Expression of GAD65 and Islet Cell Antibody (ICA512) Autoantibodies Among Cytoplasmic ICA+ Relatives Is Associated With Eligibility for the Diabetes Prevention Trial–Type 1

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More than 71,000 relatives of type 1 diabetic patients have been screened for cytoplasmic islet cell antibodies (ICAs), GAD65 autoantibodies (GAAs), and ICA512 autoantibodies (ICA512AAs). Among those 71,148 relatives, 2,448 were cytoplasmic ICA+, and the remainder were ICA−. Of the ICA+ group, 1,229 (50.2%) were positive for GAAs and/or ICA512AAs. Among ICA− relatives, 1,897 (2.76%) were positive for GAAs and/or ICA512AAs. Given the large number of relatives positive for cytoplasmic ICA and negative for "biochemically" determined autoantibodies, and the converse, we analyzed the proportion of ICA+ relatives found eligible to participate in the intervention phase of Diabetes Prevention Trial–Type 1 (DPT-1). To be eligible for the parenteral insulin DPT-1 trial, a relative had to have first-phase insulin secretion below the 1st percentile of cut-points (for parents) or below the 10th percentile (for siblings and offspring). To be eligible for the oral insulin trial, a relative had to have first-phase insulin secretion above cut-points (>1st percentile for parents, >10th percentile for siblings/offspring) and be positive for anti-insulin autoantibodies. For both trials, DQB1*0602 was an exclusion criteria, cytoplasmic ICA positivity had to be confirmed, and an oral glucose tolerance test had to result in nondiabetic levels. Of 572 relatives found to be eligible for trial entry, 442 (77.3%) were positive for GAAs and/or ICA512AAs, although overall only 50.2% of ICA+ relatives were positive for GAAs and/or ICA512AAs. The positive predictive value for trial eligibility for ICA+ relatives with GAAs or ICA512AAs who completed staging was 51.0%. In contrast, only 11.9% of ICA+ but GAA− and ICA512AA− relatives were found to be eligible by DPT criteria for trial entry. Positivity for biochemically determined autoantibodies among cytoplasmic antibody–positive relatives is associated with eligibility for the DPT-1 study.
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Type 1A diabetes is strongly associated with the presence of islet cell–related autoantibodies, autoantibodies that usually precede by years the development of overt diabetes (1,2) The detection of cytoplasmic islet cell antibodies (ICAs), as measured by indirect immunofluoresence on sections of normal human pancreas, has been associated with increased risk of type 1A diabetes in first-degree relatives (3,4) and school children (5–7). Within the last 8 years, investigators have cloned a series of islet-related autoantigens and developed radioassays for autoantibodies reacting with these "biochemically" defined autoantigens (7–15). International workshops (16–18) have compared assays for anti–insulin autoantibodies (IAAs), anti-GAD65 autoantibodies (GAA), and anti–ICA512 autoantibodies (ICA512AA); such assays are now performed in laboratories throughout the world. These assays can be set up with cutoffs, allowing high sensitivity, with specificities >99th percentile of healthy control values. The most important risk factor for the development of type 1A diabetes is the expression of multiple anti-islet autoantibodies (2,19), especially in the presence of loss of first-phase insulin release (FPIR) on intravenous glucose tolerance tests (IVGTTs) (5,20–23). In particular, expression of two or more of GAA, ICA512AA, or IAA is associated with a high risk of progression to type 1A diabetes (2,16,23). These assays can be performed in 96-well filtration plates with counting on a 96-well beta counter. Thus the assays are semiautomated and relatively inexpensive. In the present study (an ancillary study of Diabetes Prevention Trial–Type 1 [DPT-1]), we screened >71,000 relatives of type 1 diabetic patients for cytoplasmic ICAs, GAAs, and ICA512AAs. We analyzed the association between cytoplasmic ICA positivity and GAA and/or ICA512AA positivity. In addition, we studied the prognostic value of

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DPT-1, Diabetes Prevention Trial–Type 1; FPIR, first-phase insulin release; GAA, GAD65 autoantibody; IAA, insulin autoantibody; ICA, islet cell antibody; ICA512AA, ICA512 autoantibody; IDS, Immunology of Diabetes Society; IVGTT, intravenous glucose tolerance test; JDFU, Juvenile Diabetes Foundation Units; mIAA, microinsulin autoantibody.

TABLE 1
Demographic characteristics of DPT-1 relatives screened for ICAs, GAAs, and ICA512AAs

