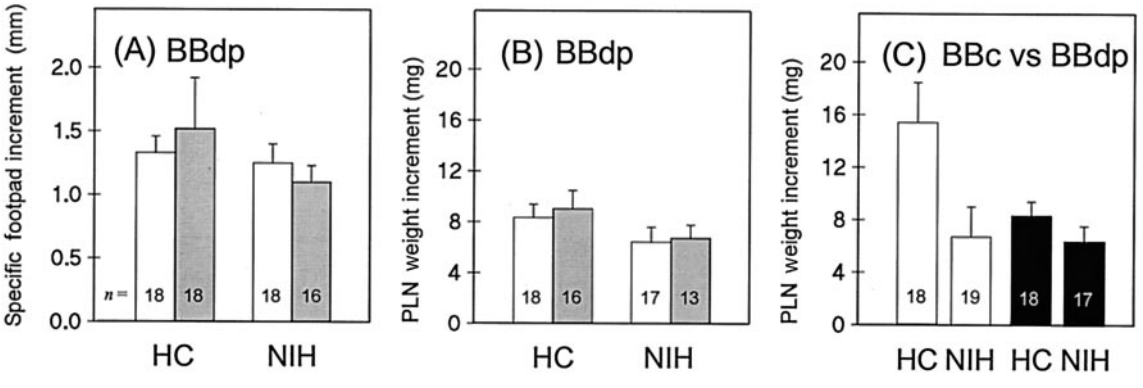

FIG. 2. Effect of oral antigen treatment in the neonatal period and subsequent weaning diet on the DTH reaction and popliteal lymph node weight in control and diabetes-prone BB rats. BBc and BBdp rat pups were hand-fed between 4 and 7 days of age either PG or NIH. Animals were weaned at 23 days onto a diabetes-retardant HC diet or the diabetes-promoting NIH diet. At 60 days of age, animals received an injection of autoclaved PG alone in the right footpad (control) or PG + NIH in the left footpad (treated). Footpad thickness was measured at 0 and 24 h; after 7 days, animals were killed and popliteal lymph nodes were dissected out, trimmed of fat, and weighed. Values are mean ± SD. The bars in *A* and *B* represent oral antigen fed in week 1 of life; □, PG; ▨, NIH. *A*: Specific footpad increment (left-right footpad swelling, mm). *B*: Specific PLN increment (left-right PLN weight, mg). *C*: A comparison of the effect of chronic exposure to different weaning diets on increase in PLN weight 1 week after injection of NIH-07 antigens in the left footpad of BBc (□) and BBdp rats (■).

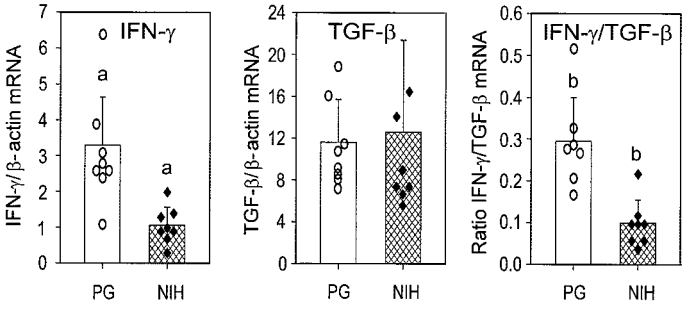

FIG. 3. Oral exposure to NIH antigens and gut cytokines in BBdp neonates. Pups were fed PG or NIH antigens on day 5 and killed 24 h later. Gut tissue was removed and frozen in liquid N_2. RNA was extracted from the tissue, and cytokines were measured using semiquantitative RT-PCR (n = 8/group). a, P = 0.00002; b, P = 0.0003 by analysis of variance. Values are means ± SD.

7 days after subcutaneous injection of NIH-07. This end point is a sensitive measure of T-cell reactivity and previous T-cell sensitization (22). There was a clear increase in PLN weight after NIH-07 injection, indicative of a T-cell response (Fig. 2*B*). However, animals that had neonatal contact with NIH-07 antigens did not exhibit an altered PLN response.

