Fetal or Neonatal Low-Glycotoxin Environment Prevents Autoimmune Diabetes in NOD Mice

Melpomeni Peppa,1 Cijiang He,1 Masakazu Hattori,2 Robert McEvoy,1 Feng Zheng,1 and Helen Vlassara1

Advanced glycation end products (AGEs) are implicated in β-cell oxidant stress. Diet-derived AGE (dAGE) are shown to contribute to end-organ toxicity attributed to diabetes. To assess the role of dAGE on type 1 diabetes, NOD mice were exposed to a high-AGE diet (H-AGE) and to a nutritionally similar diet with approximate fivefold-lower levels of N-carboxymethyllysine (CML) and methylglyoxal-derivatives (MG) (L-AGE). Suppression of serum CML and MG in L-AGE–fed mice was marked by suppression of diabetes (H-AGE mice >94% vs. L-AGE mice 33% in founder [F]0, 14% in F1, and 13% in F2 offspring, P < 0.006) and by a delay in disease onset (4-month lag). Survival for L-AGE mice was 76 vs. 0% after 44 weeks of H-AGE mice. Reduced insulitis in L-AGE versus H-AGE mice (P < 0.01) was marked by GAD- and insulin-unresponsive pancreatic intercellular (IL)-4-positive CD4+ cells compared with the GAD- and insulin-responsive interferon-γ-positive T-cells from H-AGE mice (P < 0.005). Splenocytes from L-AGE mice consisted of GAD- and insulin-responsive IL-10–positive CD4+ cells compared with the IFN-γ–positive T-cells from H-AGE mice (P < 0.005). Therefore, high AGE intake may provide excess antigenic stimulus for T-cell–mediated diabetes or direct β-cell injury in NOD mice; both processes are ameliorated by maternal or neonatal exposure to L-AGE nutrition. Diabetes 52:1441–1448, 2003

Insulin-dependent diabetes (type 1 diabetes) is an autoimmune disease resulting from T-cell–mediated destruction of pancreatic islet β-cells (1). However, the initial events of this process are incompletely understood. While both MHC and non-MHC genes have been implicated in the islet autoimmunity of both mice and humans (2,3), T-cell autoantigens, such as GAD or insulin, as well as environmental factors remain as strong candidates (2,4–7). The interplay between heritable aberrations, and environmental influences can lead to disruption of immune tolerance, T-cell activation, and β-cell injury (2,3,6,7). Diet is a strong environmental determinant of type 1 diabetes (8–14), but the underlying mechanisms involved are not fully elucidated (10).

The reactive, dicarbonyl derivatives of glucose-protein or glucose-lipid interactions, termed advanced glycation end products (AGEs), such as N-carboxymethyllysine (CML) and methylglyoxal (MG), are implicated in a wide range of diseases related to diabetes and aging (15,16), including β-cell injury and apoptosis, via reactive oxygen species (ROS)-dependent processes (17,18). AGE receptors found on immune cells mediate cell activation and cytokine expression, and in T-cells, IFN-γ secretion (19–21).

Diet constitutes an important exogenous source of highly reactive AGE (22–24). A direct correlation has been shown between ingested AGE and that found in circulation (25–27). Excess oral AGE intake results in an imbalance of AGE homeostasis, leading to significant diabetes-like pathology (25–33). On the contrary, β-cell dysfunction and diabetic complications are suppressed by AGE inhibitors (34,36) or by restriction of dietary AGE (25–33). In addition, a significant reduction of circulating AGE levels was observed in renal failure patients under peritoneal dialysis treatment after 4 weeks on a low-AGE content diet (35). Recently, food-derived AGE, rich in MG and CML derivatives, among others, were found to be potent inducers of oxidative stress and inflammatory cell activation in a manner reversible by antioxidants and anti-AGE agents, such as aminoguanidine (36). Furthermore, in type 2 diabetic db/db mice, dietary AGE restriction suppressed β-cell damage, thereby maintaining normal glucose homeostasis (31).

Based on the above, we investigated whether early exposure to AGE taken in with regular nutrients has a role in the pathogenesis of type 1 diabetes in NOD mice, a model of human diabetes (3,5).

RESEARCH DESIGN AND METHODS

Mouse dietary formulas. CML-BSA and methylglyoxal-BSA derivatives (MG) in rodent food were assessed by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies anti-CML-KLH (AC0) (Alton, Northvale, NJ) (ELISA-1) or anti-MG-ovalbumin (MG3D11) (ELISA-2), as described (36,37).

