Rapid Publications

Prevalence of Antibodies to Nucleic Acids in Insulin-dependent Diabetics and Their Relatives

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SUMMARY

We have studied nucleic acid antibodies in 23 insulin-dependent diabetics, 27 of their first degree relatives, and 23 normal, unrelated controls. We have found a significantly increased prevalence of antibody to ss-DNA, ds-RNA (reovirus), and synthetic RNA (Poly A · Poly U, Poly I · Poly C) in the unaffected relatives as well as in the diabetics when compared with normal controls. There was no relationship in HLA.

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Recently, we reported a high prevalence rate of antibodies against nucleic acids in insulin-dependent diabetic (IDD) patients in contrast to age-matched and non-age-matched controls.[1] The high titers of antibody, especially to double stranded (ds-) RNA, appeared to be related to the recent onset of illness. We speculated that an immune response might have taken place against viral agents implicated in beta-cell damage after a varying incubation period. In general, antibody titers seemed to fall in most cases after several years of illness, but some patients seemed to retain high antibody titers even after IDD for 5 yr. These observations prompted us to screen for these antibodies in the nondiabetic members of HLA (histocompatibility) tissue typed families with two or more IDD sibs with varying duration of illness. We also searched for associations of the antibody titers with sex, HLA haplotypes, and several clinical features.

METHODS

Patients. Twenty-three IDD patients (ages 9–34 yr, 12 males) from 12 families with at least 2 IDD sibs participating in the Minnesota Diabetes Genetic Study were tested. Patients and their relatives filled out questionnaires on clinical information. Duration of illness was from 2 wk to 34 yr (median of 10 yr). Fifteen normal parents (ages 41–66 yr, 8 males) and 12 normal siblings (ages 15–36 yr, 7 males) were also tested. None of the diabetics or their nondiabetic relatives had other autoimmune diseases except for one case of thyroiditis and one of Addison's disease. Specifically, there were no cases of lupus or other connective tissue disorders.

Methods. Serum antibodies to single stranded (ss-) DNA and double stranded (ds-) RNA were determined by a passive hemagglutination method.[1] The synthetic ds-RNA included polyadenylic-polyuridylic acid (Poly A · Poly U) and polyinosinic-polycytidylic acid (Poly I · Poly C). The native ds-RNA was extracted from either reovirus or statolon virus.[1] Serum antibody titers of 1:16 or above were considered positive. HLA A and B antigens were determined as described before.[2] Equality of percentages of positive titers were tested based on the method of the arc sine transformation, yielding a t statistic,[3] following a factorial analysis of variance using Tukey's method of one degree of freedom for nonadditivity[4] for error estimation.

RESULTS

Table 1 shows the number of IDD patients, unaffected first degree relatives, and normal controls with positive titers ($\geq 1:16$) for nucleic acid antibodies. There was a marked increase in the prevalence of ss-DNA (diabetics 20/23, relatives 15/27, controls 2/23) and Poly A · Poly U antibodies (diabetics 18/23, relatives 12/27, controls 4/23) in both patients and relatives. The differences were statistically significant between patients and controls (ss-DNA, $P < 9 \times 10^{-9}$, Poly A · Poly U, $P < 0.005$), but not between patients and unaffected relatives. The prevalence of ds-RNA (reovirus) and Poly I · Poly C antibody titers were also increased significantly in diabetics and relatives compared with normal controls ($P < 9 \times 10^{-4}$, $P < 0.05$, $P < 5 \times 10^{-5}$ and $P < 1 \times 10^{-4}$, respectively). In no case was there a significant difference between patients and relatives, although all antibody titers (except for ss-RNA) were more often positive in the pa-

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TABLE 1
Prevalence of antibodies to nucleic acids in multiplex diabetic families*

Subjects	N	ss-DNA	ss-RNA	ds-RNA (Reovirus)	ds-RNA (Statolon)	Poly A· Poly U	Poly I· Poly C
Diabetes	23	20 (86.9)†	2 (8.7)	11 (47.8)‡	6 (26.1)	18 (78.3)§	12 (52.2)‖
1st Degree relatives	27	15 (55.5)¶	3 (11.1)	7 (25.9)#	5 (18.5)	12 (44.4)**	9 (33.3)††
Parents	15	10 (66.7)	2 (13.3)	5 (33.3)	3 (20.0)	8 (53.3)	6 (40.0)
Sibs	12	5 (41.7)	1 (8.3)	2 (16.7)	2 (16.7)	4 (33.3)	3 (25.0)
Controls	23	2 (8.7)	0 (0.0)	2 (8.7)	1 (4.3)	4 (16.7)	1 (4.3)

* Subjects with antibody titer 1:16 or above (% in parentheses).
Statistical significance comparing patients or relatives with controls: † $P < 9 \times 10^{-9}$; ‡ $P < 9 \times 10^{-4}$, § $P < 9 \times 10^{-6}$; ‖ $P < 5 \times 10^{-5}$; ¶ $P < 6 \times 10^{-4}$; # $P < 0.05$, ** $P < 0.005$; †† $P < 1 \times 10^{-4}$.

tients. The parents had generally higher prevalence of antibodies than the sibs, but the differences were not significant. Mothers, however, had significantly higher titers than daughters for Poly A · Poly U ($P < 0.05$) and Poly I · Poly C ($P < 0.05$). In general, females tended to have higher prevalence and higher titers than males.

There were no significant associations between nucleic acid antibody titers and HLA (single antigens or haplotypes, including HLA identity to the diabetic sibs), age of the patient, duration of disease, or complications of diabetes.

DISCUSSION

The most surprising finding in this study was the high prevalence of most of the nucleic acid antibodies in unaffected relatives of diabetics. In addition, we confirmed our previous finding[1] of high prevalence of ss-DNA, ds-RNA, Poly I · Poly C, and Poly A · Poly U antibody titers in IDD patients. Whether the high prevalence in unaffected relatives is representative of all diabetic families or is unique to diabetic multiplex families (? indicative of higher genetic liability) is not known. The increased prevalence in parents versus sibs, although not statistically significant, suggest an age-related prevalence. Unaffected relatives of diabetics have been found to have higher than expected prevalence of islet cell and other organ specific antibodies.[5] However, the prevalences reported have always been below 10%, much lower than those reported here for nucleic acid antibodies. High prevalence of extracellular membrane depositions of albumin in unaffected relatives of diabetics has also been reported by us.[6] Thus, it appears that a large number of subclinical immune abnormalities are present in 1st degree relatives of IDD patients, although the great majority of these subjects do not develop IDD.

Both the albumin deposition[6] and the islet cell antibodies[5] have been reported to be associated with HLA. Our inability to find a relationship between nucleic acid antibodies and HLA may reflect either small sample size or an absence of association.

The presence of nucleic acid antibodies in IDD families adds support to the autoimmune theory of IDD.[5] The increased prevalence of reovirus ds-RNA antibodies (and possibly statolon) may be relevant to the evidence that certain RNA viruses may be diabetogenic in man.[5] It will be important to study prospectively nondiabetic sibs of diabetics for viral and immunologic parameters to determine the causal relationship, if any, between these factors and IDD.

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