Elimination of Dietary Gluten Does Not Reduce Titers of Type 1 Diabetes–Associated Autoantibodies in High-Risk Subjects

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OBJECTIVE — Removal of the dietary wheat protein gluten protects against autoimmune diabetes in animal models. Furthermore, elimination of dietary gluten reduces the frequency of type 1 diabetes in patients with celiac disease. Herein we test the hypothesis that gluten is the driving antigen for type 1 diabetes–associated islet autoimmunity.

RESEARCH DESIGN AND METHODS — Seven autoantibody-positive, first-degree relatives of patients with type 1 diabetes were placed on a gluten-free diet for 12 months followed by gluten reexposure for 12 months. Gliadin antibodies as well as the diabetes-related antibodies insulin autoantibody (IAA), GAD antibody (GADA), and tyrosin phosphatase IA2 antibody (IA-2A) were measured every 3 months; oral glucose tolerance tests were performed every 6 months. Changes in autoantibody titers were compared with those observed in a matched historical cohort.

RESULTS — A reduction in IgG gliadin antibody titers was observed during the gluten-free period, but titers of diabetes-associated autoantibodies changed independently of gluten exposure. Type 1 diabetes–associated islet autoantibody levels at the end of the gluten-free diet period were not significantly different from those before commencement of the diet ($P = 0.2$) or at the end of the gluten reexposure period ($P = 0.4$). Changes in individual subjects were identified, but no differences were noted between the gluten-free and the gluten re-exposure periods, and the changes were similar to those observed in the historical control cohort ($P = 1.0$). Major titer reductions (>50%) in the gluten-free period were observed in only one subject for all antibodies. Type 1 diabetes developed in this subject and in a second subject during the gluten reexposure period.

CONCLUSIONS — The findings do not support the hypothesis that gluten is a driving antigen in type 1 diabetes.

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Dietary gluten is the etiologic agent of celiac disease (CD) (1). CD-associated autoimmunity and disease activity are correlated with gluten exposure, and removal of gluten from the diet reduces titers of circulating CD-associated autoantibodies and restores intestinal mucosa histology and function, indicating that CD autoimmunity is driven by gluten (2). Dietary gluten has also been postulated as an etiologic agent in other autoimmune diseases, particularly type 1 diabetes (3–5). In animal models, autoimmune diabetes incidence is significantly reduced if nonobese diabetic mice are never exposed to gluten (4). In humans with CD, the frequency of type 1 diabetes, other autoimmune diseases, and associated autoantibodies is directly related to age at diagnosis of CD, suggesting that early elimination of gluten may prevent the manifestation of other autoimmune diseases (5). In accordance with this, it was recently reported that 11 patients with untreated CD and at least one diabetes-related autoantibody experienced complete loss of these autoantibodies within a 12-month period of gluten-free therapy (6).

Furthermore, there are anecdotal reports of the disappearance of other autoantibodies after elimination of dietary gluten in different autoimmune diseases (7,8). These observations suggest that gluten may be a driving antigen for autoimmunity also in type 1 diabetes. To test this, we placed nondiabetic children with type 1 diabetes–associated autoantibodies on a gluten-free diet for 12 months, followed by a normal diet for 12 months, and measured autoantibody levels. The findings of the study do not support the hypothesis that gluten drives autoimmunity in type 1 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

Subjects were recruited from the Munich Diabetes Family Study (9). They were considered eligible if they were a sibling or offspring of a patient with type 1 diabetes, were positive for at least two islet autoantibodies (insulin autoantibody [IAA], GAD autoantibody [GADA], and tyrosin phosphatase IA2 antibody [IA-2A]) in at least two consecutive serum samples, were aged <6 years, and had a normal result of an oral glucose tolerance test (OGTT). Nine relatives were eligible. Seven accepted and two declined participation in the study (Table 1). HLA-DR

