Islet Cell Antibodies and Histocompatibility Antigens (HLA) in Insulin-Dependent Diabetics and Their First-Degree Relatives

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SUMMARY

We have studied HLA A, B, D, and DR and cytoplasmic islet cell antibodies (CICA) in 282 insulin-dependent diabetics (IDD) and 806 of their nondiabetic first-degree relatives. Fifty percent of the patients were positive in the first year of disease, but only 19% among those with disease for longer than 5 yr. No associations were found between CICA and HLA types, age at diagnosis, or family history. In the relatives of IDD patients CICA was present in $^{11}/_{428}$ (2.6%) of the sibs of the probands, $^{18}/_{311}$ (5.8%) of the parents, and $^{4}/_{67}$ (6.0%) of the children. DIABETES *31*:585–588, July 1982.

A lively controversy has focused on the possible genetic heterogeneity of insulin-dependent diabetes mellitus (IDD). Several investigators have discussed the evidence suggesting that IDD is genetically heterogeneous,[1] and we have shared this view.[2] Others have favored genetic homogeneity, implying that clinical and other evidence for heterogeneity merely reflects variable expressivity of the IDD susceptibility genotype[3] through its interaction with the environment. Some of the most important lines of evidence for genetic heterogeneity in IDD stem from immunologic studies.[4] Over 50% of newly diagnosed IDD patients have cytoplasmic islet cell antibodies (CICA); this percentage decreases progressively and only 10–20% remain positive after 5 yr of disease.[5] Moreover, in patients with disease for 5 yr or longer, HLA (histocompatibility) B8 positive patients were reported to have CICA significantly more often than those who were B8 negative,[6–12] although at least one report did not confirm this relationship.[13] These results seemed to be compatible with the hypothesis that at least some forms of IDD are due to autoimmune mechanisms, and has served as one of the bases for the development of a genetic model of IDD.[14] This model proposes the existence of an IDD locus linked to HLA and with three alleles, one normal and two mutant, one of which is in linkage disequilibrium with B8. Therefore, there would be an autoimmune, B8 positive type of IDD as opposed to other less well characterized forms of disease. It has also been suggested that first-degree relatives of IDD patients have an increased frequency of CICA, which may be a predictor of clinical disease.[7]

In view of the importance of these findings we have studied HLA and CICA in 282 IDD patients and 806 of their first-degree relatives during the Diabetes Genetic Study at the University of Minnesota.

SUBJECTS AND METHODS

Subjects. We studied for CICA 282 insulin-dependent, ketosis-prone diabetics and 806 of their nondiabetic first-degree relatives. Analysis of duration of disease and age at diagnosis versus HLA and CICA was done only in 271 patients since information was not available on the other 11. One hundred and eighty-five patients (160 probands) belonged to families ascertained randomly, i.e., without intended selection, from the lay membership of the American Diabetes Association Minnesota Affiliate, Inc. This group of families included only 13% of multiplex kindreds, a number compatible with reasonable absence of bias of ascertainment. The additional patients came from multiplex families ascertained for studies of affected sib pairs and therefore biased. The analysis was done on both the whole sample and on the less biased subset of patients. All patients were Caucasian and approximately 80% were of Northern European ethnic stock. All patients and relatives were typed for HLA antigens A and B. Dw antigens were studied in 97 of those patients and DR in 153. The mean age for the patients was 26.4 ± 14 yr (mean + SD) and the age at diagnosis was 15.1 ± 10 yr; there were 47% males.

Methods. Sera from IDD and their nondiabetic first-degree relatives were stored at −20°C until tested for CICA. These

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were detected by indirect immunofluorescence (IF) using fresh human group O pancreas from heart-beating cadaver transplant donors as the tissue substrate. The tissue was snap frozen in isopentane precooled with liquid nitrogen immediately after removal, stored at −70°C, and sectioned at 4 μm. Sections were fixed in acetone for 10 min, washed three times in phosphate buffered saline (PBS pH 7.35–7.45), and overlaid with sera for 30 min at room temperature in a moist chamber. After washing with PBS, the sections were stained with fluorescein-isothiocyanate (FITC) tagged goat anti-human Ig (λ-, μ-, α-chain specific) (Cappel) for 30 min. All sera were tested at dilutions of 1:1, 1:2, and 1:4. After washing with PBS, the slides were mounted in a mixture of 9 parts glycerin and 1 part PBS and were evaluated by two independent observers using a Zeiss transmission fluorescence microscope. The intensity of the staining was quantitated as follows: 0, no positive staining; trace, staining just above background, 1–3+, increasing degrees of positive intensity. The HLA antigens A and B,[15] Dw,[16] and Dr[17] were studied by methods described before.

