Antibodies to Nucleic Acids in Juvenile-onset Diabetes

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SUMMARY

Antibodies to single stranded (SS-) and double stranded (DS-) DNA and RNA were determined by a passive microhemagglutination assay in sera from 80 children with juvenile-onset diabetes mellitus (JDM) and 129 children with asthma. The latter group was chosen for comparison with the JDM group because of their increased susceptibility to viral infection and the nonautoimmune nature of the disease. We found that JDM patients had increased titers of antibodies to SS-DNA (61.3 per cent), synthetic polyadenylicpolyuridylic acid (Poly A-U) (78.8 per cent), synthetic polyinosinicpolycytidylic acid (Poly I-C) (62.5 per cent), and DS-RNA of statolon virus (51.3 per cent) and reovirus (27.3 per cent), respectively, in contrast to asthmatics (15.5 , 34.9, 3.9, 20.2, and 2.3 per cent, respectively) or to healthy controls. The difference of the incidence of antibodies among the groups is statistically significant ($P < 0.001$). Presence of SS-DNA antibody found in two thirds of cases of JDM further support the increased prevalence of autoimmune phenomenon in that disease. Furthermore, the increased prevalence of DS-RNA antibodies in patients with JDM, found especially in cases of recent onset, is suggestive of an active immune response against the underlying viral replications that may have led to beta cell injury in islets of pancreas. DIABETES *27*:1105-11, November, 1978.

The presence of antibodies to nucleic acids has been reported frequently in patients with autoimmune diseases. In systemic lupus erythematosus (SLE), antibodies to SS-DNA, DS-DNA, and DS-RNA were found in the majority of cases with active diseases.[1-4] The same observation was made in an animal model of SLE, the hybrid of New Zealand black mice (NZB/NZWF1).[5] In other autoimmune diseases, such as Sjögren's syndrome, rheumatoid arthritis, myasthenia gravis,[1-4,6] and scleroderma,[7] lower but still significant incidences of antibodies were also found. Whereas antibodies to DNA denote the autoimmune nature of the disease, the finding of antibodies, especially to DS-RNA, suggests that persistence of latent viruses underlies the initial pathogenesis of these diseases.

Recent studies on JDM have revealed a variety of autoimmune phenomena in these patients, especially the presence of antibodies to islet cells of the pancreas[8-11] and to other organs.[12-14] Although the autoimmune process has been considered one of the essential mechanisms to lead to beta cell injury and its subsequent failure, the role of the genetic susceptibility to disease and of any prior viral insult to beta cells is still unclear. In conducting a serologic survey of JDM, we found a significant increase in the incidence of antibodies to SS-DNA and to both synthetic and native DS-RNA. These findings have practical and theoretic significance.

Patients. Sera from 80 patients (38 men and 42 women) with JDM were studied. All were insulin dependent, and 65 were attending the Pediatric Diabetes Service at the University of Maryland Hospital; fifteen were referred from local hospitals and from practicing physicians. The ages of the patients at the onset of the disease ranged from 3 to 22 years. The following associated diseases were found among the patients: one had Down's syndrome, one had SLE, one had Hashimoto's thyroiditis, one had nephrotic syndrome, and two had asymptomatic goiters. The results of the study were compared with those obtained in sera from 129 age- and sex-matched asthmatics who attended the Pediatric Allergy Service of the Uni-

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versity Hospital and from 30 healthy medical students. The asthmatic patients were chosen as one of the comparison groups because of their susceptibility to infection and because of the nonautoimmune nature of their illness.

