Tyrosine Phosphatase–Related Islet Antigen $2_{(256–760)}$ Autoantibodies, the Only Marker of Islet Autoimmunity That Increases by Increasing the Degree of BMI in Obese Subjects With Type 2 Diabetes

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OBJECTIVE

Since patients with type 2 diabetes and positive for type 1 diabetes–specific antibodies have wide variations in BMI, this study evaluated whether the frequency and pattern of islet autoantibody positivity is related to BMI.

RESEARCH DESIGN AND METHODS

Clinical and biochemical characteristics and islet autoantibodies including GAD and protein tyrosine phosphatases islet antigen-2 $(IA\text{-}2)_{IC}$ and IA-$2_{(256–760)}$ were evaluated in 1,850 patients with type 2 diabetes from the Non–Insulin Requiring Autoimmune Diabetes study cohort. BMI was evaluated in all patients, who were then subdivided in three groups according to BMI (<25, ≥25 to <30, and ≥30 kg/m^2).

RESULTS

Out of 1,850, 120 (6.5%) patients were positive for at least one of the following antibodies: GAD (4.1%), IA-$2_{(256–760)}$ (3.3%), or IA-2_{IC} (1.1%). GAD and IA-2_{IC} antibodies showed decreasing frequencies with increasing BMI ($P < 0.0001$ and 0.0006, respectively, for trend); in contrast, the frequency of IA-$2_{(256–760)}$ antibodies increased with increasing BMI ($P = 0.005$ for trend). Patients with type 2 diabetes positive for IA-$2_{(256–760)}$ alone showed a phenotype resembling classical obese type 2 diabetes, with higher BMI, waist circumference, and uric acid ($P < 0.005$ for all), lower thyroid peroxidase antibodies, and lower progression to insulin requirement than GAD antibody–positive patients ($P = 0.04$ and $P = 0.0005$, respectively).

CONCLUSIONS

The IA-$2_{(256–760)}$ antibody appears to represent an antibody marker that mainly identifies a clinical phenotype very similar to obese type 2 diabetes, suggesting a possible different pathogenetic mechanism.

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**The list of NIRAD follow-up investigators is available in APPENDIX.*

The presence of antibodies specific to type 1 diabetes was first described in patients with type 2 diabetes by Irvine et al. (1) in a subgroup of patients who were treated with oral hypoglycemic agents. These patients, known by the acronym of latent autoimmune diabetes in adults (LADA), show a slower progression to insulin requirement than patients with type 1 diabetes (2) but share a similar antibody pattern, albeit with significant quantitative differences (3,4). In islet autoantibody–positive patients clinically diagnosed as type 2 diabetic, GAD autoantibodies (GADAs) represent the marker most frequently detected (4,5), whereas tyrosine phosphatase islet antigen-2 antibodies (IA-2As) directed against the COOH portion of the protein were initially detected in a low number of patients (4). With respect to IA-2 autoantibodies, distinct constructs of the IA-2 were shown indeed to account for different immunoreactivities in autoimmune diabetes. The IA-$2_{IC(605–979)}$ construct showed the highest sensitivity when used to evaluate IA-2 immunoreactivity in patients with newly diagnosed type 1 diabetes (6), whereas this construct was less frequent than the IA-2 fragment IA-$2_{(256–760)}$ in patients with type 2 diabetes. Indeed, in the Non–Insulin Requiring Autoimmune Diabetes (NIRAD) study (7), IA-$2_{(256–760)}$A were detected in ~30% of GADA-positive patients, and interestingly, in 3.4% of GADA and of IA-2_{IC} antibody–negative patients (7), thus identifying a subset of type 2 diabetic subjects with signs of ongoing islet autoimmunity.

The presence of insulin antibodies, which represents a specific marker of diabetes in young patients, is inversely related to age and is rare in adults; these antibodies are therefore unlikely to be useful for screening for antibody-positive patients with type 2 diabetes (8–10). Zinc transporter 8 (ZnT8) autoantibodies have recently been considered as an additional marker of islet autoimmunity in patients with type 2 diabetes (11,12), although their prevalence is quite low in these patients (11).

In our previous studies (12,13), we demonstrated a great deal of heterogeneity within the type 2 diabetic islet autoantibody-positive population in terms of phenotypic, genetic, immunological, and biochemical characteristics. Specifically, BMI varied widely, ranging from values below normality up to extreme obesity. Recent studies provided evidence to support the hypothesis that chronic inflammation that characterizes visceral obesity could also determine acquired autoimmunity against β-cells (14).