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Abbreviations: CD, celiac disease; GADA, GAD antibody; IAA, insulin autoantibody; IA-2A, tyrosin phosphatase IA2 antibody; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of the seven first-degree relatives at risk at the beginning of the gluten-free diet*

Case	Proband	Age (years)	HLA-DRB1	HLA-DQB1	IAA (units)	GADA (units)	IA-2A (units)	OGTT 2 h pp (mg/dl)
1	Mother	1.2	01, 04	Not tested	9.7	15	1794	70
2	Mother	5.4	03, 13	02, 0603	12.3	259	1	125
3	Father	5.9	01, 0405	0501, 02	11.2	85	656	81
4	Mother	2.4	03, 0401	02, 0302	5.1	80	0	89
5	Both parents	4.3	0401, 0408	0304, 0302	24.8	105	163	74
6	Sister	4.0	03, 0402	02, 0302	14.8	1094	69	96
7	Father	2.0	01, 0401	0302, 0501	17.7	14	0	83

Data are *n*. Thresholds for IAA 1.5 units, GADA 8.5 units, IA-2A 2.5 units.

and -DQ alleles were determined using PCR-amplified DNA and nonradioactive sequence-specific oligonucleotide probes (10). OGTT was performed with 1.75 g glucose per kg body wt.

Dietary intervention

All seven subjects were placed on a gluten-free diet for 12 months and were subsequently reexposed to a normal gluten-containing diet for 12 months. Before the commencement of diet, parents of the subjects received a 2-day training course on dietary regulations of a gluten-free diet. Serum samples were collected immediately before commencement of the diet and at 3-month intervals throughout the 24-month dietary intervention period. OGTTs were performed at 6, 12, 18, and 24 months after commencement of intervention. The study was approved by the ethical committee (Bavarian Medical Board No. 98260).

Outcome measures

As a potential independent compliance marker, antibodies to gliadin were measured throughout the study. As outcome markers, autoantibodies to insulin, GAD, and IA-2 were measured in samples before and at commencement of the diet and at 3-month intervals throughout the intervention period. A successful outcome was defined as significant reduction in autoantibody titers at the end of the gluten-free diet period.

Antibody measurements

Gliadin IgG and IgA antibodies were measured using a commercial enyzme-linked immunosorbent assay (Euroimmun; Gross Grönau, Germany) in the laboratory of M. Stern (Tübingen, Germany), as previously described (11). IAA, GADA, and IA-2A were measured by radiobinding assay as previously described (9,12). To reduce interassay variation, all samples were measured in the same assay. Intra-assay variation was <10% for all assays.

Antibody positivity was defined by the 99th percentile of antibody levels in nondiabetic control children (9). Using these thresholds, these assays had sensitivities and specificities of 30 and 98% (IAA), 80 and 94% (GADA), and 58 and 100% (IA-2A), in the Diabetes Antibody Standardization Program 2000 workshop (13). Autoantibody IgG subclass and epitope-specific GADA and IA-2A were measured as previously described (14–16). IgG and IgA antibodies to tissue transglutaminase were measured by radiobinding assay as previously described (17).

Statistical analysis

Antibody levels at the end of the gluten-free period were compared with those recorded immediately before the dietary intervention using Wilcoxon's matched-pairs test. Furthermore, the frequencies of decreases, increases, and no change in individual autoantibody titers at the end of each dietary intervention (gluten-free diet and gluten reexposure period) were compared using χ^2 test for trend and Wilcoxon's matched-pairs test. Decreases in titer were defined as a reduction ≥50% (halving), and increases were defined as a rise >100% (doubling). IAA, GADA, and IA-2A were considered separately for each of the seven subjects, giving a maximum of 21 observations in each of the two diet periods.

The number of decreases at the end of the gluten-free diet period were also compared with changes found in a historical cohort of 26 siblings and offspring of patients with type 1 diabetes from the Munich Diabetes Family Study and the BABYDIAB Study (9,12), in which characteristics were similar to the intervention group (age <6 years, >1 islet autoantibody in consecutive samples, and normal result of OGTT). Development of diabetes was compared with that in the historical control group using Kaplan-Meier analysis. For all analyses, a two-tailed *P* value of 0.05 was considered significant.

