Insulin Autoantibodies: Evidence of Autoimmune Disease Among a Group of Puerto Rican Children With Newly Diagnosed Type 1 Diabetes Mellitus

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Type 1 diabetes is a chronic disease caused by a cell-specific destruction of the insulin producing cells of the pancreas. Although Puerto Rico has the highest incidence of type 1 diabetes among Latin American countries, there is scanty data on the presence of antibodies against insulin producing cells. To this end, 20 children (8 males, 12 females), ages 1 - 15 years, admitted to the University Pediatric Hospital with type 1 diabetes de novo between November 2000 and April 2001 were prospectively studied to determine the presence of serum antibodies against Islet cells (ICA), glutamic acid decarboxylase (GAD-65) and insulin autoantibodies (IAA). IAA was found to be present in 45% of the subjects with 85% of positive rate in subjects under age 5. GAD-65 was present in 66% and ICA was present in 23% of the subjects. We found evidence of autoimmunity against islet cell surface and intracellular components among a cohort of Puerto Rican children with newly diagnosed type 1 diabetes. These findings compared favorably with reports from other ethnicities.

Key Words: Diabetes mellitus, Puerto Rico, Insulin antibodies, Children.

Type 1 diabetes is a disease caused by destruction of the insulin producing ß cell of the pancreas. In the vast majority of patients this is caused by a T cell mediated autoimmune process. Evidence supporting this, is the association of certain human leukocytes antigens (HLA) with type 1 diabetes and the presence of antibodies against the surface or the cytoplasmic components of the islet cell in the serum patients at the time of diagnosis. The most extensively studied of these are the antibodies against the insulin producing cells within the islet of Langerhans (ICA). Other antibodies against the surface and the cytoplasm of the ß cell such as those against glutamic acid decarboxilase (GAD-65) and antibodies against insulin (IAA) may also be present at the time of diagnosis. (1) The combined presence of these genetic markers and one of more of the aforementioned autoantibodies in the serum of patients during and before the diagnosis of type 1 diabetes, has been instrumental in predicting with a high degree of certainty those individuals at increased risk for developing diabetes.

Puerto Rico has the highest incidence of type 1 diabetes in children under 15 years of age among Latin American countries with the same ethnic background: 18/100,000 (2). There is scarce data on the presence of antibodies against the surface and the cytoplasmic components of the islet cell at the time of diagnosis in Puerto Rican children. The goal of this study was to detect the presence of these antibodies ICA, GAD 65, IAA in a sample of Puerto Rican children at the time of diagnosis.

Methods

Twenty patients, 8 males and 12 females, with newly diagnosis type 1 diabetes mellitus (*de novo)*, admitted to the University Pediatric Hospital from November 2000 through April 2001, ages ranged from 1 to 15 years, were studied.

A blood sample was taken within the first seventy-two hours after admission to be processed for GAD-65 and IAA antibodies. A second blood sample was also taken for ICA's. All blood samples were processed at the Laboratory Corporation of America in Burlington, North Carolina, a commercial reference laboratory. The islet cell antibodies were measured with a Scimedx Corporation Immunoflou-rescence Kit that measures the

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immunoflourescence of primate pancreas sections islet cells when exposed to patient's serum. Fluorescence is reported as dilutions of the test serum compared to known standards.

The IAA and GAD-65 antibodies were measured with a Kronus Corporation Radio Immunoassay Kit. Both assays have an optimal sensitivity and specificity. They are reported in Kronus units. The GAD-65 antibody assay has a sensitivity of >1.0 U/ml, with a very low inter-assay and intra-assay coefficient of variation. The IAA assay also has a sensitivity of > 1.0 U/ml with a very low inter-assay and intra-assay coefficient of variation.

Results

The population studied was all newly diagnosed children with type 1 diabetes mellitus who were referred to the University Pediatric Hospital for management and education. They came predominately from the northern part of the island, our referral area.

Table 1. Distribution by age, sex, and autoimmune profile in the study population

Pt. No.	Age (yrs.)	Sex	IAA[1]	GAD-65[2]	ICA[3]
1	13	M	<1.0	<1.0	1:2
2	2 ½	M	2.6	<1.0	Neg
3	6	M	<1.0	ND[4]	Neg
4	3	F	6.0	18.2	ND
5	10	F	2.1	79.4	1:2
6	12	F	<1.0	26.2	Neg
7	15	M	19.7	1.7	Neg
8	8	M	<1.0	1.0	ND
9	11	F	<1.0	41.3	Neg
10	3 ½	M	2.5	<1.0	ND
11	6	F	3.0	ND	Neg
12	1 ½	F	71.8	ND	ND
13	4	F	<1.0	3.0	1:2
14	8	F	<1.0	3.4	ND
15	12	F	<1.0	38.3	Neg
16	7	F	<1.0	1.2	Neg
17	1	M	10.8	35.2	Neg
18	7	F	30.1	20.1	ND
19	7	M	<1	<1	ND
20	10	F	<1.0	52	Neg

[1] Normal insulin auto antibody (IAA): 0.0 – 0.9 U/ml
[2] Normal glutamic acid decarboxilase (GAD) value: 0.0 – 1.5 U/ml
[3] Normal islet cells antibody (ICA) value: negative if <1:1
[4] ND = not done

Table 2. Distribution by age, sex, and autoimmune profile of a subgroup of the study cohort: subjects under 5 years of age.

Pt. No.	Age	Sex	IAA	GAD-65	ICA
2	2 ½	M	2.6	<1.0	Neg.
4	3	F	6.0	18.2	N.D.
10	3 ½	M	2.5	<1.0	N.D.
12	1 ½	F	71.8	N.D.	N.D.
13	4	F	<1.0	3.0	1:2
17	1	M	10.8	35.2	Neg.