Antigens. Calf thymus DNA (Sigma Chemical, St. Louis) was the source of native or DS-DNA. Denatured or SS-DNA was prepared from DS-DNA being boiled and then cooled, as was described previously.[1] Calf liver RNA (Sigma) was the source of SS-RNA. Four DS-RNAs used in these studies were synthetic polyinosinicpolycytidylic acid (Poly I-C) and polyadenylicpolyuridylic acid (Poly A-U) (both from Sigma), and two native DS-RNAs isolated from reovirus and from statolon virus.*

Procedure. In the passive hemagglutination assay, chromium chloride ($CrCl_3$) was used for coupling the antigen to human 0(−) erythrocytes.[15,16] Red cells from the same donor were used throughout the experiment. Before the coupling, the antigens were adjusted to 1 mg. per milliliter, except for reovirus RNA, which was adjusted to 250 μg. per milliliter. Red cells, antigens, and 0.1 per cent $CrCl_3$ were mixed, incubated for 4½ minutes at room temperature, and then washed thoroughly three times with normal saline. After the coupling, the test was performed using the passive hemagglutination method as described previously.[15,16] All sera were decomplementized by incubation at 56° for 30 minutes. The sera were diluted serially in microtiter plates (Cook Engineering, Alexandria, Virginia). The coated cells and sera were incubated at room temperature for two hours. In order to facilitate the reading, the plates were tilted at a 60° angle for 15 minutes and the results of hemagglutination were read from a reflecting mirror.[16] The titer was recorded as the reciprocal of the end point. A titer of less than 1:8 was generally considered insignificant. For each plate, two controls were always observed: uncoated cells with the same sera and coated cells alone. Both of the controls should be read negative all the time. Immunoabsorbents were prepared by conjugation of antilight-chain antiserum to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) by the method described previously.[17] Immunoglobulins were absorbed from 12 test sera by exposure to the immunoabsorbent for 60 hours at 4° C. using constant agitation. Quantitation of the serum immunoglobulins by single radial diffusion[18] revealed that 96 per cent of IgG, 90 per cent of IgA, and 99 per cent of IgM were removed. Simultaneous quantitation of albumin showed no change of its concentration, indicating the specificity of the immunoabsorption. The specificity of the antibody was confirmed by the successful removal of its activity in the positive sera by using respective antigens. Each sample was run in duplicate and repeated at a later date to confirm the reproducibility of the assay. The chi-square test was used for statistical evaluation of data.

*Reovirus was a gift from Dr. R. Douchart, Lilly Research Lab, Indianapolis, and statolon virus a gift from Dr. A. Shatkin, Roche Institute, New Jersey.

RESULTS

There was a significantly higher prevalence and titer of antibodies to all nucleic acids in the sera of JDM patients when compared with those of asthmatic patients (see figures 1 to 4 and table 1). In an attempt to further define the nature of the serum factor(s), immunoglobulins were removed from 40 positive sera by incubation with an antilight-chain immunoabsorbent. All absorbed sera failed to show any binding activities. In order to demonstrate the specificities of antibodies, sera with the antibody activity to a given antigen were absorbed serially with red cells coated

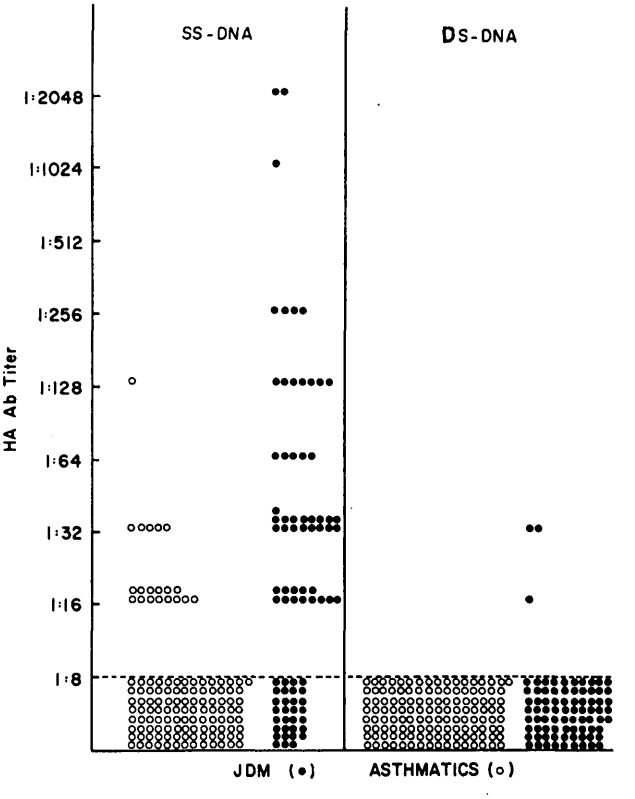

FIG. 1. Antibody titers to single stranded (SS-) and double stranded (DS-) DNA.