We used semipurified standard diet, AIN-93G (Bio-Serv, Frenchtown, NJ), which is normally exposed to heat (at 100°C × 20–60 s and at 125°C × 20–30 min), containing 355 CML-like AGE units/mg and 18 nmol/mg MG-derivatives/mg, herein termed “high-AGE diet” (H-AGE). The identical chow mix, prepared without the second step of heating, contained fivefold less CML-like AGE and 4.5-fold less MG-derived epitopes, as compared with H-AGE chow, and is thus termed “low-AGE” (L-AGE) formula (Table 1).

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CML, N-carboxymethyllysine; dAGE, diet-derived AGE; ELISA, enzyme-linked immunosorbent assay; GALT, gut-associated lymphoid tissue; bβE, hematoxylin and eosin; HAGE, high-AGE diet; IFN, interferon; IGTT, intraperitoneal glucose tolerance test; IL, interleukin; LAGE, low-AGE diet; MG, methylglyoxal; PI, proinsulin; pLy, pancreatic lymphocytes; ROS, reactive oxygen species; sAGE, serum AGE; SI, stimulation index; sLy, splenic lymphocytes; UA, urinary albumin. © 2003 by the American Diabetes Association.
TABLE 1

Characteristics of diet formulas

<table>
<thead>
<tr>
<th>Nutrients (%)</th>
<th>AIN recommendations†</th>
<th>H-AGE</th>
<th>L-AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>7.2</td>
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<tr>
<td>Carbohydrate</td>
<td>65</td>
<td>58.6</td>
<td></td>
</tr>
<tr>
<td>AGE content</td>
<td>4</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>CML (units/mg)†‡</td>
<td>535</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>MG (nmol/mg)¶</td>
<td>18</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*See ref. 38. †Ingredients: casein, cornstarch, dextrose, sucrose, cellulose, soybean oil, t-butyhydroquinone, salt mix, vitamin mix, l-cystine, choline bitartrate; CML determined by 4G9 mab (37). ¶MG determined by MG-3D11 mab (36).

Macronutrients (protein 18.4%, carbohydrate 58.6%, fat 7.2%) were identical in these diets, and micronutrients were mostly in excess of the daily requirements, following standard processing (38). Both formulas were prepared by the manufacturer and kept at 4°C.

Mice. Because female NOD mice have a much higher rate of diabetes than the male NOD mice, only female mice were studied. Prediabetic NOD founder (F0) mice (20 females and 12 brother-sister mating pairs aged 6 weeks), obtained from Dr. M. Hattori (Joslin Diabetes Center, Boston, MA), were housed in a pathogen-free environment. Upon arrival, F0 mice as well as the first (F1, n = 55) and second (F2, n = 51) generation offspring were housed at the Center for Laboratory Animal Science, Mount Sinai School of Medicine. Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee.

Baseline body weights and blood and urine samples were obtained, and mice were randomly assigned to either H-AGE or L-AGE diet and followed for up to 56 weeks of age. Of the F0 mice, 22 females and 8 mating pairs were placed on L-AGE diet, and 12 females and 4 mating pairs were placed on the H-AGE diet, immediately upon arrival (6 weeks of age). After weaning (3 weeks), the F1 (n = 55) and F2 (n = 51) generations’ offspring were assigned to diets: 21 of F1 and 30 of F2 female NOD mice continued on the L-AGE diet, while 18 of F1 and 21 of F2 female NOD mice continued on the H-AGE diet. An additional 16 females of F2 offspring from L-AGE-fed mothers were switched to the H-AGE diet, after weaning.

Food and water intake were measured daily for 1 week and weekly thereafter. Body weight and urinary glucose levels (Dextrostix; Bayer, India-nopol, IN) were checked once per week until onset of glucosuria and by tail vein blood thereafter (Glucometer Elite; Bayer, Mishawaka, IN). Overt diabetest was evidenced by persistent hyperglycemia (>250 mg/dl) for over a week. Diabetic mice were treated with insulin (1–3 units of combined regular-NPH insulin, 1:1) twice daily to maintain blood glucose levels at 150–200 mg/dl for up to 56 weeks.

Twelve-hour fasting blood samples (by retroorbital puncture) and 24-h urine samples were collected at 1- to 2-month intervals and stored at −80°C. Subgroups from F1 on either diet were sacrificed at 9, 12, and 16 weeks for collection of blood and tissue specimens.