The correlation between BMI and the risk of autoimmune diseases has been recently evaluated (15). T helper (Th) 17 cell expansion seems to be a prominent element of proinflammatory diseases in obesity and has been found to be connected to autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, multiple sclerosis, psoriasis, and type 1 diabetes. The unstable Th17 cells are likely converted into either the Th1 or Th2 phenotype, causing Th1- or Th2-mediated autoimmune diseases. In light of these findings and in an attempt to further characterize this heterogeneous form of diabetes, we aimed at evaluating whether the frequency and pattern of the humoral islet immune response in autoantibody-positive patients with type 2 diabetes is related to BMI.

RESEARCH DESIGN AND METHODS

A total of 1,850 patients, all recruited in central Italy (13) from the NIRAD study cohort of 5,330 consecutive cases of type 2 diabetes (13), was evaluated in the current study.

Inclusion criteria for the NIRAD study were: *1*) diagnosis of diabetes according to the American Diabetes Association (16); *2*) no insulin requirement and no evidence of ketosis from diagnosis to screening time; and *3*) disease duration between 6 months and 5 years. Exclusion criteria included: *1*) prior insulin therapy, *2*) pregnancy; and *3*) the presence of any other severe diseases.

The following parameters were evaluated: clinical and biochemical characteristics (age at diagnosis, duration of disease, BMI, waist circumference, fasting blood glucose, total and HDL cholesterol, triglycerides, HbA_{1c}, and uric acid) as well as autoantibodies directed against GAD, IA-$2_{IC(605–979)}$, and IA-$2_{(256–760)}$ proteins.

ZnT8, tissue transglutaminase (tTG), and thyroid peroxidase (TPO) antibodies were analyzed only in patients positive for GADA and/or IA-2A and in a twofold number of type 2 diabetic patients matched for sex and duration of disease. GADAs as well as IA-2_{IC}, IA-$2_{(256–760)}$, ZnT8, and tTG antibodies were measured by previously described immunoradiobinding assays using the correspondent in vitro translated [^{35}S]methionine-labeled protein (7,13,17,18).

As far as the IA-$2_{(256–760)}$ antibodies assay is concerned, the cutoff defined as values >99th percentile of 360 healthy control sera is 0.046. The intra- and interassay coefficients of variation are 5.7 and 10.3%, respectively. The specificity is 99% and the sensitivity is 30% for type 1 diabetes <18 years of age and 40% for type 1 diabetes >18 years of age. TPO antibodies were measured using a radioimmunoassay (Medipan, Berlin, Germany) (13).

BMI was evaluated in all patients, who were then subdivided in three groups according to BMI (<25, ≥25 to <30, and ≥30 kg/m^2). When BMI subgroups were considered, 331 patients had BMI <25 kg/m^2, 722 patients had BMI ≥25 to <30 kg/m^2, and 797 patients had BMI ≥30 kg/m^2.

Clinical and biochemical characteristics and the presence and levels of antibodies were described for each of the three BMI subgroups and compared.

A subgroup of autoantibody-positive patients (n = 53) was followed-up for 7 years from the time of first antibody screening to evaluate their progression toward insulin requirement. Initiation of insulin therapy was part of a protocol treatment in the NIRAD study, and the participating centers were blinded to antibody results. Insulin requirement was defined as the clinical need to start insulin therapy in patients whose glycemic control, measured at each scheduled visit, became unacceptable (HbA_{1c} ≥58 mmol/mol or ≥7.5%) despite treatment with a maximally tolerated combination of insulin sensitizers (metformin and/or thiazolidinediones) and sulfonylurea therapy.

The study was approved by all local ethics committees, and written informed consent was obtained from all patients.

Statistical Analysis

Statistical analysis was performed using SAS v9.3 software. Data were expressed as means ± SD. The Shapiro-Wilk test was used to test the normality of distribution of continuous variables. When data were not normally distributed, logarithmic transformation was performed.