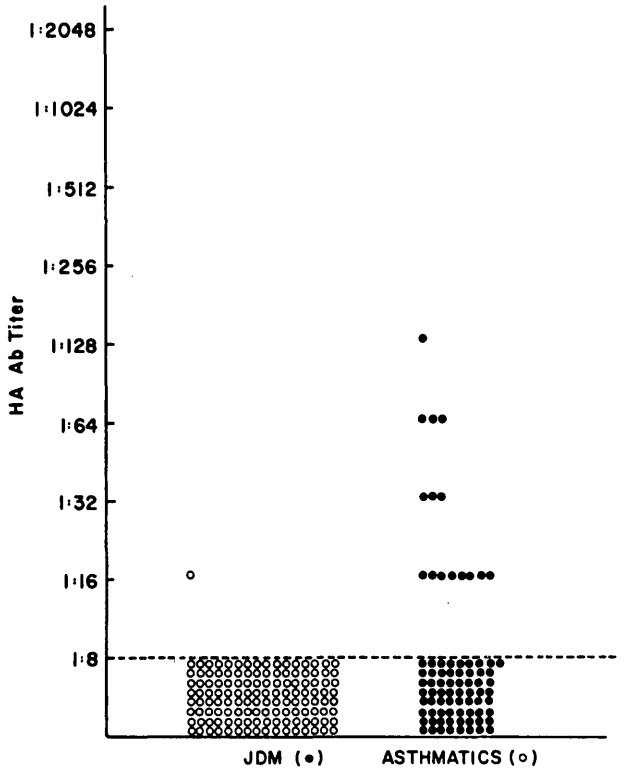

FIG. 2. Antibody titers to single stranded (SS-) RNA.

with other nucleic acid antigens. Since no loss of antibody titer was found, it was confirmed that the antibody lacked cross-reactivity. The frequency of antibody to any of the antigens in sera of 30 normal individuals was less than 6.0 per cent at the titer of 1:16 or above and was less than 3.0 per cent at the titer of 1:64 or above.

Among the three JDM patients who showed antibodies to DS-DNA, only one had clinical disease of SLE and had antinuclear antibodies. The other two had no associated diseases or any autoantibodies at the time the study was conducted. Among the seven JDM patients who showed antibody to SS-RNA, one had apparent scleroderma-like lesions characterized by atrophy of skin, wasting of muscles, and mild joint contractures.

The difference between JDM and asthmatic patients was most apparent in the prevalence of antibodies to SS-DNA and DS-RNA (in both the synthetic and native forms). The difference between the groups was more striking when the prevalence of antibodies at a higher titer (above 1:64) was compared (see table 1).

When the presence of various antibodies among JDM patients was subject to analysis by sex and by the age of the onset of the disease (either above or less than