Metabolic studies. At 16 weeks of age, an intraperitoneal glucose tolerance test (IGTT, 5% dextrose solution; 2 mg/g body wt) was performed in groups of 12 mice from four different litters after an overnight fast (13–15 h). Blood glucose (Elite Glucometer; Bayer) and insulin levels were measured before and at half-hour intervals up to 120 min. Insulin levels were measured by ELISA (Ultra-Sensitive Mouse Insulin Kit; Alpco Diagnostics, Windham, NH). Serum and urine AGE levels were determined by ELISA-1, as described (36,37). Urinary albumin (UA) levels were measured with an anti-mouse albumin–based ELISA kit (Bethyl Laboratories, Montgomery, TX); urinary creatinine was assessed by a standard colorimetric method (Stambo Laboratory, San Antonio, TX). Renal function was expressed as the albumin-to-creatinine ratio (28).

Immunohistology and flow cytometry. After asphyxiation by exposure to CO2, pancreatic tissues were fixed in 10% formaldehyde, and 4-μm paraffin sections were stained with hematoxylin and eosin (H&E). A staff pathologist analyzed 15–20 islets/pancreas in a blinded fashion, using a grading system, in which 0 = no evidence of infiltration, I = peri-islet infiltration, II = 25%, III = >25–45%, IV = >50% infiltration of each islet, and V = complete loss or only remnants of islets seen (39).

For flow cytometry, pancreatic and spleen tissues from 16-week-old mice (n = 8/group) were processed for lymphocyte isolation (40). Suspensions of pancreatic lymphocytes (pLy) were obtained after gentle mincing with fine scissors and digesting with collagenase type V (4 mg/ml) and DNase type II (200 units/ml) (Sigma Chemicals, St. Louis, MO) in RPMI-1640 for 15 min at 37°C. After centrifugation, single-cell suspensions were obtained by continuous pipetting in a solution containing trypsin/EDTA (1 ml/ml (Gibco/BRL) and DNase type II (2,000 units/ml) at 37°C for 10 min. Single-cell suspensions of splenic lymphocytes (sLy) were obtained by gently pressuring the spleens through wire mesh screens and rinsing with RPMI-1640 supplemented with 10% FBS and 2% penicillin/streptomycin (Gibco/BRL, Gaithersburg, MD). Red cells were lysed by 0.84% ammonium chloride.

For labeling pancreatic lymphocytes, anti-CD45RTC, anti-IL-4 PE (Phar- mingen, San Diego, CA), and anti-IFN-γ antibodies (Pharminogen) were used. After a 6-h incubation at 37°C in the presence of monoclonal (0.1 μg/ml Sigma) to block cytokine secretion, cells were stained with the relevant anti-CD4 antibody at 4°C for 30 min, permeabilized with saponin 0.1%, and stained with each antibody or isotype control. Cells (5 × 10⁵/sample) gated on a window encompassing the sLy and pLy population were analyzed by flow cytometry (EPICS Profile II Analyzer; Coulter, Hialeah, FL) (40).

Proliferation and lymphokine expression assays. pLy (5 × 10⁵/well) from 16-week-old F1 mice (n = 6/group) were incubated for 72 h at 37°C with antigen-presenting cells (APC’s) (6 × 10⁵/well) (sLy) from the H-AGE fed mice, treated with mitomycin (1 μg/ml Sigma) and incubated at 37°C for 20 min) in the presence or absence of ConA, the immunodominant peptide of GAD65 (217-236) and mouse proinsulin (PI 9-23) (10 μg/ml), both of which were obtained from the Peptide Synthesis Core (Department of Molecular Biology, Mount Sinai School of Medicine). Proliferative responses for pLy were confirmed and expressed as stimulation index (SI) (calculated as cpm in the presence of stimulant/cpm without stimulant).

In addition, sLy (1 × 10⁵/well) was incubated for 72 h at 37°C in the presence or absence of ConA (10 μg/ml), GAD65 (10 μg/ml), or PI (10 μg/ml). Proliferative response was determined 72 h after incubation with IL-2/Thr. sLy from 16-week-old mice (n = 12/group) were also incubated with various doses of anti-CD3 (Pharmingen), and proliferative response was measured 48 h after incubation with IL-2/Thr. ConA was used in all T-cell experiments as a non-specific stimulant to estimate proliferative response. To evaluate sLy gene expression for IFN-γ, IL-4, and IL-10, total RNA isolated from the spleens of 12-week-old female NOD mice (n = 5/group) was reverse transcribed to cDNA, and RT-PCR was performed as described. β-Arctin was amplified at the same time for each sample, and the data were expressed as the ratio of cytokine to β-arctin mRNA (41).