For normally distributed continuous variables, mean values between two groups were compared by Student *t* test. Frequency differences were compared using the χ^2 test (with Yates continuity correction) or Fisher exact test when appropriate. The Cochran-Armitage test was used to test for trends between the frequencies of GADA, IA-$2_{(256–760)}$A, and IA-2_{IC}A in each BMI subgroup. A Venn diagram was used to show the autoantibody frequencies. A *P* value <0.05 was considered as statistically significant.

RESULTS

Of the 1,850 patients with type 2 diabetes, 120 (6.5%) were positive for at least one of the autoantibodies to GAD (4.1%), IA-$2_{(256–760)}$ (3.3%), or IA-2_{IC} (1.1%).

Figure 1 shows the distribution of GAD, IA-$2_{(256–760)}$ and IA-2_{IC} autoantibody frequencies according to BMI. Overall, the frequency of GADA, IA-$2_{(256–760)}$A, and IA-2_{IC}A significantly decreased as patients' BMI increased ($P <$ 0.0001, P = 0.014, and $P <$ 0.0001, respectively, for trends across BMI subgroups). GADA was the most frequent antibody in patients with BMI <25 kg/m^2, whereas IA-$2_{(256–760)}$A was the most frequent in patients with BMI ≥ 30 kg/m^2.

However, when only the 120 GAD and/or IA-$2_{(256–760)}$ and/or IA-2_{IC} antibody–positive patients were considered, GADA and IA-2_{IC}A showed decreasing frequencies with increasing BMI ($P <$ 0.0001 and P = 0.0006, respectively, for trend), while IA-$2_{(256–760)}$A frequency significantly increased with increasing BMI (P = 0.005 for trend), with these antibodies becoming the most frequent antibody and, in ~70% of cases, the only islet autoantibody in patients with a BMI ≥ 30 kg/m^2 (Fig. 2).

The antibody combinations in autoantibody-positive patients according to BMI is shown in Fig. 3. Among 49 patients with BMI <25 kg/m^2, 25 were positive for GADA alone, and 11 were positive for all three antibodies; conversely, in patients with BMI ≥ 30 kg/m^2, 7 patients were positive for GADA, and 1 was positive for the three antibodies. Six patients with BMI <25 kg/m^2 and 21 patients with BMI ≥ 30 kg/m^2 were positive only for IA-$2_{(256–760)}$A.

In Table 1, the clinical and biochemical characteristics of type 2 diabetic patients subdivided according to GAD and/or IA-$2_{(256–760)}$ antibody positivity are reported and compared. Four groups of type 2 diabetic patients are shown: patients positive for both GAD and IA-$2_{(256–760)}$ antibodies [GADA$^+$/IA-$2_{(256–760)}$A$^+$], patients positive for GAD and negative for IA-$2_{(256–760)}$ antibodies [GADA$^+$/IA-$2_{(256–760)}$A$^-$], patients positive for IA-$2_{(256–760)}$ antibodies and negative for GADA [GADA$^-$/IA-$2_{(256–760)}$A$^+$], and patients negative for both antibodies [GADA$^-$/IA-$2_{(256–760)}$A$^-$].

Patients positive for both antibodies [GADA$^+$/IA-$2_{(256–760)}$A$^+$], as expected, showed the highest degree of autoimmunity; IA-2_{IC}, ZnT8, and TPO antibodies were significantly higher in these patients compared with the other groups (IA-2_{IC} and ZnT8: $P \leq 0.005$ for all; TPO: $P \leq 0.03$ for all). Considering the biochemical and anthropometric characteristics, we observed that, with the only exception the age at diagnosis (P = 0.016), patients positive for both antibodies [GADA$^+$/IA-$2_{(256–760)}$A$^+$] showed a phenotype substantially similar to that of patients positive for GADA only [GADA$^+$/IA-$2_{(256–760)}$A$^-$]; they differed, instead, from the other two groups, GADA$^-$/IA-$2_{(256–760)}$A$^+$ and GADA$^-$/IA-$2_{(256–760)}$A$^-$, regarding BMI ($P <$ 0.0001 for both) and waist circumference (P = 0.02 and P = 0.01, respectively). Fasting glucose and uric acid were significantly different between GADA$^+$/IA-$2_{(256–760)}$A$^+$ and GADA$^-$/IA-$2_{(256–760)}$A$^-$ (P = 0.04 and P = 0.01, respectively).