RESULTS

Compliance with gluten-free diet

All parents reported complete compliance during the study, and this was supported by gliadin antibody results. IgG gliadin antibodies were detected before the commencement of the diet in three subjects (cases 1, 3, and 5; Fig. 1). In each of these subjects, gliadin antibody levels decreased significantly under the gluten-free diet and increased again immediately after reexposure to gluten, suggesting that they were a suitable compliance marker and that these subjects complied with the diet within the study. IgA gliadin antibodies and IgG and IgA autoantibodies to tissue transglutaminase were not detected in the seven subjects.

Change in autoantibody levels during the intervention period

Overall, type 1 diabetes-associated islet autoantibody levels at the end of the gluten-free diet period were not significantly different from those before commencement of the diet ($P = 0.2$; Wilcoxon's matched-pairs test) or to autoantibody levels at the end of the gluten reexposure period ($P = 0.4$). Changes in individual subjects were identified, but these were not different between the gluten-free and the gluten reexposure periods and were similar to those observed in the historical

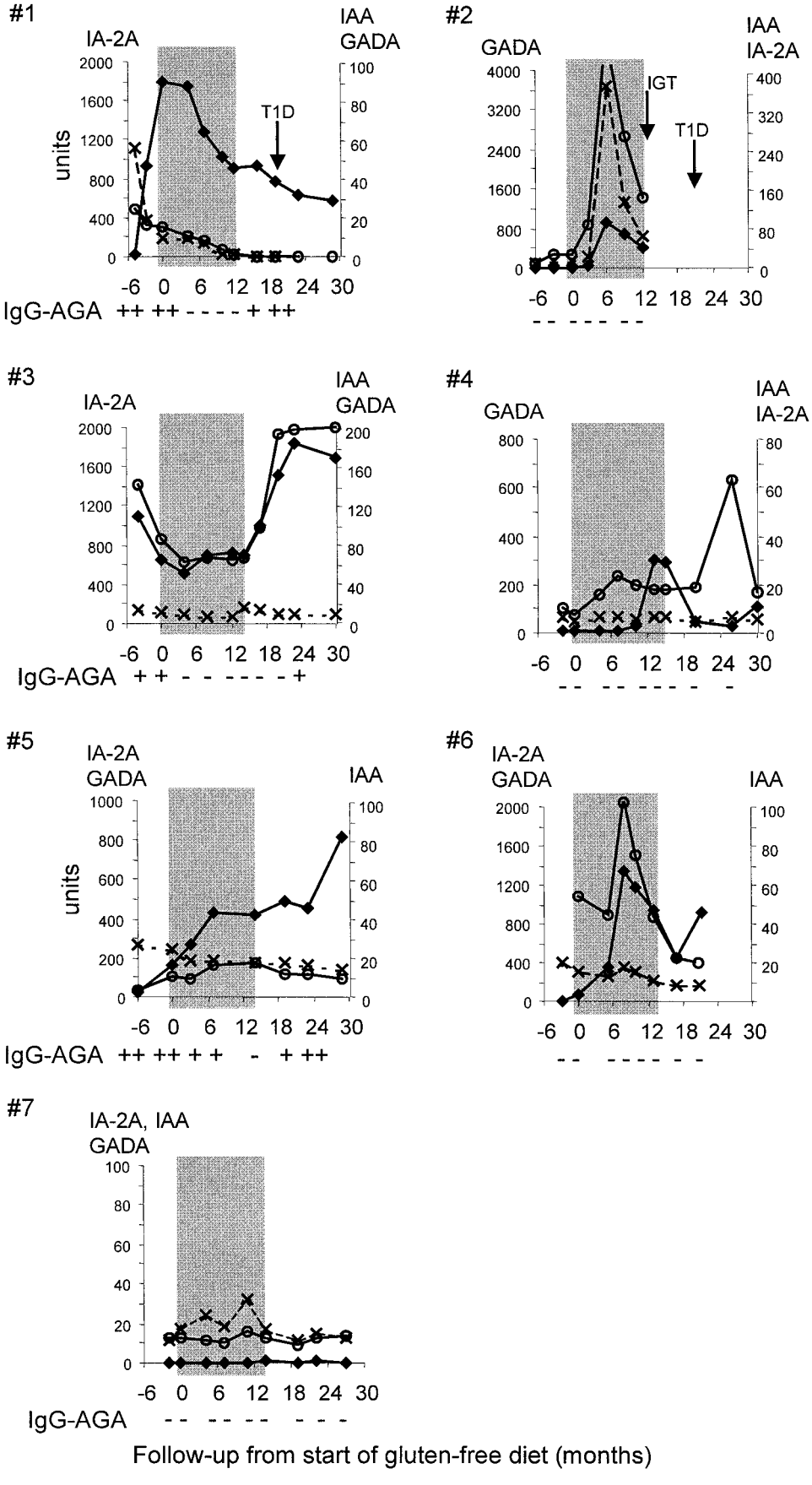

Figure 1—*Course of islet autoantibodies in subjects undergoing gluten-free diet. Autoantibodies to insulin (***x***), GAD (○) and IA-2 (◆) were measured by radiobinding assays. The thresholds for positivity were 2, 10, and 5 units, respectively. The period under gluten-free diet is shaded. IgG-gliadin antibodies were measured by enzyme-linked immunosorbent assay with a commercial kit. ++, OD >0.2; +, OD 0.087–0.2; –, OD <0.087. Type 1 diabetes developed in two subjects (cases 1 and 2) during the gluten reexposure period.*