Statistical analysis. All values are expressed as means ± SE. Two-tailed unpaired t test (Mann-Whitney) or Student’s t test was used as needed to evaluate differences between means of grouped data from mice fed with H-AGE and L-AGE diets. Differences were considered significant if P < 0.05.

RESULTS

Dietary AGE content and serum AGE levels. Food and water intake were similar among age- and diabetes-matched groups during the study period. There were no significant differences in body weights between H-AGE and L-AGE diet groups. The only weight loss was that preceding death in type 1 diabetic mice (Table 2).

Based on equivalent food intake, H-AGE fed mice ingested approximately fivefold more AGE than the L-AGE–fed mice. This was clearly reflected in the fasting serum AGE (sAGE) of both diabetic and nondiabetic groups throughout the study period (Fig. 1A and B). Baseline sAGE levels in NOD mice decline in a time-dependent manner in both diabetic and nondiabetic groups fed the L-AGE diet (P < 0.025) (Fig. 1A and B). Greater AGE ingestion by the H-AGE-fed mice was associated with greater urinary AGE excretion compared with L-AGE–fed mice in both the diabetic and nondiabetic groups (P < 0.002) (Table 2).

Dietary AGE and diabetes incidence and survival. A striking reduction in the cumulative incidence of type 1 diabetes soon became evident in the AGE-restricted
groups, unlike those placed on regular (H-AGE) diet. Of 16 F₀ H-AGE-fed mice, 15 (94%) developed diabetes by 25 weeks of age, whereas only 6 of 30 L-AGE-fed mice (33%) became diabetic (P = 0.006) (Fig. 2A) or with a delay of 15 weeks (40 weeks of age) (Fig. 2D). From the F₁ group (n = 55), 11 of 18 H-AGE-fed mice (61%) were diabetic by 25 weeks, as compared with only 3 of 21 (14%) of L-AGE mice developing diabetes by ~40 weeks (P < 0.006) (Fig. 2B) or with a 15-week lag (Fig. 2E). Similar results were obtained with the F₂ offspring: 13 of 21 (62%) H-AGE-fed mice were diabetic by 25 weeks, as compared with 4 of 30 (13.3%) L-AGE-fed mice (L-AGE vs. H-AGE, P = 0.002; *P < 0.025).

Dietary AGE and β-cell function. At ~16 weeks of age, prediabetic L-AGE F₁ mice exhibited significantly lower fasting blood glucose and higher plasma insulin levels compared with age-matched H-AGE F₁ mice (P < 0.021 and P < 0.009, respectively) (Fig. 3A and B). Also, a significantly lower glycemic response to IGTT (Fig. 3A), with greater insulin responses to glucose, were observed in the L-AGE mice compared with H-AGE mice (Fig. 3B).

Histological examination of pancreas. Pancreata of 9-week-old prediabetic mice (n = 8/group, F₁) and 40-week-old diabetic (n = 10, F₀) and nondiabetic (n = 15, F₀) mice from either diet were evaluated. At 9 weeks, islets H-AGE-fed from mice revealed severe inflammatory infiltration, essentially obliterating the normal islet architecture (>50% of the islet surface affected, grades III–V) (39). In contrast, islets from the L-AGE–fed group showed only occasional islets with lymphocytic infiltration (<24% of each islet, grade <II) (Fig. 4A and B). Quantitative analysis revealed an insulin score of 4.1 ± 1.3 for the H-AGE–fed group versus 1.2 ± 0.5 for the L-AGE–fed group (P < 0.01).

At 48 weeks, compared with the islets from diabetic H-AGE mice (100% grade >III or >50% of each islet), only 20% of islets from the diabetic L-AGE mice were affected as seriously, the remaining 80% exhibiting mild insulitis, grades I–II. Of the nondiabetic H-AGE–fed mice, 50% exhibited severe insulitis (>grade III), as compared with only 10% of L-AGE with same degree of damage and the rest showing only mild or no insulitis (grades I and II).