When comparing GADA$^+$/IA-$2_{(256–760)}$A$^-$ and GADA$^-$/IA-$2_{(256–760)}$A$^+$ among themselves, we observed that BMI, waist circumference, total cholesterol, and

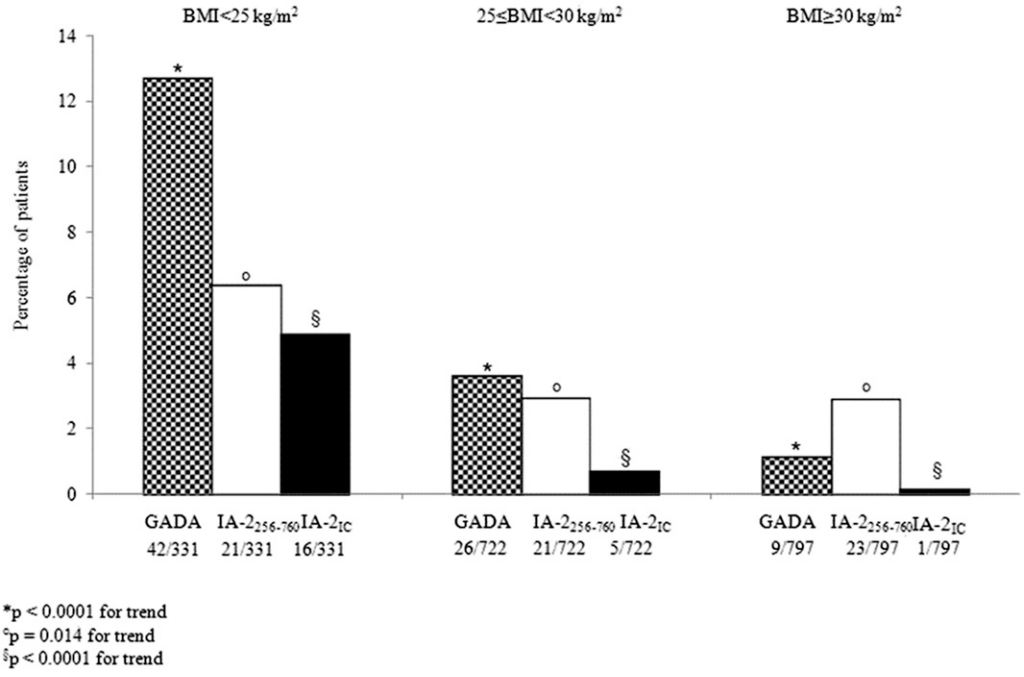

Figure 1—Frequencies of GADA and protein tyrosine phosphatase IA-2As (IA-$2_{256–760}$A and IA-2_{IC}A) in 1,850 type 2 diabetic patients according to BMI.