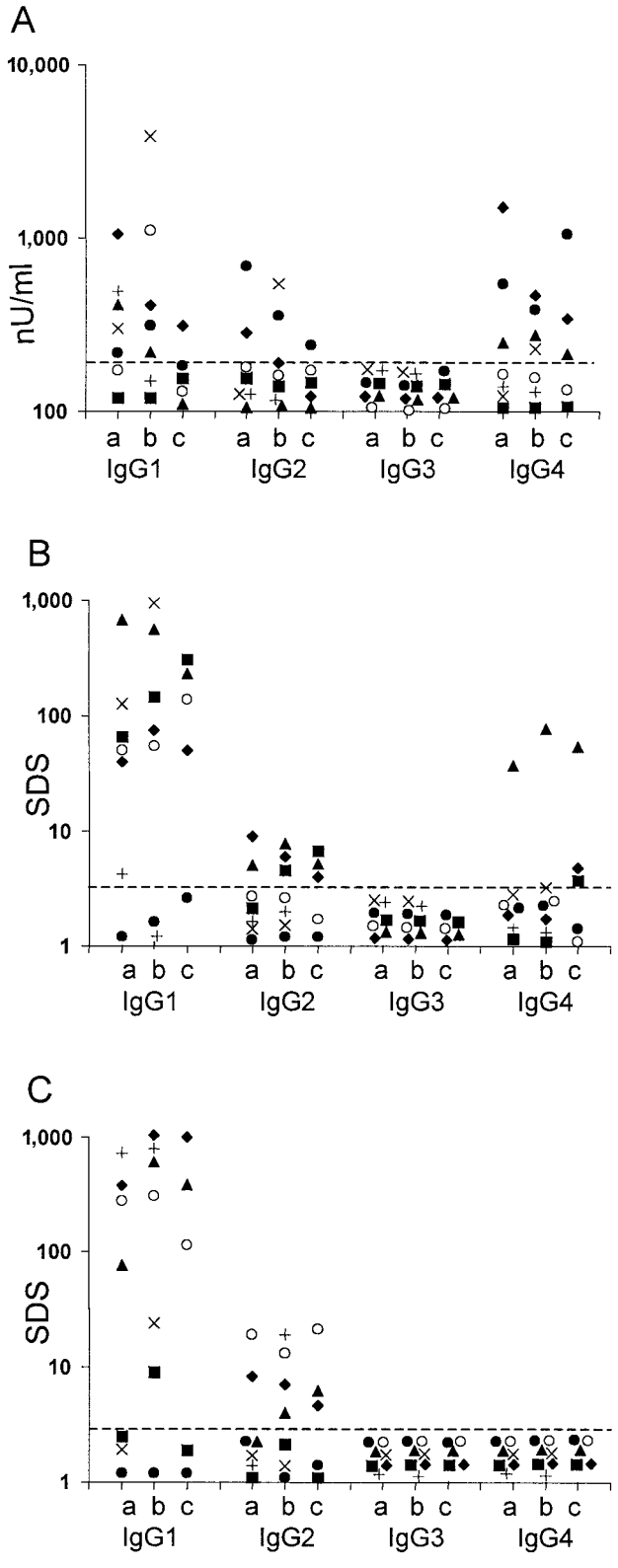

Figure 2—*Levels of each antibody subclass of IAA (A), GADA (B), and IA-2A (C) in the seven treated high-risk relatives before (a) and after (b) the 12-month gluten-free diet period and 12 months after reexposure to gluten (c). The dotted lines represents the threshold for positivity. IAA subclasses are expressed in nU/ml serum; GADA and IA-2A subclasses are shown in standard deviation scores.*

control cohort. Major reductions (>50%) in the gluten-free period were observed in only one subject (case 1 for IAA, GADA, and IA-2A). Type 1 diabetes later developed in this subject during the gluten reexposure period. In all other subjects, type 1 diabetes–associated antibodies either increased (case 2 for IAA, GADA, and IA-2A; case 4 for GADA and IA-2A; case 5 for IA-2A; and case 6 for IA-2A) or remained stable (cases 3 and 7) during the gluten-free period. The gluten reexposure period was characterized by development of type 1 diabetes in two subjects (cases 1 and 2), markedly increased levels of GADA and IA-2A at 3 months after reintroduction of gluten in one subject (case 3), and markedly elevated levels of GADA 12 months after reexposure in one subject (case 4).