Subject characteristics	Subjects tested for ICAs, GAAs, and ICA512AAs (n = 71,148)	Total DPT-1 subjects tested for ICA (n = 79,119)	ICA+	GAA+	ICA512AA+	GAA+ and ICA512AA+
Sex						
Male	31,264 (43.9)	34,743 (43.9)	3.98	4.22	1.66	1.21
Female	39,867 (56.0)	44,359 (56.1)	3.06*	3.82‡	1.22*	0.85*
Unknown	17 (0.0)	17 (0.0)	5.88	0	0	0
Ethnicity						
White	56,641 (79.6)	63,177 (79.9)	3.62	4.16	1.50	1.08
Hispanic	7,773 (10.9)	8,552 (10.8)	2.71*	3.35†	1.14§	0.71‡
Black	1,916 (2.7)	2,174 (2.7)	3.28	3.88	0.75‡	0.54§
American Indian	222 (0.3)	234 (0.3)	3.70	2.31	0.46	0.46
Asian/Pacific Island	686 (1.0)	779 (1.0)	3.29	4.34	1.50	1.05
Other	1,161 (1.6)	1,288 (1.6)	2.46	2.99	1.32	0.88
Unknown	2,749 (3.9)	2,915 (3.7)	2.83	2.87	0.99	0.81
Age (years)						
0–5	8,443 (11.9)	9,448 (11.9)	3.00	3.45	1.58	1.07
6–11	18,497 (26.0)	20,504 (25.9)	3.72‡	4.02§	1.87	1.33
12–17	13,974 (19.6)	15,453 (19.5)	4.07*	4.61*	1.93	1.50§
18–29	8,395 (11.8)	9,379 (11.9)	3.64§	3.77	1.53	1.20
30–45	21,839 (30.7)	24,335 (30.8)	3.01	3.90	0.70*	0.42*
Relationship to proband						
Sibling	27,585 (38.8)	30,507 (38.6)	4.36	4.95	2.04	1.53
Offspring	17,321 (24.3)	19,274 (24.4)	3.16*	3.61*	1.22*	0.86*
Parent	15,776 (22.2)	17,729 (22.4)	2.72*	3.61*	0.69*	0.41*
Non–first-degree relatives	10,073 (14.2)	11,214 (14.2)	2.48*	2.30*	0.90*	0.60*
Unspecified relation	393 (0.6)	395 (0.5)	8.38	12.04	8.12	6.02

Data are n (%) or %. *$P < 0.0001$; †$P < 0.001$; ‡$P < 0.01$; §$P < 0.05$. All P values were calculated in comparison to the first subgroup (male, white, age 0–5 years, sibling) with corresponding group of each column.

biochemically determined autoantibodies relative to eligibility for DPT-1 among ICA+ relatives.

RESEARCH DESIGN AND METHODS

Subjects. With nine coordinating clinical centers and >370 affiliate and satellite centers, the DPT-1 has screened relatives throughout the U.S. and Canada for cytoplasmic ICAs. To have been eligible for screening, an individual must have been a first-degree relative of a type 1 diabetic patient and between ages 2.5 and 45 years or have been a second-degree relative and between ages 2.5 and 20 years. Individuals were eligible for participation in the trial between ages 3 and 45 years. If a relative was found to be ICA+ (≥10 Juvenile Diabetes Foundation units [JDFU]; see below), they were staged with repeat ICA testing, HLA-DQ typing, an IVGTT, determination of IAAs, and oral glucose tolerance testing.

Of the group screened before 30 June 2000 for ICAs, 71,148 initial samples from DPT-1 subjects were also screened for GAAs and ICA512AAs. Sex, ethnicity, age, and relationship to proband of this completed screening group and all DPT-1 eligible subjects as a whole are summarized in Table 1. Of 71,148 relatives, 2,448 were found to be ICA+ based on initial screening samples; 500 of these were staged and found to be eligible for a DPT-1 intervention (Fig. 1). An additional 72, whose initial ICA sample was negative, were then found to be ICA+ on follow-up and were determined to be trial-eligible. Thus, 572 relatives were trial-eligible at the time of our analysis.

The results of the determination of IAAs, DQ typing, and IVGTT are available for all individuals (n = 572) who were eligible for the parenteral insulin DPT-1 trial and for the oral insulin trial. Subjects, or their parents, gave informed consent, and oversight was provided by institutional review boards.

ICA assay. Cytoplasmic ICAs were determined on frozen sections of human pancreas by the DPT-1 ICA Core Laboratory (in Gainesville, FL, from February 1994 to September 1997; in New Orleans, LA, from September 1997 to December 1998) (7) Samples were considered positive at ≥10 JDFU. In the recent Immunology of Diabetes Society (IDS) Combinatorial Autoantibody Workshop (Orvieto, Italy, November 1995), this ICA assay had a specificity of 100%, with a sensitivity of 74.4% for new-onset patients aged <30 years.

GAA and ICA512AA assay. GAA and ICA512AA levels were measured simultaneously by combined GAA and ICA512AA radioassay, as previously described, in the DPT-1 GAA and ICA512AA Core Laboratory (Denver, CO; full-length GAD65 and ICA512bdc cDNA clones) (24). The assay was performed in 96-well filtration plates with autoantibody bound [^{3}H]GAD65 and [^{35}S]ICA512 precipitated with protein A Sepharose. The cut-points were set at indexes of 0.032 (mean ± 2 SD for GAAs) and 0.071 (mean ± 6 SD for ICA512AAs), the 99th and 100th percentile, respectively, of 198 normal controls. The interassay coefficients of variation were 6.5 and 9.6%, respectively, for GAA and ICA512AA assays, with the samples of index values ~1. In the IDS Combinatorial Workshop, for patients younger than age 30 years, assay specificity was 99 and 100% and sensitivity was 83.7 and 74.4% for GAAs and ICA512AAs, respectively.