Pancreatic lymphocyte populations. The total number of pLy was markedly suppressed in 16-week-old L-AGE–fed mice, as compared with the H-AGE–fed mice (L-AGE 0.10 ± 0.15 × 10⁷ vs. H-AGE 7.7 ± 5.6 × 10⁷, P < 0.001, respectively) (Fig. 5A). Also, CD4⁺ pLy were approximately threefold lower in the L-AGE compared with H-AGE-fed mice (L-AGE 8.3 ± 4.4% vs. H-AGE 23.4 ± 6.1%, P = 0.000) (Fig. 5B). A fivefold greater percentage of CD4⁺ pLy was positive for IL-4 in the L-AGE group, whereas the reverse was observed in the H-AGE–fed group (L-AGE 25.9 ± 3.22% vs. H-AGE 5.52 ± 1.15%, P < 0.009) (Fig. 5C). In contrast, a threefold greater percentage of CD4⁺ from the H-AGE group were IFN-γ positive, as...
The splenic T-cell CD4+ to CD8+ ratio (3.5:1) was not different between the diet groups. Thus, splenic T-cells from 12-week-old mice were tested for lymphokine mRNA expression: L-AGE mice exhibited low levels of IFN-γ and low IL-4 but a high IL-10–expression: L-AGE mice exhibited low levels of IFN-γ (0.04 ± 0.008, IL-4 0.07 ± 0.002, IL-10 2.98 ± 0.6), as compared with sLy from H-AGE–fed mice (IFN-γ 1.75 ± 0.5, IL-4 0.22 ± 0.06, IL-10 0.38 ± 0.04, P < 0.001, respectively) (Fig. 6D).

**Proliferation of pLy and sLy.** The pLy from H-AGE-fed mice showed a significantly greater proliferative response to GAD (10 μg/ml) and to PI (10 μg/ml) compared with that of pLy from L-AGE mice (P < 0.011 and P < 0.005, respectively) (Fig. 5D).

By comparison, L-AGE mouse–derived sLy proliferated more rigorously than sLy from H-AGE mice in response to GAD (10 μg/ml) and to PI (10 μg/ml) (P < 0.02, P < 0.017, respectively) (Fig. 6B), as they did in response to anti-CD3 (P < 0.005) (Fig. 6C).

**DISCUSSION**

The current study demonstrates that restricted exposure to diet-derived glycotoxins leads to marked and sustained blockade of type 1 diabetes in the genetically susceptible NOD mouse. The earlier in life the restriction was applied, the greater the decrease in incidence of disease. These effects were marked by suppression of islet infiltration by β-cell cytotoxic T-cells and islet toxicity. In addition, significantly delayed onset, reduced severity of disease, and marked increase in overall survival occurred.

While these effects were readily attributable to the food, they were not due to differences in nutrient composition, as the diets used were of a single origin, with equivalent energy profiles (38). A prominent feature, however, was their distinctly different glycotxin content. A large proportion of CML or MG derivatives found in the regular or H-AGE diet did not develop in the L-AGE preparation, which resulted from the shorter heat exposure during processing. Indeed, methods of food processing (heating, sterilizing, and ionizing) impact on diverse unstable tissue-toxic species (25–27). AGE are found capable of β-cell damage (17,18,31,34), as well as of altered immune cell activation (19–21). Thus, the

![FIG. 2. Incidence of type 1 diabetes in F0, F1, and F2 generations of female NOD mice exposed to L-AGE or H-AGE diet. A: By 6 weeks of age, F0 mice were placed on H-AGE (n = 16) or L-AGE diet (n = 30). B: After weaning (3 weeks), F0 offspring were placed on H-AGE (n = 18) or L-AGE diet (n = 21). Also, a subgroup of F1 mice (n = 16) from L-AGE mothers was switched to H-AGE diet (L- to H-AGE). C: After weaning (3 weeks), F2 offspring were placed on an H-AGE (n = 21) or L-AGE diet (n = 30). A–C: Cumulative incidence of type 1 diabetes. D–F: Incidence of type 1 diabetes in F0, F1, and F2 mice on H-AGE diet (○) on H-AGE diet from L-AGE mothers (L- to H-AGE) (●) and L-AGE diet (●). Glucose incidence was monitored for 56 weeks. *P = 0.000 (A), *P = 0.006 (B), *P = 0.000 (C) for H-AGE or L-AGE (L- to H-AGE diet) vs. L-AGE groups.