We also tested for a priming/tolerance-inducing effect of NIH-07 antigens when fed only after weaning. In diabetes-resistant BBc rats, the PLN response to NIH-07 antigens was much higher if the animals had never received the NIH-07 diet as compared with rats that were fed NIH-07 from weaning (Fig. 2*C*) ($P < 0.01$). By contrast, BBdp rats showed no evidence of developing tolerance to NIH-07 diet fed from weaning.

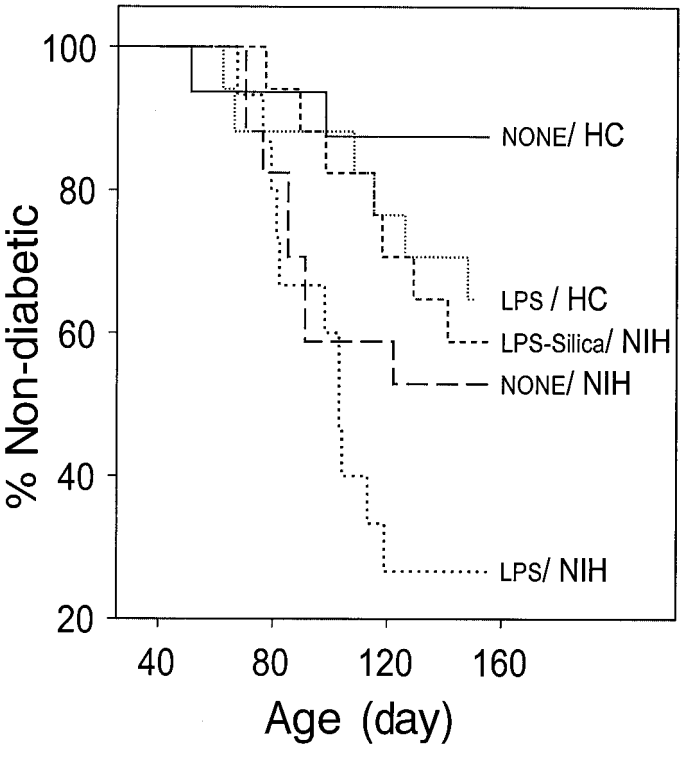

FIG. 4. Diabetes in BBdp rats fed in the neonatal period with LPS and silica. Neonates were fed PG + LPS or PG + LPS/silica between days 4 and 7 and weaned onto NIH-07 or HC at day 23. Diabetes incidence was monitored up to 155 days. Number of animals per group (oral administration diet): HC diet alone, 17; NIH-07 diet alone, 17; LPS/HC, 15; LPS/NIH, 15; LPS+silica/NIH, 17.

TABLE 2
Insulitis score in the pancreas of adult BBdp rats exposed in the neonatal period to LPS or LPS + silica

	Diet			
	HC	NIH	NIH	NIH
Neonatal oral administration	None	None	LPS	LPS + silica
Insulitis rating	1.8 ± 0.9*†	2.9 ± 1.2*	3.7 ± 0.7†‡	2.2 ± 1.1‡
n	16	16	15	17

Data are means ± SD values with the same symbols are significantly different. *P = 0.04, †P = 0.001, ‡P = 0.001 by analysis of variance with least significant difference.

IFN-γ and TGF-β in the gastrointestinal tract of neonates fed NIH-07. As there seemed to be little effect of the neonatal treatment on DTH, this suggested that the benefit observed was unlikely to be the result of the induction of systemic tolerance but might be related to the local immune response in the gastrointestinal tract. To test this proposition, we measured IFN-γ and TGF-β gene expression in the gut of neonates. BBdp rat pups aged 5 days were fed either PG or PG + NIH (n = 8/group) and killed at 24 h, and their tissues were harvested. Cytokine gene expression was measured using semiquantitative RT-PCR. The animals that received NIH-07 showed no change in TGF-β mRNA expression in the gut, but IFN-γ was downregulated compared with those fed PG alone, resulting in a decreased IFN-γ/TGF-β ratio, which indicated a change in local gut immune bias in response to NIH-07 diet in the first week of life (Fig. 3). Transcripts of interleukin (IL)-12p40 were also measured but were found to be below detection limits (data not shown).