ICA512bdc and ICA512ic autoantibody assays. A subset of 2,151 samples from these DPT-1 samples was randomly selected, and two different con-

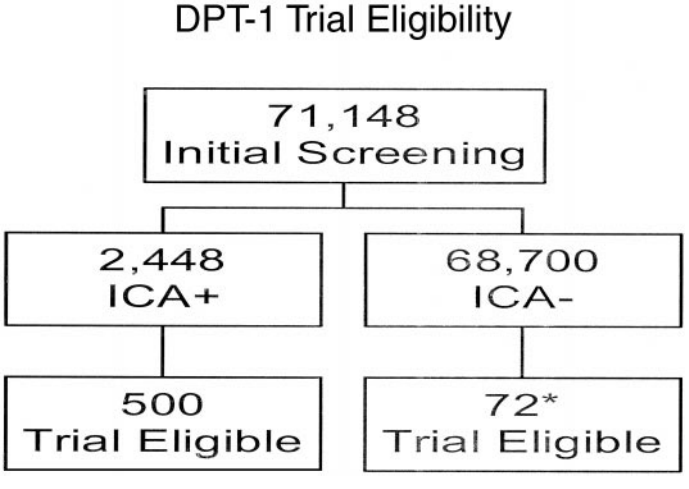

FIG. 1. Summary of number of individuals screened for DPT-1 found to be cytoplasmic ICA+ or ICA− and to be trial eligible on staging. *Converted to ICA+ after negative ICA screening sample and thus staged.

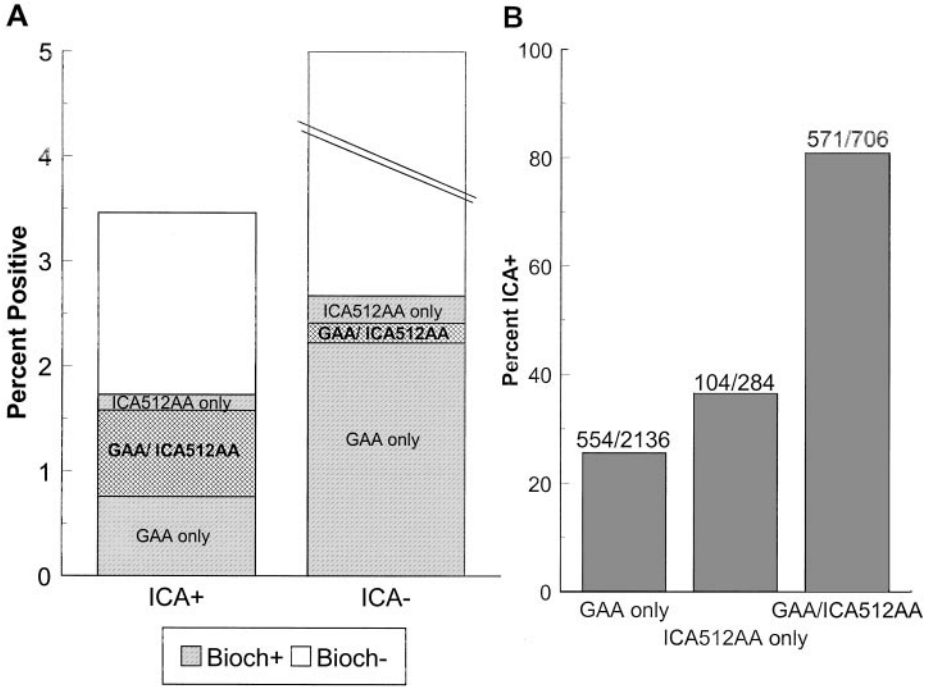

FIG. 2. *A*: Of 71,148 relatives of type 1 diabetic patients originally screened, 2,448 (3.44%) were ICA+. Of these ICA+ relatives, 1,229 (50.2%; 1.73% of total population screened) were GAA+ and/or ICA512AA+. Of the 68,700 ICA− relatives, 1,897 (2.76%; 2.67% of total population screened) were GAA+ and/or ICA512AA+. *B*: ICA positivity among relatives with indicated biochemical (Bioch) autoantibody positivity.

structs of ICA512 (IA-2)—ICA512bdc (amino acids: 256–556:630–979) and ICA512ic (complete intracellular domain of ICA512 molecule, amino acids: 605–979)—were used in the measurement of autoantibodies. Autoantibodies were measured in the same format as the GAA assay described above. The cut-points for positivity were set at indexes of 0.048 and 0.010, representing the 99th percentile for ICA512bdc and ICA512ic, respectively, of 198 normal controls.