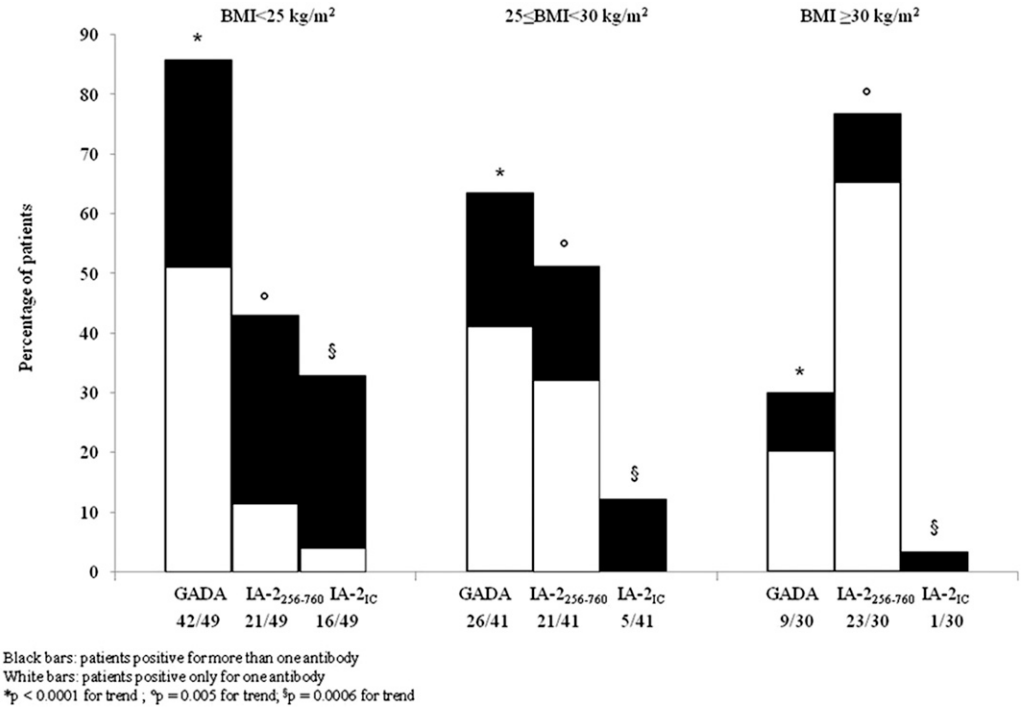

Figure 2—Frequencies of GADA and protein tyrosine phosphatase IA-2As (IA-$2_{256–760}$A and IA-2_{IC}A) in 120 autoantibody-positive type 2 diabetic patients according to BMI.

uric acid levels were significantly lower in GADA$^+$/IA-$2_{(256–760)}$A$^-$ patients than in GADA$^-$/IA-$2_{(256–760)}$A$^+$ patients ($P <$ 0.0001, P = 0.001, P = 0.01, and P = 0.003, respectively). Furthermore, HDL cholesterol and TPO antibodies were significantly higher in GADA$^+$/IA-$2_{(256–760)}$A$^-$ patients than in GADA$^-$/IA-$2_{(256–760)}$A$^+$ patients (P = 0.04 for both). On the other hand, findings were similar when GADA$^-$/IA-$2_{(256–760)}$A$^+$ patients and GADA$^-$/IA-$2_{(256–760)}$A$^-$ patients were compared, as no statistically significant differences were observed, with the only exception IA-2_{IC}A frequency (P = 0.002), showing that patients positive for IA-$2_{(256–760)}$A only have a phenotype substantially resembling that of a classical obese patient with type 2 diabetes. Among 120 type 2 diabetic patients positive for GADA, IA-2_{IC}A, and IA-$2_{(256–760)}$A subdivided into three groups according to BMI ($<$25, $\geq$25 to $<$30, and $\geq$30 kg/m^2), decreasing frequencies of ZnT8 antibody with increasing BMI (P = 0.01 for trend) were observed (data not shown).

In the prospectively evaluated subgroup of antibody-positive patients in whom the risk of progression to insulin therapy during a 7-year follow-up was analyzed (n = 53), a significantly higher number of GADA$^+$/IA-$2_{(256–760)}$A$^+$ and GADA$^+$/IA-$2_{(256–760)}$A$^-$ patients (61.5%, n = 8 of 13; and 62.5%, n = 15 of 24) required insulin treatment compared with GADA$^-$/IA-$2_{(256–760)}$A$^+$ (n = 0 of 16) patients (P = 0.0001, odds ratio 51.00 [95% CI 2.51–1,035.75]; and P = 0.0005, odds ratio 47.32 [2.52–888.45], respectively).

CONCLUSIONS

In the current study, we demonstrated that in patients with type 2 diabetes, the prevalence of the type 1 diabetes–specific GAD, IA-$2_{IC(605–979)}$, and IA-$2_{(256–760)}$ autoantibodies significantly decreased as BMI increased from $<$25 to $\geq$30 kg/m^2. Of particular interest, we observed that, in islet autoantibody-positive patients, the frequency of IA-$2_{(256–760)}$A significantly increased as BMI increased so that it became the most prevalent antibody in patients with BMI $\geq$30 kg/m^2. In addition, presence of the IA-$2_{(256–760)}$A, when not associated with GADA (as was the case in $\geq$80% of antibody-positive patients with BMI $>$25 kg/m^2), identified a phenotype resembling a classical obese patient with type 2 diabetes, with significantly higher BMI, waist circumference, total cholesterol, and uric acid level and a lower frequency of HDL cholesterol and TPO antibody positivity than patients positive for GADA alone.