Modification of diabetes outcome by LPS and silica. To examine further the concept of neonatal gut immunomodulation, we administered orally a nonspecific immunostimulatory compound, LPS, to neonates in the same manner as described above. LPS treatment increased diabetes incidence from ~50 to 73% (Fig. 4). Although this increase was not statistically significant, it may have been biologically relevant as we observed the same enhancement in diabetes incidence when LPS was administered to BBdp neonates that were weaned onto a WG-based diet (F.W.S., unpublished data). Furthermore, there was an increase in islet infiltration as determined by standard light microscopy. Rats that were fed an HC diet had a lower insulitis rating compared with those that were fed NIH-07 alone or orally exposed as neonates to LPS and weaned to NIH (Table 2). When silica particles were administered concurrently, the LPS-induced increase in diabetes cases was blocked (LPS versus LPS/silica group, 41 vs. 73%; P = 0.02, Kaplan Meier log rank). This was also accompanied by a significant decrease in insulitis score (2.2 ± 1.1 vs. 3.7 ± 0.7; P = 0.001) (Table 2). In addition, age of onset was delayed in the LPS-silica/NIH group compared with none/NIH (110 ± 23 vs. 85 ± 17 days; P = 0.02), suggesting a possible benefit.

To examine for a local effect of these treatments on gut immunity, we measured gut cytokines in pups that were untreated (fed with dam's milk alone), fed LPS or LPS + silica in PG at day 5, and killed 24 h later. IFN-γ mRNA

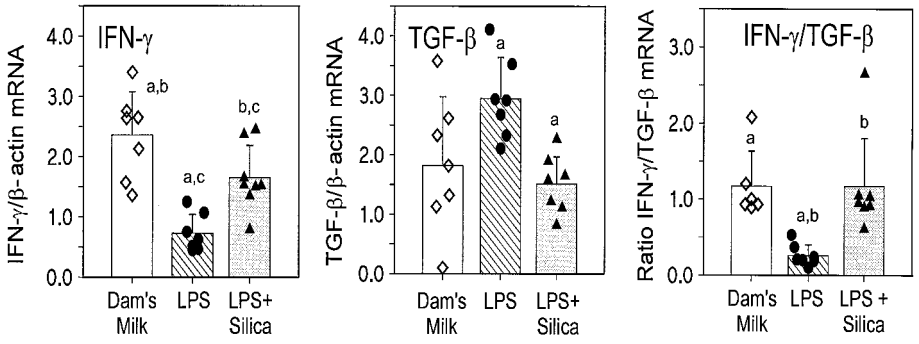

FIG. 5. Effect of immune modulators on gut cytokines in BBdp neonates. LPS or LPS/silica was fed (in PG) to BBdp neonates on day 5, and animals were killed 24 h later. Cytokines in gut tissue were measured using semiquantitative RT-PCR (n = 8/group). Values are means ± SD. Bars that share the same letter are significantly different at $P \leq 0.01$.

expression decreased in response to oral LPS ($P < 0.001$) and was significantly restored in the LPS/silica group (Fig. 5). TGF-β mRNA expression in the small intestine of neonates varied less in response to oral dosing of LPS or LPS + silica (Fig. 5). As a consequence, LPS induced a significant drop in the IFN-γ-to-TGF-β ratio of gene expression, which was restored in the LPS/silica group (Fig. 5). Again, mRNA for IL-12p40 was not detected.

DISCUSSION

The possibility that type 1 diabetes may be related to antigens encountered via the gut lumen has been discussed since the mid-1980s, when evidence first appeared that diet could affect the spontaneous development of diabetes in BB rats and studies in patients implicated early exposure to breast milk substitutes as a risk factor. More recently, there have been several indications that the gut may be abnormal in some people who develop diabetes.