![FIG. 3. β-Cell function of NOD mice exposed to L-AGE or H-AGE diet. After glucose challenge (IGTT, 5% dextrose solution, 2 mg/g body wt ip) in 16-week-old nondiabetic female F1 mice from H-AGE–fed (●) and L-AGE–fed groups (●) (n = 12/group). Glucose (A) and insulin (B) responses were estimated at half-hour intervals up to 120 min. Data are the mean ± SE of 12 measurements per time point per group. A: *Glucose in H-AGE vs. L-AGE at 0 min, P = 0.021; at 30 min, P = 0.036; at 60 min, P = 0.009; at 90 min, P = 0.009; and at 120 min, P = 0.009. B: *Insulin in H-AGE vs. L-AGE at 0 min, P = 0.009; at 30 min, P = 0.009; and at 60 min, P = 0.009.
possibility that food-derived AGE contribute to autoimmune diabetes was raised, herein viewed from the perspective of the diabetes-protective effect of a diet low in AGE applied to diabetes-prone NOD mice.

Diabetes suppressive effects under L-AGE feeding were significant in founder mice, initiated at 3 or 6 weeks of age, and were extended throughout two generations of offspring, F1 and F2, if kept on the L-AGE maternal regimen, yielding a diabetes-free rate of ~86% and greatly increased survival rates (by ~76%) for up to 56 weeks of age. As with other diabetogenic factors (8–14), type 1 diabetes reduction (~33 to 14%) coincided with initiation to the L-AGE diet during the perinatal period. Indeed, autoimmune cell infiltration of islets occurs soon after the 3-week weaning in NOD mice (42,43). The greatest disease prevention (~14%) was associated with exposure to a low in AGE maternal environment, suggesting that toxic AGE are transportable via the placenta. However, the protective maternal effect of the L-AGE diet was reversed after crossing over to the H-AGE diet, as readily as the normally H-AGE maternal effect was reversed by the L-AGE diet, when applied at weaning (3 or 6 weeks). These data confirmed the plasticity inherent in this period and reinforced the importance of “dose,” “time,” and “duration” of exposure to toxic factor(s) (12).

Thus, the majority of mice exposed to L-AGE environment maternally or at weaning displayed modest insulitis and no diabetes for >1 year. A milder insulitis and a milder diabetes characterized L-AGE mice that did become diabetic, as compared with those with severely damaged islets and overt diabetes seen in H-AGE–fed mice by ~25 weeks. Further evidence was obtained by assessing β-cell function: 16-week-old prediabetic L-AGE NOD mice exhibited near-normal glucose and insulin responses to glucose challenge compared with the typically dysfunctional pattern of age-matched H-AGE–fed mice.

The diabetogenic AGE components in the H-AGE preparation used herein may include CML or MG-derived compounds, although this remains to be confirmed. Also, coexisting non-AGE substances in that formula cannot be ruled out (8–14). However, a plausible link may be invoked between ordinary foods, such as casein in milk, which has been implicated in type 1 diabetes (44), and common glycotoxins; heating milk during pasteurization increases its AGE content (24) and its putative β-cell toxicity. Of note, unlike most other “diabetogenic” factors,

FIG. 4. Islet morphology in non-diabetic F1 NOD mice exposed to L-AGE or H-AGE diet. A: Pancreatic tissues retrieved from 9-week-old F1 H-AGE–fed mice, stained by H&E, showed severe mononuclear cell infiltration and disruption of pancreatic islet architecture. B: Islets of age-matched F1 NOD mice from the L-AGE–fed group did not exhibit these changes. Magnification ×400.

