PAPERS AND ORIGINALS

Viruses and the aetiology of diabetes: a study in identical twins

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Summary

Sera were collected from 49 pairs of identical twins, 27 of whom were discordant (only one twin affected) and 22 concordant (both diabetic) for insulin-dependent diabetes. All were tested for antibodies to mumps, cytomegalovirus, rubella, Coxsackie virus types B1-5, and *Mycoplasma pneumoniae*. The diabetic co-twins had no more antibodies to any of the viruses than the non-diabetic co-twins of the discordant pairs. Antibodies to Coxsackie B2, rubella virus, and *M pneumoniae* were found more often in the discordant than in the concordant twins. In 30 of the 71 diabetic twins symptoms began when they were aged 4-6 years or 10-15 years. More concordant than discordant twins were diagnosed during the months January to March.

Hence there was no direct evidence of a virus aetiology of juvenile onset diabetes in these twins, and the difference in antibody titres between the concordant and discordant twins was in keeping with a genetic difference between them. The age and time of onset suggested that environmental factors may be important in causing diabetes in the twins.

Introduction

Diabetes may be the result of a combination of hereditary and environmental factors. Viruses have recently attracted increasing interest as possible aetiological agents, but although their role has been clearly shown in experimental diabetes in animals¹

their importance in the aetiology of diabetes in man is still uncertain.

The fact that the incidence of juvenile onset diabetes varies with age, season, year, and geographical area²⁻⁴ suggests that environmental factors may be important. Epidemiological studies suggest a possible link between juvenile diabetes and mumps,⁴⁻⁶ Coxsackie B4 virus,⁷ congenital rubella,⁸ and *Mycoplasma pneumoniae* infection.⁹ Serological studies also have suggested that diabetes may sometimes be associated with Coxsackie B4 infection,¹⁰ and though there is little direct evidence of the involvement of the islets of Langerhans in virus infection histological evidence of B-cell damage has been reported in Coxsackie B and cytomegalovirus infections.¹¹

A series of monozygotic twins, some of whom were concordant for diabetes (both twins diabetic) and the remainder discordant (only one twin affected), provided a study group in which intra-pair genetic differences were eliminated. If viruses do play a causal part in juvenile-onset diabetes one would expect to find that the affected twin in the discordant pairs showed evidence of previous infection more often than the unaffected co-twin whereas the co-twins in the concordant pairs showed similar patterns of previous virus infections.

We describe here an investigation into virus antibody titres in identical twins, discordant and concordant for diabetes, and include data for age and seasonal incidence.

Methods

Forty-nine pairs of identical twins were studied. They were the more accessible members of a larger series of monozygotic twins, which has been described.¹² Monozygosity was established by clinical assessment and confirmed in every pair by typing for the blood groups ABO, CDE, MN, S, P, Lu^a, K, Le^a, and Fy^a. The error in diagnosing monozygosity by this method does not exceed 3%.¹³

Serum was collected from each of the 98 twins, in 40 pairs samples from the co-twins were collected on the same day. Virus antigens were kindly provided by the Standards Laboratory, Central Public Health Laboratory, Colindale. Sera were tested for complement-fixing antibodies¹⁴ against mumps S and V, cytomegalovirus, and *M pneumoniae*. Rubella haemagglutination-inhibiting antibodies were titrated by the method of Stewart *et al*¹⁵ on sera pretreated with heparin and manganous chloride.¹⁶ Antibodies to Coxsackie B virus types 1-5 were titrated by a conventional neutralisation test in tissue culture

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using 100 medium tissue culture infective dose (TCID₅₀) virus challenge. We also recorded the age at diagnosis of the diabetic twins in the group reported here and the month of onset of symptoms in all insulin-dependent twins in the whole series¹² for whom accurate information was available (24 discordant and 42 concordant diabetics).

Results

Of the 49 pairs 27 were discordant for diabetes and 22 were concordant. All the diabetics were dependent on insulin. Table I shows the clinical features of the twins in each group. The mean duration of diabetes was slightly longer in the co-twins of the concordant pairs (15 and 12 years) than in the affected twins of the discordant pairs (9 years). More striking, however, was the difference in the duration of discordance in the two groups. In the concordant pairs the mean period between the diagnosis of diabetes in each twin was three years; in the discordant pairs the mean time since the development of diabetes in the affected twin was nine years. The twins in 18 of the pairs were living apart, but the differences in antibody titres between twins in these pairs were similar to those found in the 31 pairs of twins who were living together.

Low antibody titres are known to persist for many years after virus infection; we took titres of 1/8 or more to indicate past infection. There were no significant differences between antibody titres in the diabetic and non-diabetic co-twins of discordant pairs (table II), and, in particular, titres of antibodies to Coxsackie B4 were no higher in the affected twins. Furthermore, there were no intra-pair differences in the concordant pairs between the first and second twin to be diagnosed (table II). There were, however, overall differences between the concordant and discordant twins. In the discordant twins significant antibody titres to Coxsackie B2 virus, rubella virus, and *M pneumoniae* were commoner than in concordant twins (table II). Thus 33 out of 54 discordant twins compared with 15 out of 44 concordant twins had

antibodies to Coxsackie B2 virus. All the discordant twins but only 36 of the 44 concordant twins were positive for rubella virus, and 44 out of 52 discordant twins compared with 23 out of 42 concordant twins were positive for *M pneumoniae*. There was no difference between the two groups in respect of Coxsackie B1 B3 B4 and B5 mumps, or cytomegalovirus titres.

Age at diagnosis of diabetes in 71 twins.

