Pancreatic Alpha Cell Autoantibodies and Glucagon Response to Arginine

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SUMMARY

The frequency and significance of cytoplasmic pancreatic alpha cell autoantibodies (ACA) were investigated in 2102 healthy controls, 879 patients with insulin-dependent diabetes mellitus (IDDM) who were negative for islet cell autoantibodies (ICA), and 1567 relatives of IDDM patients. ACA were found in approximately 1 in 200 people of all ages and were not significantly associated with IDDM, the IDDM-associated HLA phenotypes DR3 and DR4, or thyrogastric or adrenal autoantibodies. Of 11 ACA-positive patients studied by arginine stimulation tests, none had frank glucagon deficiency. Thus, ACA do not appear to be associated with defective alpha cell function or with IDDM.

DIABETES *33*:435–437, May 1984.

A variety of autoantibodies to the pancreatic islets have been described. Cytoplasmic islet cell autoantibodies (ICA), first noted in 1974,[1] react with all cells of the pancreatic islet. At the time of diagnosis, 70–80% of children with insulin-dependent diabetes mellitus (IDDM) have ICA.[2] In 1976,[3] Bottazzo and Lendrum subsequently described autoantibodies that reacted solely with the cytoplasm of the glucagon-secreting (alpha) cells of the islet (pancreatic alpha cell autoantibodies, ACA, Figure 1), or solely with the cytoplasm of the somatostatin-secreting (delta) cells.

Our study was designed to evaluate the frequency of ACA in various population groups, to learn the relationship of ACA to other autoantibodies and HLA phenotype, and to assess the glucagon status of those individuals found to possess ACA.

Autoantibodies against endocrine glands are characteristically found to precede or accompany the clinical onset of the respective associated clinical diseases; however, autoantibodies may be present without apparent consequence.[4] This may be due to limited duration of follow-up, as ICA, thyroid microsomal (TMA), and gastric parietal cell (PCA) autoantibodies may be present for years before the development of their associated diseases.[1,5–9] From our previous studies of asymptomatic patients with organ-specific autoantibodies,[4,7–9] we anticipated that one-third to one-half of all ACA-positive individuals would display a derangement in glucagon secretion.

MATERIALS AND METHODS

All studies were approved by the Health Center Institutional Review Board. ACA were sought in sera from 2102 healthy controls, 879 patients with IDDM who were negative for ICA, and 1567 relatives of IDDM patients (approximately 80% were first-degree relatives). ACA and ICA were determined in undiluted sera by indirect immunofluorescence methods,[2] using unfixed blood group O, 4-μm cryocut sections of human pancreas. Goat antihuman IgG fluorescence isothiocyanate conjugate was used. Autoantibody localization to the alpha cell was confirmed by four-layer immunofluorescence methods employing specific antipancreatic glucagon antisera (30K).[3] To identify associated autoimmune phenomena, TMA, PCA, and adrenal (AA) autoantibodies were sought using indirect immunofluorescence methods as previously described.[2] Undiluted sera were used for AA determinations, while TMA and PCA were identified using 1:4 dilutions of sera. Four patients were available for longitudinal study of the persistence of ACA. Adequate volumes of sera were available from six patients for determination of ACA titer. Nine subjects with ACA were HLA-typed by standard microcytotoxicity techniques.[10]

Glucagon responses to arginine were studied in a representative group of patients with ACA (N = 11). After an overnight fast, 10% arginine-HCl solution was infused in a dose of 0.5 g/kg (maximum dose 30 g) over 30 or 40 min. Blood samples for glucose, glucagon, and insulin were drawn every 5–10 min from 10 min before to 20 min after

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Received for publication 6 October 1983.