FIG. 5. Pancreatic T-cell responses in nondiabetic NOD mice exposed to L-AGE or H-AGE diet. A: Total infiltrating pancreatic T-cells isolated from pancreata of 16-week-old female NOD mice were measured in H-AGE– or L-AGE–fed mice (n = 8/group), *P < 0.001. B: The percentage of CD4+ pancreatic T-cells was assessed by anti-CD4+ fluorocyanin isothiocyanate (FITC) using fluorescence-activated cell sorter (FACS) analysis, *P < 0.000. C: The cytokine profile of CD4+ T-cells was evaluated by labeling with anti-IFN-γ or anti-IL-4 antibodies (FACS analysis), *P < 0.009, respectively. D: Proliferation of pancreatic T-cells (5 × 105/well), incubated for 72 h at 37°C with GAD and insulin (10 μg/ml), was estimated by 3H-TdR (1 μCi/well), *P < 0.01 for GAD and <0.005 for insulin. Data are the means ± SE of eight measurements/group in H-AGE (□) and L-AGE (■) mice.
AGES are known to contribute to other diabetic organ toxicity (15, 25–33). Of interest, CML and MG-related derivatives were negligible in L-AGE diet, relative to the H-AGE diet; otherwise, these two preparations were identical (37). While no class of heat-enhanced compounds has been implicated in the etiology of type 1 diabetes thus far, AGES are known to manifest an intriguing array of cellular effects, namely oxidant stress, nuclear factor (NF)-κB activation, or apoptosis and death (15, 17, 34, 36), and may constitute such a class.

With regard to the targeted tissue, diabetogenic factors are thought to act through the immune system, principally via β-cell cytotoxic T-cells (1, 2, 6, 7, 10). The hypothesis introduced herein is no exception. A marked suppression of total pLy was observed in the L-AGE–fed mice (~15-fold) compared with the H-AGE NOD group fed a regular diet. In type 1 diabetes in humans and NOD mice maintained on common diets, such as the H-AGE food, cytotoxic CD8+ and CD4+ cells, which are generated at the gut-associated lymphoid tissue (GALT) early in life, dominate islet infiltrates and disease induction (45, 46). However, in the present studies, islets from the L-AGE–fed mice displayed a pattern of predominantly IL-4–positive CD4+ cells. There were virtually no infiltrates in islets from two generations of mice exposed to L-AGE nutrients during the fetal stage or early in life. In these mice, there was also a marked unresponsiveness of pancreatic T-cells to β-cell antigens, insulin, and GAD. This might indicate blocked autoreactive T-cell recruitment due to low local expression of these antigens under a “safer” AGE-poor diet (47). The vigorous proliferative response to the same antigens by sLy from the L-AGE–fed mice, however, suggested the opposite. Of interest, sLy in these mice consisted largely of CD4+ IL-10–positive and IFN-γ–negative T-cells, suggesting that active and able Th2 T-cells were released systemically under L-AGE feeding. In fact, the rigorous sLy response to GAD and insulin pointed to preexisting memory to these self-antigens (47). It can be thus speculated that the L-AGE diet provided the young GALT system with a low dose of certain AGE-related antigen(s), which enabled Th2 cells to actively suppress nondeleted autoreactive T-cells (45–47). The opposite might be expected in an environment of excess AGE antigen(s), as provided by the H-AGE diet, whereby cytotoxic T-cells prevail.

Because normally dying β-cells liberate cellular components that stimulate autoreactive T-cells (7), the notable absence of such clones from L-AGE–derived islets was surprising. An alternate source and mode of presentation of such “self-antigens” might be postulated. For instance, animal- or plant-derived GAD peptides (48, 49) could be modified by, and or absorbed together with, AGE epitopes derived from heat-processed food. The specific steps of such a mechanism are uncertain at this time. However, a rarely discussed but relevant property of glycotoxins is their receptor-dependent action on primed T-cells to produce IFN-γ (21). It is plausible that early exposure of GALT to excess diet-derived AGE-peptides (e.g., AGE-GAD peptides) results in a population of nondeleted autoreactive T-cells, which in a predisposed setting, as in the NOD mouse, offsets the balance in favor of tissue-specific GAD-cytotoxic T-cell clones and β-cell damage (2, 6, 7, 10, 45, 46).

The above data could also be consistent with toxicity rendered directly upon the β-cell (7, 17, 18, 31, 34), as well as with the view that initial antigen-target tissue interactions may require both sustained supply and high doses of AGE to the β-cell in order to cause diabetes (2, 6, 7, 10, 12), as immune infiltration and insulitis alone are not necessarily associated with diabetes.

In summary, low-AGE diets may be effective for maintaining the necessary balance against autoreactive T-cell responses, as well as for preventing direct β-cell injury. The mechanistic hypothesis proposed herein is consistent with the recent significant increase in type 1 diabetes (2, 6, 7, 50), in view of the worldwide assimilation of Western-type AGE-enriched dietary patterns (2, 6), including those involved in the fetal stages and in early life. If confirmed clinically, dietary AGE restriction may prove an effective, low-cost, noninvasive strategy for diabetes prevention.
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