We have also found that none of the 16 patients positive for IA-$2_{(256–760)}$A alone progressed to insulin therapy requirement during 7 years of follow-up compared with 61.5% (8 of 13) of patients positive for both antibodies and 62.5% (15 of 24) of patients positive for GADA alone. Based on these findings, if we accept to define LADA as proposed by Fourlanos et al. (19), a form of diabetes diagnosed as type 2 but that presents positivity for at least one antibody associated to type 1 diabetes [indeed, we previously demonstrated (7) that autoantibodies to IA-$2_{(256–760)}$ are present in 30% of type 1 diabetic children and 40% of type 1 diabetics adult patients], we have to conclude that LADA is a quite heterogeneous disease, ranging from patients with high GADA titer and "clear signs of ongoing autoimmunity" to patients positive only for IA-$2_{(256–760)}$ and with a relatively mild autoimmune reactivity. Furthermore, as previously emphasized by Brooks-Worrel and Palmer (20), islet autoantibodies used so far to identify LADA patients are only those islet autoantibodies first identified in type 1 diabetes. The original detection of IA-$2_{(256–760)}$ autoantibodies in patients phenotypically resembling

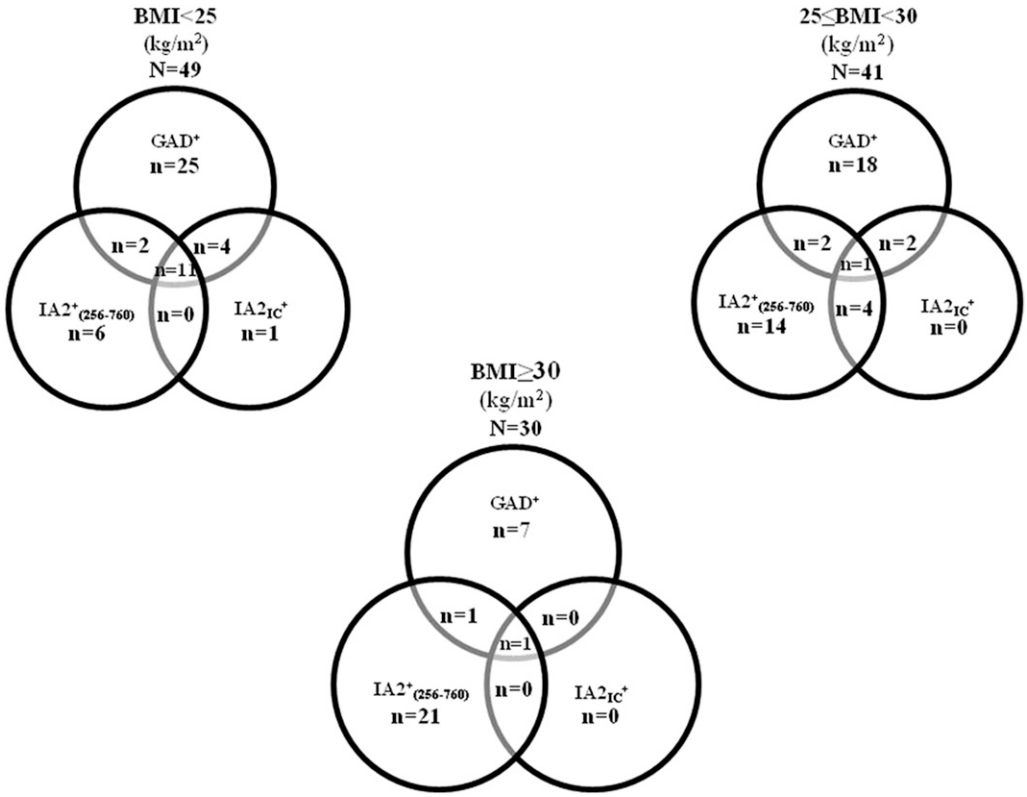

Figure 3—Venn diagram of autoantibody combinations [GADA and protein tyrosine phosphatase IA-2As (IA-$2_{256–760}$A and IA-2_{IC}A)] in 120 autoantibody-positive type 2 diabetic patients, subdivided according to BMI.

type 2 diabetic (6) could help in classifying LADA more accurately, recognizing the wide heterogeneity of this form of diabetes.

Our results, suggesting a lower degree of islet destructive autoimmunity in subjects positive only for IA-$2_{(256–760)}$ and demonstrating a higher frequency of these antibodies in obese patients with type 2 diabetes, raise questions about the real meaning of such autoantibodies directed against this specific IA-2 epitope.