Microinsulin autoantibody assay. A subset of 6,420 samples were randomly selected for microinsulin autoantibody (mIAA) assay analysis. The details of the mIAA assay protocol have been previously described (25). Briefly, in buffer (with 1.0% bovine serum albumin), ^{125}I-labeled insulin (Amersham) was incubated at 4°C overnight with 5 μl of serum (at a 1:25 dilution) with and without cold human insulin. After incubation, the assays followed the standard protocol for precipitation with 50% protein A/8% protein G-Sepharose (Pharmacia), washing, and measurement of radioactivity in counts per minute (25). An index was determined based on the difference in counts per minute between wells without and with cold insulin, with a positivity criterion of 0.010, which was the 99th percentile of 106 normal controls. The interassay coefficient of variation was 16% (n = 6) at medium-low positive levels, and the intra-assay coefficient of variation was 12% (n = 10).

IAA assay. IAAs were determined with a fluid phase radioassay using polyethylene glycol precipitation in the DPT-1 IAA Core Laboratory (Boston, MA) (26) The cut-point was 39 nU/ml (mean ± 2 SD), which was the 99th percentile of 151 normal controls. The interassay coefficient of variation was 10.3% at low positive values. In the IDS Combinatorial Workshop, the assay had a specificity of 91% and sensitivity of 49%.

IVGTT. The IVGTT was performed according to the ICARUS (Insulin Carotids US Scandinavica) protocol (27). The 1 + 3 min insulin was used as the index of FPIR. Insulin levels were determined in the DPT-1 β-Cell Function Core Laboratory (Seattle, WA) (6). Eligibility for the parenteral insulin trial of DPT-1 required an FPIR <60 μU/ml for ages <8 years, <100 μU/ml for ages ≥8 years, and <60 μU/ml for parents of diabetic patients.

HLA-DQ typing. HLA-DQ typing was determined as part of the DPT-1 study using sequence-specific oligonucleotide probes in the DPT-1 HLA Core Laboratory (Denver, CO) (28). Individuals with HLA-DQA1*0102, DQB1*0602 were excluded (noneligible) from the DPT-1 trial.

Statistical analysis. Categorical variables were analyzed using χ^2 tests or Fisher's exact tests, depending on cell size. Continuous variables were compared using Student's t test or Wilcoxon's rank-sum test, depending on the distribution of the variable of interest. An analysis of variance was used for comparisons of a continuous variable across more than two levels. Statistical analyses were performed using SAS, True Epistat, and Prism Software.

RESULTS

Of 71,148 initial screening samples measured for GAAs, ICA512AAs, and cytoplasmic ICAs, 2,448 (3.44%) were ICA+ and 68,700 (96.56%) were ICA−. Among the 2,448 ICA+ relatives, 1,229 (50.2%, 1.73% of total relatives screened) were positive for GAAs and/or ICA512AAs (554 [22.6%] GAAs only, 104 [4.3%] ICA512AAs only, and 571 [23.3%] GAAs and ICA512AAs). Among ICA− relatives, 1,897 (2.8% of ICA−, 2.67% of total relatives screened) were positive for GAAs and/or ICA512AAs (1,582 GAAs only, 180 ICA512AAs only, and 135 GAAs and ICA512AAs). The prevalence of GAAs and ICA512AAs among ICA+ and ICA− relatives is summarized in Fig. 2*A*. Figure 2*B* displays ICA positivity among individuals with GAAs and/or ICA512AAs. In total, 39.3% (1,229 of 3,126) of the individuals positive for GAAs and/or ICA512AAs were ICA+, 25.9% (554 of 2,136) of individuals with GAAs alone were ICA+, 36.6% (104 of 284) of individuals with ICA512AAs alone were ICA+, and 80.9% (571 of 706) of individuals with both GAAs and ICA512AAs were ICA+. Table 1 summarizes positivity for each autoantibody relative to sex, ethnicity, age, and relationship to proband.

Two different constructs are often used for the determination of ICA512 (IA-2) autoantibodies in international workshops: ICA512bdc (amino acids 256–556:630–979) and ICA512ic (amino acids 605–979). A random subset (n = 2,151) of DPT sera were evaluated with radioassays using both constructs, As illustrated in Fig. 3, 14 of 2,151 (0.65%) samples assayed with the alternative splice variants ICA512bdc and ICA512ic gave discordant results. Each construct was positive for 7 of 2,151 samples, with the other construct negative, whereas 29 samples were positive for both constructs. Given the similar sensitivity, the ICA512bdc construct was used in the current study.

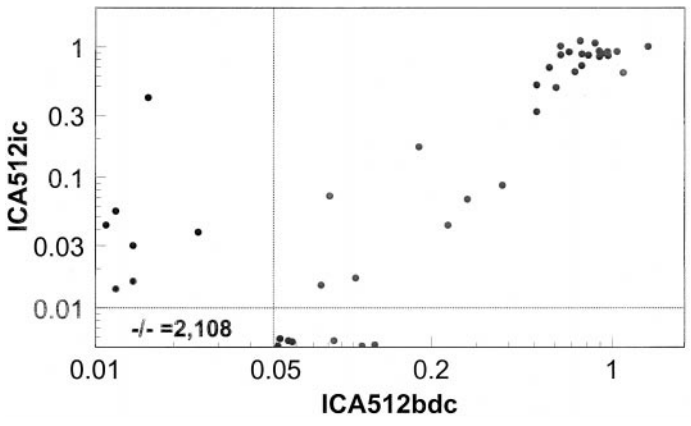

FIG. 3. Two different constructs of ICA512 (IA-2)—ICA512bdc (amino acids 256–556:630–979) and ICA512ic (complete intracellular domain of ICA512 molecule, amino acids 605–979)—were used in the measurement of autoantibodies. *N* = 2,151 DPT samples. Note that 2,108 were sera negative (−/−) with both constructs. *x*-Axis, ICA512bdc; *y*-Axis, ICA512ic.