RESULTS

The frequency of CICA for the total sample of 271 diabetics was 50% for those patients with disease for 1 yr or less, 41% for those with disease for 5 yr or less, and 18.7% when the disease had lasted for longer than 5 yr.

HLA versus CICA. No associations were found between any of the HLA B types and CICA positivity both for those patients with less than 5 yr and more than 5 yr of duration of disease; the results are shown for B8 in Table 1. Since the hypothesized IDD susceptibility locus seems more closely linked to the D region of chromosome #6, we also studied Dw and DRw antigens. As shown in Table 1 no associations between CICA and HLA DR antigens were detected. The results were equally negative for Dw antigens. These analyses were repeated for the patients ascertained without intended bias, i.e., excluding those ascertained through multiplex kindreds. Again no relationships were found between CICA and HLA.

Age at diagnosis versus CICA. No relationship was found between age at diagnosis of the disease (below or above 5 yr) and CICA positivity (Table 2). Other cutoff levels such as 10 yr of age also failed to yield associations. Figure 1 shows the relationships between duration of disease and CICA positivity, controlling for age at diagnosis (below or over 5 yr) for our data and that of Neufeld et al.[18] We did not find a significant difference in CICA frequency in relation to age at diagnosis as they did, although our numbers are smaller. More detailed information on the search for relationships between CICA and HLA in random and total IDD proband samples is shown in the APPENDIX.

We also searched for an association between microangiopathy and CICA in those patients with duration of disease less than 5 yr and 5 yr or longer. Proliferative retinopathy was defined as the presence of neovascularization diagnosed by an ophthalmologist and nephropathy as a serum creatinine of 2.0 mg/dl or above. No associations

TABLE 1
Islet cell antibodies versus HLA types and duration of disease (DOD)

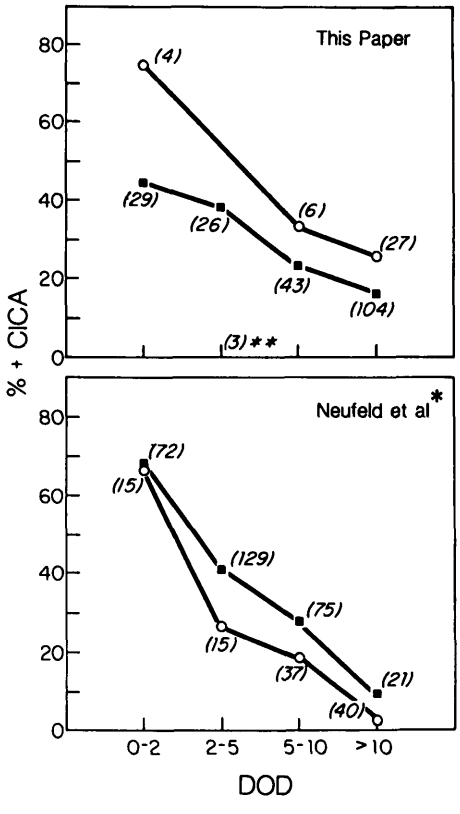
	DOD ≤ 5		DOD > 5		All	
	No.	Percent	No.	Percent	No.	Percent
B 8/x*	8/21	38	12/65	18	20/86	23
8/15	3/10	30	2/13	15	5/23	22
15/x	7/17	41	8/42	19	15/59	25
x/x	14/30	47	12/69	17	26/99	26
All	32/78	41	34/189	18	66/267	25
* x is any antigen other than 8 or 15.						
DR 3/x*	2/9	22	3/20	15	5/29	17
3/4	4/9	44	4/36	11	8/45	18
4/x	11/30	37	5/40	13	16/70	23
x/x	1/5	20	0/4		1/9	11
All	18/53	34	12/100	12	30/153	20

* x is any antigen other than 3 or 4.