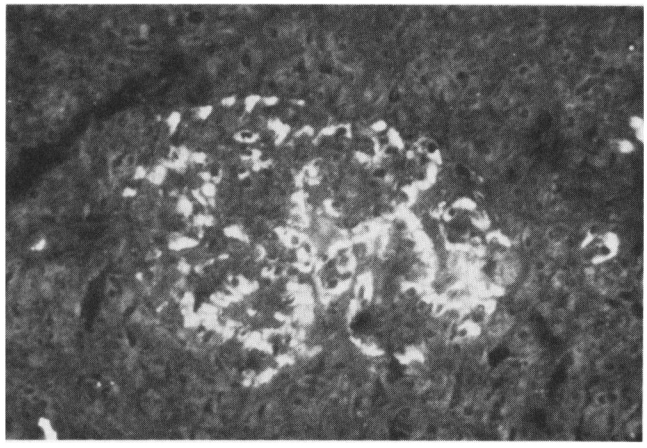

FIGURE 1. Photomicrograph of a section of human pancreas showing IgG ACA by indirect immunofluorescence.

the initiation of the arginine infusion, and every 10–20 min thereafter, until termination of the test at 60–90 min after initiation of the arginine infusion. Plasma pancreatic glucagon and insulin (Serono) concentrations were determined by radioimmunoassay.

RESULTS

Nineteen sera of the 4547 sera examined for ACA were positive. The frequency of ACA in relatives of patients with IDDM was slightly, but not significantly, greater than that in the general population (Table 1). It is of interest that no ICA-negative patients with IDDM were found to have ACA, including patients who were initially positive for ICA and lost this antibody with time. In addition to the above patients, two other individuals with ACA were identified during evaluation of their sera for ICA. One patient had type I autoimmune, polyglandular syndrome consisting of mucocutaneous candidiasis, hypoparathyroidism, adrenal insufficiency, and primary hypogonadism;[11] the other patient had non-insulin-dependent diabetes mellitus (NIDDM), and chronic renal insufficiency. One patient with IDDM was found coincidentally to have autoantibodies reacting solely with the somatostatin-secreting delta cells.

Additional autoantibodies were sought in all 21 ACA-positive sera (Table 2). Not unexpectedly, the patient with type I autoimmune, polyglandular syndrome had AA and PCA.[11] The patient with NIDDM was negative for all autoantibodies tested.

Thyrogastric autoantibodies (TMA and/or PCA) were not significantly increased in frequency in ACA-positive patients from the general population over their respective control population (0% versus general population, 16%, P = 0.11). Likewise, the frequency of thyrogastric autoantibodies in ACA-positive relatives of IDDM patients was not significantly higher than in relatives of IDDM patients in general (45% versus 26%, P = 0.18).

HLA-DR3 and/or -DR4 was found in 3 of 4 (75%) white patients with ACA from the general population; however, this was not significantly different from the frequency of DR3 and/or DR4 in the general white population (46% [67/145], P = 0.22). Further, in 403 relatives of IDDM patients that were HLA-typed, 82% had DR3 and/or DR4, a frequency significantly higher (P < 0.05) than the 62% frequency (5/8) of DR3 and/or DR4 in ACA-positive relatives of IDDM patients.

Of the four patients longitudinally studied over a mean of 2 yr, only one sera became ACA-negative. Initially, this patient's sera was only weakly positive for ACA. Of the sera titered, 3 were positive at 1:1, and one each was positive at 1:4, 1:8, and 1:16.

The group studied by arginine infusion tests included the patient with NIDDM and the patient with type I autoimmune, polyglandular syndrome. Overall, glucagon concentrations rose rapidly with arginine infusion, peaked on an average at 40 min, and fell toward baseline after termination of the infusion. The mean (± SEM) fasting glucagon concentration (excluding the NIDDM patient) was 141 ± 19 pg/ml. The patient with NIDDM and the patient with type I autoimmune, polyglandular syndrome both had baseline hyperglucagonemia (390 pg/ml and 307 pg/ml, respectively). After arginine stimulation, the NIDDM patient was markedly hyperresponsive (peak glucagon value, 1640 pg/ml) as compared with the remaining 10 patients (peak glucagon value, 447 ± 53 pg/ml, mean ± SEM). The maximal change of glucagon over baseline in these latter 10 patients was 307 ± 39 pg/ml (mean ± SEM).

One patient had a marginal increase in glucagon concentration over baseline of only 62 pg/ml and on repeat testing 126 pg/ml. This 37-yr-old white female tolerated a 30-h fast without difficulty and maintained a normal blood glucose and appropriate concentrations of insulin, glucagon, other anti-insulin hormones, and plasma metabolic intermediates throughout (data available upon request).