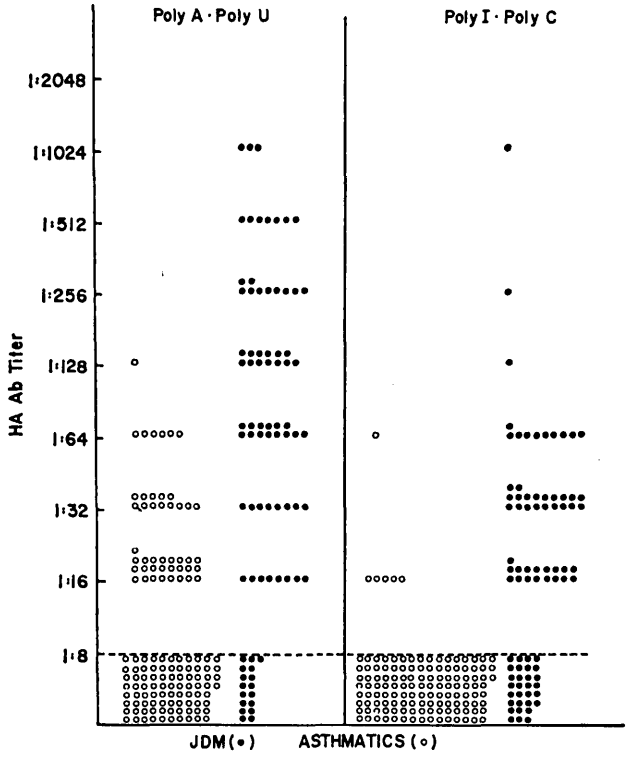

FIG. 3. Antibody titers to synthetic double stranded (DS-) RNA.

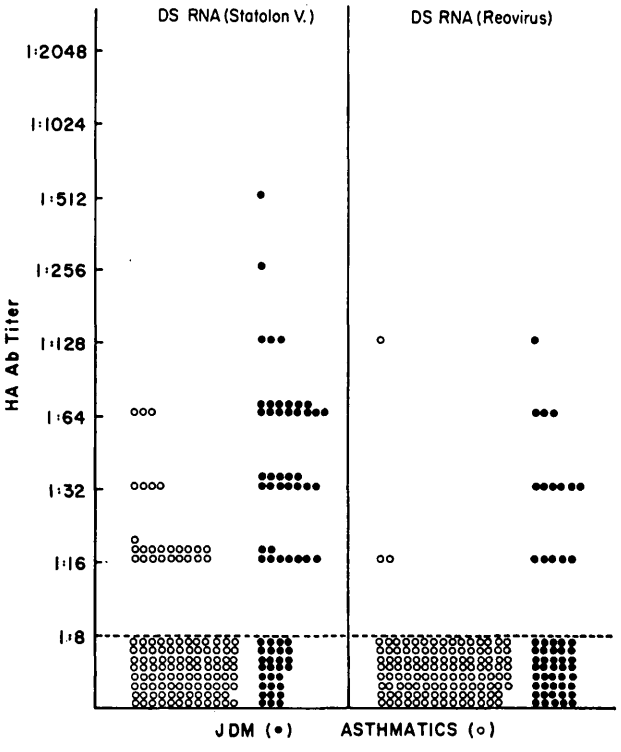

FIG. 4. Antibody titers to native double stranded (DS-) RNA.

TABLE 1

Frequency of serum antibodies to nucleic acid

Antigens		SS-DNA		DS-DNA	SS-RNA	Poly A-U		Poly I-C		Statolon v.		Reovirus
Patients		⩾ 1:16	⩾ 1:64	⩾ 1:16	⩾ 1:16	⩾ 1:16	⩾ 1:128	⩾ 1:16	⩾ 1:64	⩾ 1:16	⩾ 1:64	⩾ 1:16
Asthmatics	No.	20/129	1/129	0/129	1/129	45/129	1/129	5/129	1/129	26/129	3/129	3/129
	Per cent	15.5	0.8	0.0	0.8	34.9	0.8	3.9	0.8	20.2	2.3	2.3
JDM	No.	49/80	19/80	3/80	15/80	63/80	33/80	50/80	13/80	40/78	19/78	15/55
	Per cent	61.3	23.8	3.8	18.8	78.8	41.3	62.5	16.3	51.3	24.4	27.3
P value		A	A	C	C	A	A	A	A	B	B	B

A, < 0.001; B, 0.005 > P >0.002; C, < 0.01. JDM, juvenile-onset diabetes; SS, single stranded; DS, double stranded; Poly A-U, polyadenylicpolyuridylic acid; Poly I-C, polyinosinicpolycytidylic acid.