TABLE 2
Age at diagnosis (AADx) versus duration of disease (DOD) for cytoplasmic islet cell antibody positive diabetic patients

	AADx < 5 yr		AADx ≥ 5 yr		All	
DOD	No.	Percent	No.	Percent	No.	Percent
0–2	3/5	60	15/34	44	18/39	46
2–5	1/5	20	13/34	38	14/39	36
5–10	2/7	29	12/43	28	14/50	28
>10	6/25	24	16/118	14	22/143	15
All	12/42	29	56/229	24	68/271	25

FIGURE 1. Results of the analysis of islet cell antibody (CICA) positivity versus duration of disease (DOD) in our data (upper panel) and that of Neufeld et al. (lower panel). Diabetics with age at diagnosis before 5 yr (○——○) and after 5 yr of age (■——■) are shown for both sets of data. *Neufeld, et al.[18] **There were only three subjects (all three CICA negative) at 2–5-yr duration of disease for the group with early onset.



were found between these complications of IDD and CICA.

Among the first-degree relatives of the probands we found that $^{18}/_{311}$ (5.8%) parents, $^{11}/_{428}$ (2.6%) sibs, and $^{4}/_{67}$ (6.0%) children were positive for CICA. Three of the 11 CICA-positive sibs of the probands did not share haplotypes with their probands and three shared only one haplotype. Thus far, none of the CICA-positive relatives has developed IDD.

DISCUSSION

Surprisingly, our results showed no association between B8, D(R)w3, or other HLA antigens and CICA in IDD patients. Our sample is the single largest reported so far,[6–12,19] and we have also studied the D region of HLA, which would be expected to enhance any previously recognized B antigen association with a presumed autoimmune indicator as CICA. The reason for the discrepancy between our results and those reported by other,[6–12] though not all,[13] groups is not apparent. A few potential confounding factors can be ruled out. Our criteria of ascertainment of IDD patients described in METHODS, and the finding of the expected excess of IDD-associated HLA antigens in our sample, indicates that we are indeed dealing only with IDD or type I diabetics. Our patients have a mean age at diagnosis (15.1 ± 10 yr) that is higher than in other reports. However, when we analyzed the subset of patients with age of diagnosis before 15 yr no association between CICA and HLA emerged. The overall percentages of CICA-positive IDD patients for the several duration-of-disease periods studied were similar to those previously reported,[5,7] suggesting that our determinations are reliable. The frequencies of CICA in relatives of patients were also similar to those reported by others. Furthermore, we have exchanged several samples with Dr. Abner Notkins from the National Institutes of Health and found that our results were concordant (personal communication).

It is well established that IDD is clinically associated with other autoimmune disorders, some of which are also associated with B8 and D(R)w3 antigens (for reviews see refs. 1 and 11). CICA were first described in IDD patients with clinical and/or laboratory evidence of other autoimmune processes.[4] Although we have not studied organ-specific autoantibodies other than CICA in our patients, only about 4% of the patients or their first-degree relatives reported the clinical presence of autoimmune diseases. This result seems lower than the 9% recently reported by Walker et al.[20] Thus, it may be that our IDD sample includes fewer patients with autoimmunity and that the association between CICA and HLA applies primarily to IDD patients with other underlying (not necessarily clinically detectable) immune processes. It is also possible that autoimmune abnormalities associated with IDD, and reflected in the association HLA/CICA, are more common in the European countries where the positive reports were originated, than in the American upper Midwest. In this context, studies from Scandinavia, from where most of our population is extracted, would be of interest. Data analyzed during the 8th International Histocompatibility Workshop[21] also failed to show any relationships between HLA and CICA.

Neufeld et al.[18] have reported that children who develop IDD before age 5 yr are significantly less often CICA positive than the other IDD children. We were unable to confirm this finding, although our sample of children with earlier onset of disease was much smaller than that reported by Neufeld et al.[18] and this may explain the discrepancy. Our inability to demonstrate associations between CICA and HLA should not be construed as evidence against the probably autoimmune nature of at least some types of IDD. Recent evidence has suggested that complement-fixing CICA[22] or cell-membrane ICA[23] may be more relevant to the pathogenesis of IDD. It will be important to search for associations between the latter and HLA.