DISCUSSION

In our general U.S. population, ACA frequency (0.4%) was similar to the frequency of ACA reported from Britain (0.6%).[3] ACA were not significantly more common in ICA-negative IDDM patients or in relatives of IDDM patients. Therefore, ACA do not appear to be associated with IDDM. This is in marked contrast to the association of ICA with IDDM. Over-

TABLE 1
Demographics of population studied and ACA frequencies

	N	Age (yr, mean ± SD)	B/W ratio	Sex ratio (M/F)	Thyrogastric autoantibody-positive	Adrenal autoantibody-positive	HLA-DR3- and/or -DR4-positive	ACA positive	
								N	%
General population	2102	42 ± 22	0.40	0.77	16%	0.7%	46% (67/145)	8	0.4*
IDDM patients (ICA-negative)	879	18 ± 12	0.23	1.1	26%	1.5%	96% (232/242)	0	0
Relatives of IDDM patients	1567	33 ± 15	0.10	0.65	26%	1.8%	82% (330/403)	11	0.7*

B, Black; W, White.
*P = 0.2.

TABLE 2
Characterization of ACA-positive individuals

	N	Age (yr, mean ± SD)	B/W ratio	Sex ratio (M/F)	Thyrogastric autoantibodies	Adrenal autoantibodies	HLA-DR3- and/or -DR4-positive
ACA-positive patients from general population	8	36 ± 14	0.33	1.0	0%	0%	75% (3/4)
P (cf. general population)					NS	NS	NS
ACA-positive relatives of IDDM patients	11	37 ± 18	0.22	1.2	16%	9%	62% (5/8)
P (cf. relatives of IDDM patients)					NS	NS	<0.05

The overall age range of ACA-positive patients was 6–79 yr.
This table excludes the patients with NIDDM and type I autoimmune, polyglandular syndrome.

all, Neufeld et al.[2] found 36% of IDDM patients to be ICA-positive, and summarizing several reports, Nerup and Lernmark[12] found ICA in 4% of first-degree relatives. In our own recent studies, we found that 0.4% of the general population were ICA-positive while ICA were present in 2.7% of relatives of IDDM patients.

As a group, patients with ACA frequently had other autoantibodies (24%, present study; 31%, Bottazzo and Lendrum[3]). However, these high frequencies were a reflection of the patient groups studied, and when matched to suitable controls, ACA were not significantly associated with thyrogastric or adrenal autoantibodies or indeed with HLA-DR3 and/or -DR4.

No ACA-positive patient had frank glucagon deficiency. Furthermore, the patient with the lowest glucagon responses to arginine tolerated a 30-h fast without difficulty. Although a study of glucagon responses to arginine in normal (ACA-negative) controls was not performed, ACA-positive patients, excluding the patients with NIDDM and type I autoimmune, polyglandular syndrome, had appropriate fasting and peak glucagon concentrations when compared with literature controls.[13] With respect to the NIDDM patient, inappropriate hyperglucagonemia is characteristic of diabetes and uremia.[13–15] Although pharmacologic doses of glucocorticoids[16] can produce basal and stimulated hyperglucagonemia, the patient with type I autoimmune, polyglandular syndrome was on a physiologic replacement dose of cortisol and the etiology of her basal hyperglucagonemia was unclear.

ACA-positive individuals will be identified as the number of sera screened for ICA increase. From this study, it would appear that such ACA-positive people are not at risk for hypoglucagonemia and subsequent hypoglycemia. Only two ACA-positive patients had previously been studied, and both were found to have normal glucagon responses to arginine infusion.[17]

In summary, ACA are very unusual autoantibodies, as there was no apparent pathologic correlate to their presence. Furthermore, no clear-cut associations between ACA and IDDM, autoantibodies (TMA, PCA, and AA), or particular HLA phenotypes were established.

ACKNOWLEDGMENTS

This study was supported by contracts with the Department of Health and Rehabilitative Services of the State of Florida for a Regional Diabetes Program for Children and a Diabetes Research, Education and Treatment Center; the Division of Pediatric Endocrinology; and the Department of Pathology. Dr. Winter is supported by NIH Fellowship Award 1 F32 AM07052-01.

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