An intriguing hypothesis is that such humoral autoimmune response may be secondary to a chronic inflammation process associated to β-cell damage, which occurs in obese patients with type 2 diabetes (20). Visceral obesity is one of the main risk factors for type 2 diabetes. Indeed, evidence supports the concept that chronic inflammation (low-grade inflammation), which characterizes visceral obesity, is involved in the pathogenesis of insulin resistance and type 2 diabetes (21), through the contribution of several proinflammatory cytokines, interleukin (IL)-1β (22), IL-6 (23), leptin, and tumor necrosis factor-α (24), implicated in disrupting insulin signaling, causing insulin resistance.

Increasing evidence has demonstrated that this chronic inflammation, characterized by islet-associated expression of cytokines, including IL-1 (25) and IL-6 (26), hyperglycemia, chemokines (27), and dyslipidemia (28) act together in determining an inflammatory process in the islets of patients with type 2 diabetes. The presence of these inflammatory mediators in patients with type 2 diabetes has been also implicated in β-cell apoptosis (29). Cellular inflammation in the islets of obese patients with type 2 diabetes is therefore characterized by the presence of cytokines, apoptotic cells, amyloid deposits, and eventually fibrosis and immune cell infiltration. The presence of immune cells (20,26) suggests that immune-mediated islet damage may indeed be a component of type 2 diabetes.

Low-grade inflammation that characterizes obese patients could also determine acquired autoimmunity against β-cells; however, this adaptive autoimmune response, although present, may not necessarily have a pathogenic role in the development of type 2 diabetes (14). Indeed, the inflammatory process in adipose tissue seems to be similar in all insulin-resistant individuals with obesity, regardless of whether or not they progress to develop type 2 diabetes (30).

Taking into account that the development of islet autoimmunity in obese patients with type 2 diabetes may be related to the established systemic inflammation associated with obesity, the real meaning of the specific immune reactivity against the 256–760 domain of IA-2 protein needs to be further investigated. Tyrosine phosphatase–related IA2 protein (amino acids 1–979), one of the major autoantigens in type 1 diabetes and target of both humoral and T-cell reactivity, is an enzymatic inactive transmembrane glycoprotein localized in the secretory granules of peptide-secreting endocrine cells (31). Although the real function of this protein is largely unknown, there is evidence that its association with other proteins may favor a link between secretory granules with a cytoskeleton, thus affecting exocytosis. To date, IA-2As are detected using sensitive and specific

Table 1—Clinical and biochemical characteristics and antibody frequencies in type 2 diabetic patients grouped according to GADA and protein tyrosine phosphatase [IA-$2_{(256–760)}$A] positivity

	1	2	3	4
	$GADA^+/IA\text{-}2_{(256–760)}A^+$ (n = 20)	$GADA^+/IA\text{-}2_{(256–760)}A^-$ (n = 58)	$GADA^-/IA\text{-}2_{(256–760)}A^+$ (n = 42)	$GADA^-/IA\text{-}2_{(256–760)}A^-$ (n = 1,730)
Age at diagnosis (years)	44.0 ± 15.6	53.5 ± 14.3	54.6 ± 11	56.4 ± 11.2
Duration of disease (months)	21.5 ± 20.3	23.5 ± 20.7	24.6 ± 19.8	24.7 ± 19
BMI (kg/m^2)	24.9 ± 3.9	26.1 ± 4.2	30.8 ± 6.4	30.1 ± 5.7
Waist circumference (cm)	92.2 ± 7.6	91.2 ± 13.7	101.1 ± 13.7	99.2 ± 15.8
Fasting glucose (mg/dL)*	169.1 ± 57.8	163.2 ± 68	143.6 ± 45.8	146.3 ± 43
Total cholesterol (mg/dL)	174.9 ± 65.8	152.1 ± 58.8	183.7 ± 56	179.6 ± 73.2
HDL (mg/dL)	51.4 ± 11	54.0 ± 28.2	44.7 ± 12.4	48.6 ± 18.7
Triglycerides (mg/dL)*	147.8 ± 69.5	162.0 ± 70.6	184.6 ± 83.2	180.8 ± 95.6
HbA_{1c} (%)	7.7 ± 1.9	7.7 ± 2.3	7.1 ± 1.6	6.9 ± 3.7
HbA_{1c} (mmol/mol)	61.0 ± 21.1	61.0 ± 21.5	54.0 ± 20.7	52.0 ± 23.8
Uric acid (mg/dL)	4.1 ± 2.1	4.0 ± 0.8	5.2 ± 1.2	5.2 ± 1.5
$IA\text{-}2_{IC}^+$	13/20 (65)	7/58 (12)	1/42 (2.4)	0/1,730 (0)
$ZnT8^+$	10/20 (50)	5/58 (8.6)	0/42 (0)	2/240 (0.83)
$tTGA^+$	0/20 (0)	2/58 (3.4)	0/42 (0)	1/240 (0.41)
TPO^+	13/20 (65)	20/58 (34.4)	6/42 (14.2)	20/240 (8.3)