Gorsuch et al.[24] reported that $^{10}/_{248}$ (4%) of nondiabetic sibs of IDD probands were ICA positive and all shared at least one HLA haplotype with their probands. We found a slightly lower frequency of CICA-positive sibs (2.6%) and $^{3}/_{11}$ did not share HLA haplotypes with the proband. Neither the overall positivity rate nor the proportion of discordancy differs significantly between these studies, though the latter difference is marginally nonsignificant ($0.05 < P < 0.10$) and merits further study in larger samples.

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APPENDIX: ISLET CELL ANTIBODIES AND HISTOCOMPATIBILITY ANTIGENS (HLA) IN INSULIN-DEPENDENT DIABETICS AND THEIR FIRST-DEGREE RELATIVES

TABLE 1

Islet cell antibody positivity in different HLA DR types according to duration of disease in random IDD probands

	DOD* < 1	1–5	5–10	>10	Total
3/4	2/2 (100%)	1/5 (20%)	2/9 (22%)	1/13 (7%)	6/29 (20%)
3/x	—	2/5 (40%)	1/3 (33%)	2/9 (22%)	5/17 (29%)
4/x	3/6 (50%)	8/22 (36%)	3/11 (27%)	2/20 (10%)	16/59 (27%)
x/x	0/2 (0%)	1/3 (33%)	0/1 (0%)	0/2 (0%)	1/8 (12%)
All	5/10 (50%)	12/35 (34%)	6/24 (25%)	5/44 (11%)	28/113 (24%)

* Duration of disease.

TABLE 2

Islet cell antibody positivity in different HLA DR types according to duration of disease in all IDD probands (random and biased)

	DOD < 1	1–5	5–10	>10	Total
3/4	2/2 (100%)	2/7 (28%)	2/10 (20%)	2/26 (7%)	8/45 (17%)
3/x	0/1 (0%)	2/8 (25%)	1/5 (20%)	2/15 (13%)	5/29 (17%)
4/x	3/7 (42%)	8/23 (34%)	3/14 (21%)	2/26 (7%)	16/70 (22%)
x/x	0/2 (0%)	1/3 (33%)	0/1 (0%)	0/3 (0%)	1/9 (11%)
All	5/12 (41%)	13/41 (31%)	6/30 (20%)	6/70 (8%)	30/153 (19%)

TABLE 3
Islet cell antibody positivity in different HLA B types according to duration of disease in random IDD probands

	DOD < 1	1–5	5–10	>10	Total
8/15	0/1 (0%)	3/7 (42%)	1/2 (50%)	0/4 (0%)	4/14 (28%)
8/x	2/3 (66%)	2/8 (25%)	6/15 (40%)	2/25 (8%)	12/51 (23%)
15/x	2/5 (40%)	3/8 (37%)	0/2 (0%)	2/17 (11%)	7/32 (21%)
x/x	5/7 (71%)	7/18 (38%)	3/16 (18%)	5/23 (21%)	20/64 (31%)
All	9/16 (56%)	15/41 (36%)	10/35 (28%)	9/69 (13%)	43/161 (26%)

TABLE 4
Islet cell antibody positivity in different HLA B types according to duration of disease in all IDD probands (random and biased)

	DOD < 1	1–5	5–10	>10	Total
8/15	0/1 (0%)	3/9 (33%)	1/4 (25%)	1/9 (11%)	5/23 (21%)
8/x	3/6 (50%)	5/15 (33%)	6/18 (33%)	6/47 (12%)	20/86 (23%)
15/x	2/5 (40%)	5/12 (41%)	2/6 (33%)	6/36 (16%)	15/59 (25%)
x/x	5/9 (55%)	9/21 (42%)	5/21 (23%)	7/48 (14%)	26/99 (26%)
All	10/21 (47%)	22/57 (38%)	14/49 (28%)	20/140 (14%)	66/267 (24%)

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