To further analyze the association between cytoplasmic ICAs and GAAs or ICA512AAs, the levels of these autoantibodies were compared within different subgroups of relatives. The levels of GAAs of ICA+ relatives (n = 1,125, median 0.548, range 0.033–1.978) were significantly higher than the levels of ICA− relatives (n = 1,717, median 0.135, range 0.033–1.983; median-test, $P < 0.001$). The levels of ICA512AAs of ICA+ relatives (n = 675, median 0.685, range 0.072–1.867) were also significantly higher than the levels of ICA− relatives (n = 315, median 0.170, range 0.072–1.388; median-test, $P < 0.001$). The ICA titers of ICA+ relatives with GAA and/or ICA512AA positive (n = 1,229) and negative (n = 1,219) subgroups were compared. The median titers of ICA were 160 and 20 for the GAA- and/or ICA512AA-positive versus the GAA- and ICA512AA-negative group, respectively ($P < 0.001$, Wilcoxon's rank-sum test).

The mIAA assay is a methodology that has recently become available and allows more rapid analysis of IAAs. With ~6,400 screening samples analyzed for mIAAs, 1.6% of the samples with no autoantibodies (including those negative for ICAs, GAAs, and ICA512AAs) were mIAA+. There were 160 ICA+ sera negative for GAAs and ICA512AAs and only 3 of 160 (1.9%) mIAA+. In contrast, 23% (28 of 122) of the ICA+ sera with GAAs and/or ICA512AAs were mIAA+ ($P < 0.0001$).

Given the large number of relatives positive for cytoplasmic ICAs and negative for biochemically determined autoantibodies (1,219 of 2,448), we compared the results of IVGTTs among the relatives who had cytoplasmic ICAs only (n = 862), ICAs with GAAs only (n = 386), ICAs with ICA512AAs only (n = 67), and ICAs with both GAAs and ICA512AAs (n = 384) (Fig. 4). The mean of FPIR (mean ± SE) was 190.0 ± 5.4 for relatives with ICAs alone, 141.0 ± 6.4 for relatives with ICAs plus GAAs only, 117.4 ± 12.8 for relatives with ICAs plus ICA512AAs only, and 108.4 ± 4.0 for relatives with ICAs plus both GAAs and ICA512AAs ($P < 0.001$, F test). Using Tukey's method with an overall α of 0.05, examination of the multiple comparisons indicated that relatives with ICAs alone had significantly higher FPIRs than relatives with ICAs plus GAAs or ICA512AAs or relatives with ICAs plus both GAAs and ICA512AAs.

To be eligible for the parenteral insulin DPT-1 trial, a relative had to have FPIR <1st percentile of cut-points (for parents) or <10th percentile (for siblings and offspring). To be eligible for the oral insulin trial, a relative had to have FPIR above cut-points (>1st percentile for parents, >10th percentile for siblings/offspring) and be positive for anti-IAAs. For both trials, DQB1*0602 was an exclusion criteria and cytoplasmic ICAs had to be confirmed. Overall, 164 ICA+ relatives were found to have diabetes during staging and were thus considered to not be trial-eligible (131 of 164 were GAA+ and/or ICA512AA+). Among the relatives with GAAs and/or ICA512AAs on their initial screening samples who completed staging, 51.0% (442 of 866) have been found to date to be eligible for trial entry. In contrast, 11.9% (130 of 1,088) of ICA+ relatives negative for GAAs and ICA512AAs were found to be eligible for trial entry ($P < 0.001$). Of 572 relatives in Table 2 who were found to be eligible for trial entry (318 for parenteral trial and 254 for oral trial), a total of 77.3% (442 of 572) were GAA+ and/or ICA512AA+, 70.8% (405 of 572) were GAA+, 43.5% (249 of 572) were ICA512AA+, and 37.0% (212 of 572) were both GAA+ and ICA512AA+. Of 1,382 relatives who completed staging and were found to not be eligible for trial entry, 286 were confirmed ICA− from later tests and only 8.7% (25 of 286) of them were GAA+ and/or ICA512AA+ compared with 50.2% (1,229 of 2,448) of the total ICA+ group. In the ICA+ group, 163 had HLA-DQB1*0602 and were thus not trial eligible. Of this group, 32 of 163 (19.6%) were GAA and/or ICA512AA positive.