The age distribution of these children ranged between 1 and 15 years of age (Table 1). A sub-cohort of 6 children under 5 years of age was analyzed separately from the rest of the children (Table 2). Twelve of the 20 children were males and 8 were females.

IAA was found to be present in 9 out of the 20 patients for a 45 % positive rate. Of the 6 children under age 5, five had a positive IAA for a 83% positive rate. GAD-65 was run in 17 of the 20 patients; thirteen were found to be positive, for a positive rate of 66%. Three of the children under 5 had a positive GAD-65 for a 50% rate. ICA was performed in only 13 of the 20 patients with only 3 patients having more than 1:2 anti islet cell antibodies for 23% positive rate.

Discussion

Type 1 diabetes mellitus is recognized as a worldwide health problem affecting approximately 150 x 10^6 people. It is felt to be one of the leading causes of serious and chronic diseases in children. The incidence of type 1 diabetes varies with its geographical region, being higher in northern European countries, Finland and Sweden where the incidence is 35/100,000 and 25/100,000 children respectively, and lower towards the warmer climates, Africa and Asia. There is one noticeable exception in the island of Sardinia where it is higher than expected based on its geographical location, an incidence of 32/100,000 children (1).

In Puerto Rico, type 1 diabetes has become an important health problem. In 1998, our Diabetic Registry showed an incidence of 18/100,000 children less than 15 years of age per year (2). This is the highest incidence in the Caribbean and in other Latin American countries with the same ethnic background, that is: Cuba 4/100,000; Mexico 1.3/100/000; Colombia 3.8/100,000; Argentina, approximately 7/100,000 (2,3) and the United States Virgin Islands 7.5/100,000 (4). The incidence was also higher than Hispanics living in Chicago and Puerto Ricans living in Philadelphia. (5,6)

There is a common agreement on the destruction of the

pancreatic beta cell as the final event leading to type 1 diabetes. Although the etiology might be multifactorial, there is mounting evidence that it is caused by a T cell mediated autoimmune process: patients dying from other causes have been found to have a lymphocytic infiltration of the islets of Langerhans and it has a close association with other autoimmune diseases such as Hashimoto's thyroiditis, Addison's disease etc. Antibodies to cytoplasmic components and to cell surface components of the beta cell such as ICA, GAD-65, and IAA have been found in the sera of patient's years before and at the time of diagnosis. (1, 7, 8)

Antibodies to insulin are felt to be the only beta cell-specific antibodies. They are the hallmark of beta cell destruction. In our cohort we found a high percentage of serum IAA positivity, almost half of those tested (45%) among newly diagnosed children with type 1 diabetes mellitus. In fact, the positive rate doubled - 83% - when we looked at the ones younger than age 5. These results compared favorably with previous reports of the presence of antibodies against insulin in children never exposed to exogenous insulin. In the USA literature, IAA's were highest in children under 5 years of age (1) this has not been found to be true in other ethnicities. (9, 10) This suggests a rather aggressive autoimmune destruction of the beta cell in children diagnosed at infancy.

GAD-65 antibodies were also present in 3 out of 4 (76%) of the tested population, which parallels what is reported in the literature. It also may vary with age, being lower in the 0 to 5 group, and highest in the older adolescent and adult (9, 10).

The number of individuals with positive ICA was much less than expected i.e. 23% vs. the 80 % reported in the literature. The difference observed might well be related to ethnicity. White children in the USA and northern Europeans are 70% to 80% ICA positive at the time of diagnosis (8, 10). This high percentage of ICA positivity is not found in African-Americans (11), Japanese (9), or Mexican-Americans (personal communication).

In summary, we found evidence that type 1 diabetes mellitus in children is related to autoimmunity directed against islet cells. In particular, for our cohort IAA was found to be the most prevalent positive marker to ascertain for diabetes risk. It would be desirable to pursue further this finding and determine if this would still hold to a population-wide study in Puerto Rico.

Conclusion

Our findings support the hypothesis that type 1 diabetes is an autoimmune disease. We found evidence of positive immune markers in newly diagnosed children with type 1 diabetes. IAA seemed to be the most prevalent antibody with highest presence under age five.

Resumen

La diabetes tipo 1 es una enfermedad crónica causada por la destrucción de la célula productora de insulina del páncreas. Aunque Puerto Rico posee la mayor incidencia de diabetes tipo 1 en latinoamérica, existen escasos datos evaluando la presencia sérica de anticuerpos contra las células productoras de insulina en esta población. El propósito de este estudio fue determinar prospectivamente la presencia de anticuerpos séricos contra los islotes de Langerhans (ICA), la decarboxilasa de ácido glutámico (GAD-65) y autoanticuerpos contra la insulina (IAA) en 20 niños (8 varones y 12 hembras), entre 1 y 15 años de edad, hospitalizados con diabetes tipo 1 *de novo* desde noviembre de 2000 hasta abril de 2001, Se encontró la presencia de IAA en un 45% de los sujetos, donde 85% de estos estaban presente en niños menores de 5 años de edad. La GAD-65 se encontraba presente en un 66% de los pacientes y en 23% del grupo los ICA estaban presentes. En este estudio se encontró evidencia sérica de autoinmunidad en contra de la célula beta, tanto de superficie como en los componentes intracelulares, en este grupo de niños puertorriqueños recién diagnosticados con diabetes mellitus tipo 1. Estos hallazgos comparan favorablemente con lo reportado en otras etnias.

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