10 years of age), two significant findings were all restricted to the antibodies to Poly A-U: (1) the antibodies were found in 89 per cent of female and 66 per cent of male patients, (2) antibodies were found in all 18 female patients who had the earlier onset of the disease (before 10 years of age). In contrast, only 50 per cent of female patients who had the onset of the disease after 10 years of age had antibodies.

Another intriguing finding was that the antibodies to synthetic RNA (both Poly I-C and Poly A-U) in JDM patients appeared to be related to the duration of the disease (table 2). When the titers of the antibody were further considered, a significant number of patients with higher titers was among the recent-onset diabetics (figure 5). The trend was present but was less apparent for antibodies to native DS-RNA.

TABLE 2

Prevalence of antibodies to synthetic DS-RNA in relation to duration of illness in 65 JDM patients

	Onset < 12 mo.		1–5 years		3–5 years		> 5 years	
Antigens	No.	%	No.	%	No.	%	No.	%
Poly I-C	18/22	81.8	5/9	55.6	3/6	50.0	3/28	10.7
Poly A-U	20/23	87.0	8/12	66.7	4/8	50.0	6/22	27.3

DS, double stranded; JDM, juvenile-onset diabetes; Poly I-C, polyinosinicpolycytidylic acid; Poly A-U, polyadenylicpolyuridylic acid.

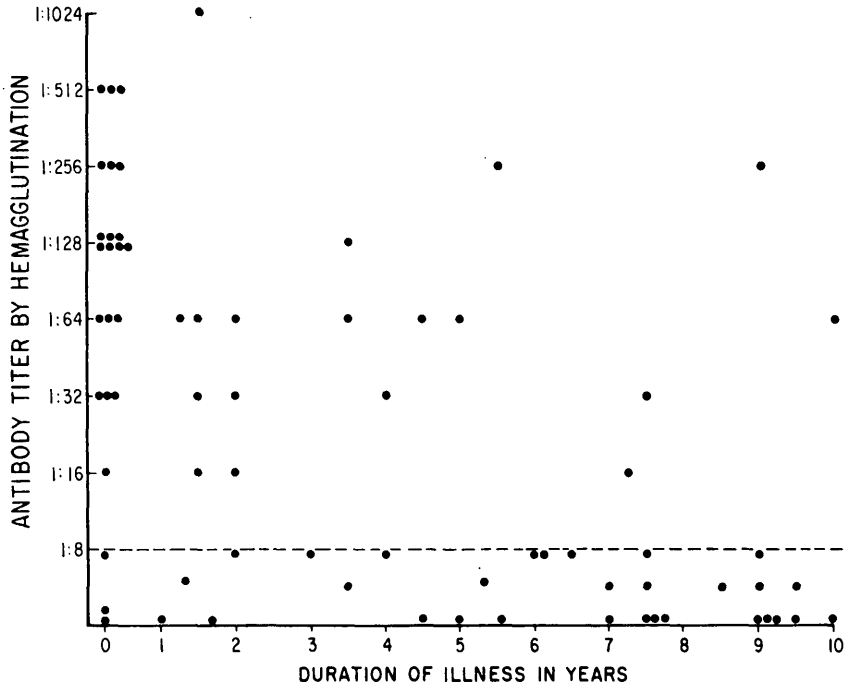

FIGURE 5

Antibodies to synthetic polyadenylic-polyuridylic acid (Poly A-U) in relation to duration of illness in patients with juvenile-onset diabetes.

DISCUSSION

The findings of the heavy infiltration of lymphocytes in islets of the pancreas, especially in the recent-onset diabetics,[19] have stimulated recent interest in the research of the autoimmune aspects of insulin-dependent diabetes.

The association of other autoimmune endocrinopathies among JDM patients were well documented previously[20-26] as well as the findings of high prevalence of anti-organ or anti-cell autoantibodies in diabetes.[8-14] However, the studies of antibodies to cell component, such as nucleic acids, in JDM have not been reported previously. Those nucleic acids, both RNA and DNA, are known to be present in the body circulation but are not generally immuogenic in normal individuals.[4] From the studies in other autoimmune disorders, the presence of the antibodies to nucleic acids may imply that (1) nucleic acids are altered in the host, (2) there is an increase of autoimmune activity in the host because of the loss of suppressor T-lymphocytes, and (3) the antibodies are the results of the immunization to viral nucleic acids.