Other autoantibodies either remained stable or decreased during the gluten reexposure period. In one subject (case 5), IA-2A levels were elevated 15 months after reexposure. Overall, at the end of the gluten-free period, there were 3 observations of reduced antibodies, 11 observations of unchanged levels of antibodies, and 7 observations of increased antibodies compared with 3 observations of decreased antibodies, 12 observations of unchanged levels of antibodies, and 3 observations of increased antibodies at the end of the gluten reexposure period ($P = 0.5$, χ^2 test for trend). In the historical control cohort, there were 10 observations of decreased antibodies (5 IAA, 4 GADA, and 1 IA-2A), 49 observations of unchanged levels of antibodies (21 IAA, 14 GADA, and 14 IA-2A), and 19 observations of increased antibodies (0 IAA, 8 GADA, and 11 IA-2A) over a 12-month period. The number of decreased antibodies in the gluten-free period (3 of 21) did not differ from that observed in the historical control cohort (10 of 78; $P = 1.0$).

Islet antibody subclasses and epitopes

Subclasses and epitope reactivity of GADA and IA-2A generally paralleled overall antibody titers and were typical of what has been reported in prediabetes (14–16). GADA were against the major central and carboxyl terminal epitopes, with no significant change in specificity during the study. IA-2A were predominantly against PTP domain epitopes. IgG1