Savilahti et al. (23) found that jejunal biopsies from patients showed increased major histocompatibility complex class II expression (HLA-DR, DQ, and DP), a sign of enhanced immune activation, that had expanded in most of the villi and crypts in addition to the normal expression seen only on the upper villi. Another study compared peripheral blood mononuclear cells of young patients with type 1 diabetes and healthy children and found that IFN-γ secretion was higher and TGF-β was lower in $\alpha_4\beta_7$ high T-cells compared with control subjects (24). The two major inducers of MHC class II expression in the inflamed gut are IFN-γ and tumor necrosis factor-α. Enhanced Th1 cytokine responses in the gut can cause gut inflammation and damage, and they are associated with increased gut permeability in celiac patients, Crohn's disease, and other chronic gut inflammatory conditions (25). Increased gut permeability was recently reported for patients with type 1 diabetes (26) and BBdp rats (12). Thus, there is suggestive evidence that the gut of patients with type 1 diabetes shows damage that may be the result of inflammatory changes. However, it is not clear yet whether an inflammatory condition in the gut is linked to the process that destroys islet β-cells.

The data presented here for BBdp rats confirm (2) that the diet received from weaning is a major modulator of the natural course of disease development. When fed a mostly cereal-based diet or a WG-based diet, between ~60 and 74% of animals developed overt diabetes as compared with <20% if rats received a diet with HC as the sole source of amino acids. The surprising finding was that exposure to WG or cereal-based diets in the first week of life, a time before gut closure when the gastrointestinal mucosa is immature, resulted in delay and even avoidance of diabetes in approximately one-third of diabetes-prone BB rats. No such protective effect was seen when an HC-based diet was administered during the same time period. Additional evidence for a critical role of gut function during the first week of life came from oral feeding with LPS. This intervention produced more diabetes cases and increased islet infiltration, whereas the concomitant administration of silica particles resulted in a significantly lower diabetes frequency than seen after LPS alone. Silica particles are toxic to macrophages or other phagocytes and hence target LPS-responsive cells. Because of the large particle size, silica is not expected to pass the intestine and reach the systemic circulation. Hence, the gut tissue itself is probably responsible for the enhancement of disease seen after exposure to LPS or suppression seen after early exposure to a cereal diet mixture or WG proteins.

This view is supported by the lack of a systemic memory response in rats exposed to the NIH-07 diet in the neonatal period. There was neither enhancement or suppression of the 24-h DTH response to cereal antigens nor a modulation of the 7-day popliteal lymph node response. The latter measurement is a highly sensitive and reliable surrogate of T-cell sensitization (22).

Evidence for a direct effect on gut immune function by exposure to non–breast milk antigens in the first week of life came from studies of cytokine gene expression. We found a significant decrease of IFN-γ but not TGF-β gene expression, which is indicative of an altered immunoreactive state after dosing with NIH-07 antigens or LPS in comparison to HC. It is interesting that silica coadministration restored the IFN-γ/TGF-β ratio to normal by decreasing IFN-γ mRNA levels, while TGF-β levels were increased.

Because IFN-γ is a key proinflammatory and TGF-β the dominant anti-inflammatory cytokine in the gut, LPS does not seem to modulate gut immune reactivity in a remarkably different way than the NIH-07 mixture. Hence, the major difference between the two treatments was that in the case of LPS, cereal antigens were not introduced at the same time but only 2 weeks later, at weaning, indicating that the timing of exposure to NIH-07 antigens is important.

These findings suggest that the gut of BBdp rats responds unfavorably to cereal antigens seen after weaning onto solid food. When the same dietary antigens are introduced to the neonate, with concomitant exposure to dam's milk, a potent immunomodulatory mixture of growth factors, cytokines, and various other peptides, the detrimental impact of weaning onto solid cereal-based

food is dampened. A particular role of the neonatal gut immune system in controlling diabetes development was recently also suggested for diabetes-prone nonobese diabetic mice (17). It was reported that neonatal administration of human insulin or a B-chain fragment had a much more potent suppressive effect on later disease development than when fed after weaning.

An additional outcome of our study was that diabetes-resistant BBc rats seemed to differ in their response to cereal-based diets. The PLN response of BBc rats to cereal antigens was significantly suppressed after continuous exposure to NIH-07 from weaning, whereas no suppression was seen in BBdp rats. The latter response was lower, which is in accord with the functional T-cell defect and lymphopenia of BBdp rats (27). Taken together, our findings lend additional support to the concept that the gut immune response is abnormal in BBdp rats and linked to the disease process.

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