Data are mean ± SD unless otherwise indicated. 1 vs. 2: age at diagnosis, $P = 0.016$; $IA\text{-}2_{IC}^+$ and $ZnT8^+$, $P < 0.0001$; and TPO^+ $P = 0.03$. 1 vs. 3: age at diagnosis and $ZnT8^+$ $P = 0.005$; BMI, $IA\text{-}2_{IC}^+$, and TPO^+, $P < 0.0001$; waist circumference, $P = 0.02$; $tTGA^+$, P = not calculable. 1 vs. 4: age at diagnosis, $P = 0.0002$; BMI, $IA\text{-}2_{IC}^+$, $ZnT8^+$, and TPO^+, $P < 0.0001$; waist circumference and uric acid, $P = 0.01$; and fasting glucose, $P = 0.04$. 2 vs. 3: BMI, $P < 0.0001$; waist circumference, $P = 0.001$; total cholesterol, $P = 0.01$; HDL and TPO^+, $P = 0.04$; and uric acid, $P = 0.003$. 2 vs. 4: BMI, waist circumference, $IA\text{-}2_{IC}^+$, and TPO^+, $P < 0.0001$; total cholesterol, $P = 0.009$; uric acid, $P = 0.0012$; and $ZnT8^+$, $P = 0.002$. 3 vs. 4: $IA\text{-}2_{IC}^+$, $P = 0.002$. *Data were log transformed.

radioimmunoassays, differing in which of the radiolabeled IA-2 constructs were used, usually chosen among the full-length IA-$2_{FL(1–979)}$, the truncated NH2 terminally spliced IA-2 variant lacking exon 13, the IA-$2_{BDC(aa256–556:630–979)}$, and the intracytoplasmic IA-$2_{IC(605–979)}$ construct (32,33). Although all of these constructs are sensitive and specific, the IA-$2_{IC(605–979)}$ construct has demonstrated the highest sensitivity in patients with newly diagnosed type 1 diabetes and in patients with pre–type 1 diabetes, suggesting its use for type 1 diabetic screening (6).

Previous studies have demonstrated that IA-2A in type 1 diabetes is directed against multiple epitopes of the intracellular cytoplasmic portion of the protein (amino acids 601–979) located in the juxtamembrane region and the COOH-terminal domain (amino acids 630–979) (34–37).

In contrast, we have recently found that, in islet autoantibody–positive type 2 diabetic patients, the immunoreactivity against IA-$2_{IC(605–979)}$ is present at lower frequency than that toward the IA-$2_{(256–760)}$ fragment.

Of note, this immunoreactivity, frequent in obese subjects, is located in a segment of the IA-2 molecule comprising the extracellular portion of the protein.

In light of such evidence, we hypothesize that islet inflammation occurring in type 2 diabetes may favor the presentation of extracellular antigens to antigen-presenting cells in an early stage of the inflammatory process.

In conclusion, we have demonstrated that autoantibodies against the IA-$2_{(256–760)}$ protein appear to represent an antibody marker that mainly identifies a clinical phenotype of patients very similar to that of classic obese type 2 diabetes. The presence of this antibody could underline a mechanism of eliciting a humoral immune response different from that occurring in "classical" autoimmune diabetes and possibly secondary to chronic systemic inflammation associated with obesity as well as to islet inflammation associated with type 2 diabetes.

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Author Contributions. R.B. wrote the manuscript and implemented the study design. M.S. and S.Z. wrote the manuscript. G.C. and L.M. performed data analysis. F.P. and C.T. were responsible for antibody testing and reviewed the manuscript. F.D. reviewed the manuscript and together with C.T. provided the IA-$2_{(256–760)}$ construct. All authors approved the final version of the manuscript. R.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

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