The antibodies to DS-DNA are known to rise in patients during the active phase of SLE and have been considered to be a pathognomonic finding for that disorder.[1-4] Immune complexes, formed from DS-DNA and its antibodies, are probably responsible for the development and the perpetuation of the vasculitis seen in SLE. As expected, the only patient in this study who had overt SLE showed antibodies to DS-DNA. The other two patients who showed low titers of antibodies did not have other antibodies such as ANA, nor did they show other clinical signs of SLE at the time their sera were tested.

The findings of the high prevalence of antibodies to SS-DNA in JDM (61.3 per cent in JDM in contrast to 15.5 per cent in asthmatics) were striking. The antibody has been reported in high frequencies in many autoimmune diseases such as SLE, rheumatoid arthritis, myasthenia gravis, and chronic active hepatitis.[1-6] A transient rise of the antibody of SS-DNA was reported in experimental animals following gram-negative infection.[27] This observation in experimental infection may help to explain the presence of antibody in some asthmatic patients. However, the origin of SS-DNA antigen, to which the antibody was formed in the majority of autoimmune disorders, remains largely speculative. We found that there was a lack of correlation between the duration of diabetes and the presence of the antibody in our patients. The findings seem to reflect the general increase and persistence of autoimmune phenomena in JDM.

Antibodies to SS-RNA were recently reported in the majority of patients with scleroderma so studied.[7] It is important to note that one of the seven JDM patients who were shown to have antibodies to SS-RNA had clinical signs of scleroderma. Since the association of scleroderma-like lesions among diabetics has been reported previously,[28] a follow-up on those seropositive patients should allow us to explore further the significance of this antibody.

The high prevalence of antibodies to DS-RNA found in the sera of JDM patients in contrast to those of normal controls and asthmatics raises intriguing questions in regard to the origin of RNA and its implication in the pathogenesis of the disease. Apparently, the rise of the antibody was not a result of the bouts of acute infection, as it was also illustrated in the results in asthmatics, who are known to have increased susceptibility to respiratory infections.[29-32] Only a limited antibody response has been reported also in persons who received immunization with live virus.[3] In the analysis of the seropositive patients, it was also found that the highest prevalence (100 per cent) was in the group of female patients who had the onset of the disease before 10 years of age in contrast to male patients or other female patients who had onset of the disease at the later age. The significance of this finding is unknown at the present.

An important adjunct to the finding of the antibody to DS-RNA in JDM patients was the relationship between the appearance of the antibodies and the duration of the disease (see table 2). The patients who showed higher titers were also found in the recent-onset diabetics (figure 5). These findings strongly suggest that the immune response to DS-RNA may be linked with the development of the disease. The RNA viruses have been implicated in the etiology of insulin-dependent diabetes in man[33-38] as well as in animal models.[39-42] Whether our findings reflect the immune response to altered DS-RNA of the hosts or to the DS-RNA of the infectious agents as was strongly implicated in SLE patients[3,5] remains speculative and requires further investigation.

The prevailing hypothesis of the development of insulin-dependent diabetes appears to favor the sequence of events that is influenced by genetic susceptibility. It may imply either that the hosts are susceptible to infectious agents, such as implicated viruses, or that the hosts have a predilection for the development of the autoimmunity. Subsequently,

damage of beta cells could be accomplished by aggressive immunocytes with or without the participation of antibodies as it was reported previously.[43] There is still a paucity of information to adequately explain the occurrence of pathologic events between the individual susceptibility and the subsequent development of the disease. Our findings provide the additional information that patients with JDM have increased immune activity to nucleic acids, presumably through autoimmune mechanisms, and the results of the study, especially the antibody to DS-RNA in recent-onset diabetics, may implicate virus(es) in the development of diabetes.

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