DISCUSSION

Large-scale trials for the prevention of type 1A diabetes, such as the DPT-1 trial, were designed before the development and detailed characterization of quantitative assays for autoantibodies reacting with ICA512 (IA-2) and GAD65. Thus, the entry criteria for the DPT-1 trial are based on the positivities of cytoplasmic ICAs and IAAs, FPIR, and HLA typing, but are not related to the presence

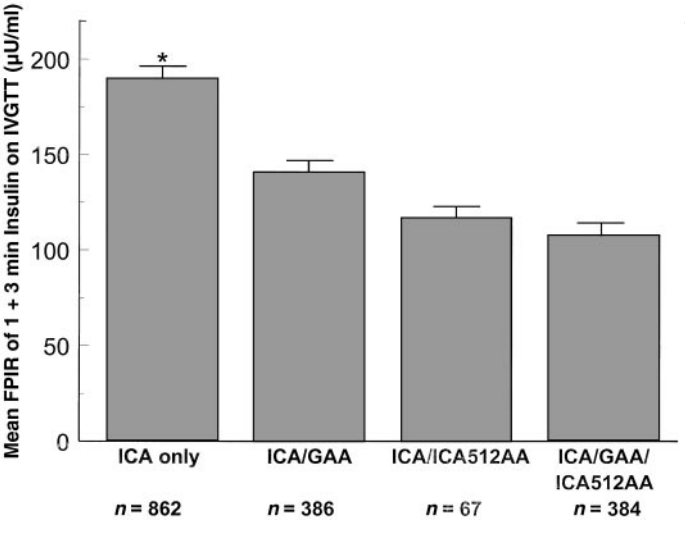

FIG. 4. The mean values of FPIR among ICA+ relatives divided by GAA and ICA512AA positivities (both negative, one positive only, or both positive). *FPIR of ICA only significantly higher than in other groups ($P < 0.001$).

TABLE 2
Subjects who completed staging for DPT-1 trial entry

	GAA+ and/or ICA512AA+	GAA− and ICA512AA−	Total
Trial-eligible			
Parenteral	243 (76.4)	75 (23.6)	318
Oral	199 (78.3)	55 (21.7)	254
Excluded from trial			
ICA not confirmed	25 (8.7)	261 (91.3)	286
Diabetes confirmed during trial	131 (79.9)	33 (20.1)	164
HLA-DQB*0602	32 (19.6)	131 (90.4)	163
Completed total staging	866	1,088	1,954
Percent eligible after staging	442/866 (51.0)	130/1,088 (11.9)	572/1,954 (29.3)

Data are *n* (%) or *n*.

or absence of GAD65 and ICA512 autoantibodies. It is thus possible to analyze the correlation between the presence of the latter two autoantibodies among ICA+ relatives and eligibility for entry into the DPT-1 trial.

In our study, 50% (1,229 of 2,448) of cytoplasmic ICA+ relatives were positive for autoantibodies reacting with either GAD65 or ICA512 (IA-2) in comparison to 2.8% (1,897 of 68,700) of ICA− relatives. Thus, as expected, cytoplasmic ICA+ relatives were enriched for relatives with positive GAD65 or ICA512 autoantibodies. Nevertheless, only 39.3% (1,229 of 3,126) of individuals who were positive for GAAs or ICA512AAs were cytoplasmic ICA+. Individuals with ICA512AAs were more often ICA+ (68.2%, 675 of 990) compared with individuals with GAAs (39.6%, 1,125 of 2,842), and approximately half of ICA+ relatives were negative for both GAAs and ICA512AAs. With 6,420 screening samples analyzed for mIAAs, only 1.9% (3 of 160) of ICA+ individuals negative for GAAs and ICA512AAs were mIAA+ versus 23.0% (28 of 122) of ICA+ individuals positive for GAAs and/or ICA512AAs.

A number of studies have indicated that the presence of cytoplasmic ICAs in the absence of both GAAs and ICA512AAs is associated with a very small risk of progression to diabetes (2,19). Both a high titer of ICAs and low FPIR are associated with a high risk of developing type 1 diabetes among first-degree relatives (2,3,29) The present data also indicate that the levels of cytoplasmic ICAs in sera positive for GAAs and ICA512AAs are significantly higher ($P < 0.001$) than when GAAs and ICA512AAs are not present. FPIR was significantly different among the ICA+ groups, according to GAA and ICA512AA positivity. The mean level of FPIR of relatives with ICA+ who were also negative for GAAs and ICA512AAs was ~200 μU/ml, significantly greater than the level for ICA+ relatives also positive for GAAs and/or ICA512AAs.

Staging for DPT-1 was designed to identify ICA+ relatives with a relatively high risk of progression to diabetes. Thus, if GAA and ICA512AA positivity on the initial screening sample identifies higher-risk relatives and the staging criteria identify a high-risk population, GAA and ICA512AA positivity should correlate with trial eligibility. Among the relatives who were found to be eligible for the trial, 442 of 572 (77.3%) relatives (76.4% for parenteral trial and 78.3% for oral trial, respectively) were positive for GAAs and/or ICA512AAs on their first screening sample. In contrast, only 50.2% of all ICA+ relatives were positive for GAAs and/or ICA512AAs, and 51.0% (442 of 866) of ICA+ relatives with GAA+ or ICA512AA+ relatives were eligible. Only 11.9% (130 of 1,088) of cytoplasmic ICA+ relatives negative for GAAs and ICA512AAs were found to be eligible for trial entry. The positive predictive value for trial eligibility for ICA+ relatives with GAAs or ICA512AAs was 51.0%. The negative predictive value for relatives not being eligible for trial participation when they were ICA+ but GAA- and ICA512AA-negative was 88.1%. These data suggest that the biochemical antibodies expressed by ICA+ relatives in the initial screening sample can be used to a large extent to predict the results of the staging process. It is likely that if future trials for the prevention of type 1A diabetes use ICA testing, it may be more efficient to stage for trial entry only those relatives positive for GAA and/or ICA512AA with eligibility. It should also be realized that the staging criteria includes one cutoff for variables set at the 10th percentile of control populations (e.g., IVGTT response of children), thus a proportion of individuals would be found eligible with this criterion.