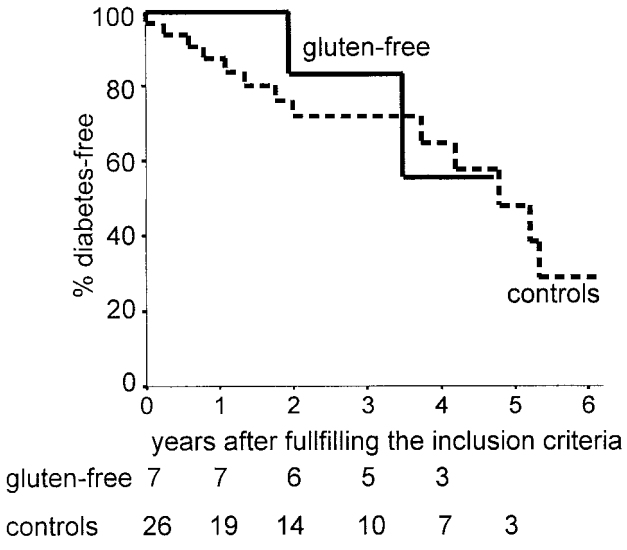

Figure 3—*Risk of progression to diabetes by Kaplan-Meier analysis in seven children under intervention compared with historical control siblings and offspring (*P *= 0.8). All children in the intervention and historical control group were aged <6 years and had multiple islet autoantibodies and a normal result of OGTT at the start of the study.*

was the predominant GADA and IA-2A subclass in all subjects (Fig. 2).

One subject also had strong IgG4 GADA (case 6) throughout the study. IAA were IgG1 and/or IgG4. A marked shift from IgG1 predominance to IgG4 predominance occurred for IAA in case 6 during the during gluten-free period, whereas a significant shift from IgG4 to IgG1 during gluten-free period and back to IgG4 during gluten reexposure period occurred in case 7.

Progression to type 1 diabetes

Progression to type 1 diabetes in the seven subjects under intervention did not differ from that observed in the historical control cohort (Fig. 3).

CONCLUSIONS — This study failed to observe a significant reduction in titers of type 1 diabetes–associated autoantibodies upon commencement of a gluten-free diet. The antibody variations observed within the seven relatives at the end of the 12-month gluten-free diet were similar to the increases and decreases in autoantibody levels found in a matched historical control population, suggesting that the removal of dietary gluten did not modulate the islet autoimmunity within the early preclinical period of type 1 diabetes (9,12,14). The onset of diabetes observed in the gluten reexposure period was also consistent with the natural history of the disease in young multiple autoantibody–positive relatives.

The findings of this study are in contrast to what has been reported in patients with islet autoantibodies and celiac disease, in whom disappearance of these antibodies was observed after commencement of the gluten-free diet (6). Such patients differ from those in our intervention cohort in that they are not first-degree relatives of patients with type 1 diabetes, not all had more than one islet autoantibody at the time of gluten removal and therefore have a lower inherent risk for developing diabetes, and they had celiac disease. The findings of our study are not inconsistent with previous findings, because we examined only subjects with high risk of diabetes and no celiac disease. Based on changes in matched untreated islet autoantibody–positive children, the current intervention in seven subjects has 90% power to significantly detect ($P < 0.05$, two-tailed test) autoantibody decreases in 50% of the events measured after the gluten-free period. We conclude, therefore, that removal of gluten from the diet in young first-degree relatives with established type 1 diabetes–associated autoimmunity does not seem to change the course of this autoimmunity.

We have not tested whether a gluten-free diet can delay onset of diabetes. Moreover, it remains untested whether total avoidance of gluten exposure can prevent or delay diabetes in man, as was the case for NOD mice (4). It should be noted that several dietary interventions have reduced incidence of diabetes in NOD mice and it is not clear whether any of these will be successful in humans. None of these studies, including those using a gluten-free diet, have examined whether the dietary modification affects autoantibodies in NOD mice or whether it is effective if introduced after the appearance of autoimmunity, as was the case in the current study. We conclude, therefore, that although avoidance of gluten can delay diabetes in NOD mice and removal of gluten may result in a reduction of diabetes-associated autoantibodies in patients with celiac disease, gluten does not drive production of islet autoantibodies in type 1 diabetes as it does in celiac disease.

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