In all, 60.7% (1,897 of 3,126) of relatives with GAAs and/or ICA512AAs were cytoplasmic ICA−. In this group, the levels of both GAAs and ICA512AAs were significantly lower than the levels of the ICA+ group. In as much as the presence of cytoplasmic ICAs initiates the staging process for DPT-1, the percentage of such relatives having low FPIR, anti-IAAs, or the HLA allele DQB1*0602 is currently unknown. If one were to consider designing trials for the prevention of type 1A diabetes in the absence of determination of cytoplasmic ICAs (i.e., by GAA and ICA512AA screening), the prognosis of ICA− GAA+ and/or ICA512AA+ relatives would be an important consideration. Studies of this group (as an ancillary study of the DPT-1) are underway.

With our current information, we believe that the design of future trials identifying relatives with a risk of diabetes similar to the oral or parenteral DPT trials might consider determination of GAAs and ICA512AAs followed by staging that might include determination of cytoplasmic ICAs. Further follow-up and analysis of the DPT-1 cohort should aid in the design of such trials; in particular, the analysis of ICA− relatives expressing biochemical autoantibodies will be required to determine whether the trials are sufficient to identify high-risk individuals without cytoplasmic ICA analysis.

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REFERENCES

1. Bingley PJ, Bonifacio E, Gale EAM: Can we really predict IDDM? *Diabetes* 42:213–220, 1993
2. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45:926–933, 1996
3. Bonifacio E, Bingley PJ, Shattock M, Dean BM, Dunger D, Gale EA, Bottazzo GF: Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes. *Lancet* 335:147–149, 1990
4. Riley WJ, Maclaren NK, Krischer J, Spillar RP, Silverstein JH, Schatz DA, Schwartz S, Malone J, Shah S, Vadheim C, Rotter JL: A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *N Engl J Med* 323:1167–1172, 1990
5. Maclaren N, Lan M, Coutant R, Schatz D, Silverstein J, Muir A, Clare-Salzer M, She JX, Malone J, Crockett S, Schwartz S, Quattrin T, DeSilva M, Vander VP, Notkins A, Krischer J: Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2 and IA-2beta predict immune-mediated (type 1) diabetes in relatives. *J Autoimmun* 12:279–287, 1999
6. Greenbaum CJ, Sears KL, Kahn SE, Palmer JP: Relationship of β-cell function and autoantibodies to progression and nonprogression of subclinical type 1 diabetes. *Diabetes* 48:170–175, 1999
7. Schatz D, Krischer J, Horne G, Riley W, Spillar R, Silverstein J, Winter W, Muir A, Derovanesian D, Shah S, Malone J, Maclaren N: Islet cell antibodies predict insulin-dependent diabetes in United States school age children as powerfully as in unaffected relatives. *J Clin Invest* 93:2403–2407, 1994
8. Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS, Eisenbarth GS: Life table analysis of progression to diabetes of anti-insulin autoantibody-positive relatives of individuals with type I diabetes. *Diabetes* 38:1320–1325, 1989
9. Ziegler A-G, Hummel M, Schenker M, Bonifacio E: Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes: the 2-year analysis of the German BABYDIAB study. *Diabetes* 48:460–468, 1999
10. Colman PG, McNair P, Margetts H, Schmidli RS, Werther GA, Alford FP, Ward GM, Tait BD, Honeyman MC, Harrison LC: The Melbourne Pre-Diabetes Study: prediction of type 1 diabetes mellitus using antibody and metabolic testing. *Med J Aust* 169:81–84, 1998
11. Bingley PJ, Bonifacio E, Williams AJK, Genovese S, Bottazzo GF, Gale EAM: Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes* 46:1701–1710, 1997
12. Gorus FK, Goubert P, Semakula C, Vandewalle CL, Schepper JD, Scheen A, Christie MR, Pipeleers DG, the Belgian Diabetes Registry: IA-2-autoantibodies complement GAD_{65}-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. *Diabetologia* 40:95–99, 1997
13. Marshall MO, Hoyer PE, Petersen JS, Hejnaes KR, Genovese S, Dyrberg T, Bottazzo GF: Contribution of glutamate decarboxylase antibodies to the reactivity of islet cell cytoplasmic antibodies. *J Autoimmun* 7:497–508, 1994
14. Falorni A, Örtqvist E, Persson B, Lernmark Å: Radioimmunoassays for glutamic acid decarboxylase (GAD65) and GAD65 autoantibodies using ^{35}S or ^{3}H recombinant human ligands. *J Immunol Methods* 186:89–99, 1995
15. Gianani R, Rabin DU, Verge CF, Yu L, Babu S, Pietropaolo M, Eisenbarth GS: ICA512 autoantibody radioassay. *Diabetes* 44:1340–1344, 1995
16. Verge CF, Stenger D, Bonifacio E, Colman PG, Pilcher C, Bingley PJ, Eisenbarth GS, participating laboratories: Combined use of autoantibodies (IA-2ab, GADab, IAA, ICA) in type 1 diabetes: Combinatorial Islet Autoantibody Workshop. *Diabetes* 47:1857–1866, 1998
17. Schmidli RS, Colman PG, Bonifacio E, participating laboratories: Disease sensitivity and specificity of 52 assays for glutamic acid decarboxylase antibodies: the Second International GADAb Workshop. *Diabetes* 44:636–640, 1995
18. Greenbaum C, Palmer JP, Kuglin B, Kolb H, participating laboratories: Insulin autoantibodies measured by radioimmunoassay methodology are more related to insulin-dependent diabetes mellitus than those measured by enzyme-linked immunosorbent assay: results of the Fourth International Workshop on the Standardization of Insulin Autoantibody Measurement. *J Clin Endocrinol Metab* 74:1040–1044, 1992
19. Kulmala P, Savola K, Petersen JS, Vähäsalo P, Karjalainen J, Löppönen T, Dyrberg T, Åkerblom HK, Knip M, Childhood Diabetes in Finland Study Group: Prediction of insulin-dependent diabetes mellitus in siblings of children with diabetes: a population-based study. *J Clin Invest* 101:327–336, 1998
20. Pietropaolo M, Peakman M, Pietropaolo SL, Zanone MM, Foley TP Jr, Becker DJ, Trucco M: Combined analysis of GAD65 and ICA512 (IA-2) autoantibodies in organ and non-organ-specific autoimmune diseases confers high specificity for insulin-dependent diabetes mellitus. *J Autoimmun* 11:1–10, 1998
21. Wiest-Ladenburger U, Hartmann R, Hartmann U, Berling K, Böhm BO, Richter W: Combined analysis and single-step detection of GAD65 and IA2 autoantibodies in IDDM can replace the histochemical islet cell antibody test. *Diabetes* 46:565–571, 1997
22. Aanstoot H-J, Sigurdsson E, Jaffe M, Shi Y, Christgau S, Grobbee D, Bruining GJ, Molenaar JL, Hofman A, Baekkeskov S: Value of antibodies to GAD_{65} combined with islet cell cytoplasmic antibodies for predicting IDDM in a childhood population. *Diabetologia* 37:917–924, 1994
23. Bingley PJ, Christie MR, Bonifacio E, Bonfanti R, Shattock M, Fonte M-T, Bottazzo G-F, Gale EAM: Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes* 43:1304–1310, 1994
24. Yu L, Rewers M, Gianani R, Kawasaki E, Zhang Y, Verge C, Chase P, Klingensmith G, Erlich H, Norris J, Eisenbarth GS: Anti-islet autoantibodies develop sequentially rather than simultaneously. *J Clin Endocrinol Metab* 81:4264–4267, 1996
25. Yu L, Robles DT, Abiru N, Kaur P, Rewers M, Kelemen K, Eisenbarth GS: Early expression of anti-insulin autoantibodies of man and the NOD mouse: evidence for early determination of subsequent diabetes. *Proc Natl Acad Sci U S A* 97:1701–1706, 2000
26. Vardi P, Dib SA, Tuttleman M, Connelly JE, Grinbergs M, Rabizadeh A, Riley WJ, Maclaren NK, Eisenbarth GS, Soeldner JS: Competitive insulin autoantibody assay: prospective evaluation of subjects at high risk for development of type 1 diabetes mellitus. *Diabetes* 36:1286–1291, 1987
27. Bingley PJ, Colman P, Eisenbarth GS, Jackson RA, McCulloch DK, Riley WJ, Gale EAM: Standardization of IVGTT to predict IDDM. *Diabetes Care* 15:1313–1316, 1992
28. Pugliese A, Kawasaki E, Zeller M, Yu L, Babu S, Solimena M, Moraes CT, Pietropaolo M, Friday RP, Trucco M, Ricordi C, Allen M, Noble JA, Erlich HA, Eisenbarth GS: Sequence analysis of the diabetes-protective human leukocyte antigen-DQB1*0602 allele in unaffected, islet cell antibody-positive first degree relatives and in rare patients with type 1 diabetes. *J Clin Endocrinol Metab* 84:1722–1728, 1999
29. Robert JJ, Deschamps I, Chevenne D, Roger M, Mogenet A, Boitard C: Relationship between first-phase insulin secretion and age: HLA, islet cell antibody status, and development of type I diabetes in 220 juvenile first-degree relatives of diabetic patients. *Diabetes Care* 14:718–723, 1991