Fig 1 shows the age at diagnosis in the 71 diabetic twins (27 discordant and 44 concordant). There were two peaks at 4-6 years and 10-15 years; the age of onset in 30 patients fell within these age groups. The month of onset of diabetic symptoms in the concordant and discordant twins is shown in table III. Symptoms started between January and March in 17 of 42 concordant twins compared with only 3 of 24 discordant twins. There were no other significant differences between the two groups.

TABLE I—Clinical features in 49 pairs of identical twins

Pair type	No of pairs	No with first-degree family history	Mean duration (and range) of diabetic discordance (years)	Diabetic status of co-twins	Mean age (and range) at testing (years)	Mean duration (and range) of diabetes (years)
Discordant	27	4	9 (1 month-29 years)	Diabetic	30 (7-62)	9 (1 month-29 years)
Concordant	22	5	3 (0-10 years)	Non-diabetic Diabetic (1st diagnosed) Diabetic (2nd diagnosed)	30 (7-62) 33 (10-66) 33 (10-63)	15 (1-44) 12 (3 months-42 years)

TABLE II—Virus antibody titres in discordant and concordant pairs of twins. Geometric mean titres are reciprocal geometric mean antibody titres of positive sera

	No of pairs tested	Discordant twins				Total No (%) positive	No of pairs tested	Concordant twins				Total No (%) positive
		Diabetic twins		Non-diabetic twins				1st Diagnosed		2nd Diagnosed		
		No positive	Geometric mean titre	No positive	Geometric mean titre			No positive	Geometric mean titre	No positive	Geometric mean titre	
Coxsackie B1	27	9	30	8	29	17 (31)	22	9	14	5	19	14 (32)
Coxsackie B2	27	17	31	16	45	33 (61)*	22	9	20	6	14	15 (34)*
Coxsackie B3	27	12	20	11	32	23 (43)	22	9	23	9	37	18 (41)
Coxsackie B4	27	13	18	17	24	30 (56)	22	15	25	13	37	28 (64)
Coxsackie B5	27	10	14	12	17	22 (41)	22	6	15	8	14	14 (32)
Mumps S	25	8	8	8	8	16 (32)	21	9	6	6	7	15 (36)
Mumps V	25	22	13	19	11	41 (82)	21	18	13	16	10	34 (81)
Cytomegalovirus	25	9	11	8	8	17 (34)	21	10	14	9	13	19 (45)
Rubella	25	25	51	25	43	50 (100)†	22	19	92	17	64	36 (82)†
M pneumoniae	26	22	10	22	11	(44 (85)‡	21	10	6	13	9	23 (55)‡

*χ² = 6.04; P<0.02.
†χ² = 7.73; P<0.01.
‡χ² = 8.71; P<0.01.

TABLE III—Month of onset of diabetic symptoms in twins

	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Discordant	2	0	1	1	0	2	1	0	6	1	2	8
Concordant	7	5	5	1	0	3	0	4	5	2	7	3

Discussion

A study of diabetes in identical twins showed that when the index twin developed diabetes under the age of 40 years half the pairs were concordant but the remainder showed persisting discordance.¹² In most concordant pairs the interval between diagnosis in the co-twins was less than three years. Since the mean period of discordance of the discordant pairs was 11 years and all the unaffected twins had a normal glucose tolerance that did not deteriorate on repeated testing they were unlikely to become diabetic. As the co-twins of each pair are genetically identical diabetes in the affected members of the discordant pairs must be mainly environmentally determined. A search for a consistent environmental difference between the co-twins of the discordant pairs, however, showed no difference in dietary carbohydrate intake, body weight, or past history of infections. With the current interest in a possible viral aetiology of juvenile onset diabetes it was natural to study viral antibody titres in the twins.

The antibody titres provided no further support for a viral aetiology, but there are several possible explanations for our findings: (1) There is a high prevalence of these antibodies in the normal population. Differences were therefore unlikely in the small number investigated in this study. (2) Only a limited range of antibodies was tested. (3) Many of the diabetic twins were seen several years after the onset of diabetes, when antibody titres might have declined to undetectable levels. Only 11 twins, 5 from discordant and 6 from concordant pairs, were seen within two years of onset. (4) In some of the discordant pairs the non-diabetic twins may have had viral infections which were insufficient to produce overt diabetes. In support of this idea there is some epidemiological evidence that there may be a delay between viral infection and the onset of clinical diabetes.^{4, 6, 17} This may represent the period during which an autoimmune process progressively destroys a critical mass of islets. In keeping with this hypothesis islet-cell antibodies have been detected in two diabetic patients as long as one year before the onset of frank diabetes.¹⁸

The more common finding of antibodies to Coxsackie B2 virus, rubella virus, and *M pneumoniae* in the discordant than in the concordant twins supports the concept of a genetic difference between the two groups, resulting in varying susceptibility to different viruses. This evidence does not directly implicate any of these three micro-organisms in the aetiology of diabetes in the discordant or concordant pairs, but each has been suspected of causing diabetes.^{8, 9, 19} Support for the possibility of a genetic difference between the concordant and discordant twins comes from data relating to histocompatibility antigens. The histocompatibility antigens HL-A8 and W 15 are associated with insulin-dependent diabetes.^{20, 21} One theory is that so-called immune-response genes in the region of the 2nd HL-A locus²² confer an increased susceptibility to pancreatropic micro-organisms. HL-A typing of the twins has shown an increased frequency of the W 15 antigen in both concordant and discordant pairs but of HL-A8 only in the concordant pairs.²³ There may therefore be genetic heterogeneity between the concordant and discordant groups.

A seasonal variation in the incidence of juvenile onset diabetes, with peaks in the autumn and winter, has been described previously. In our study both the concordant and discordant twins also showed an increased incidence in the autumn and winter. There was a difference between them, however, many more of the concordant than the discordant twins developed diabetes between January and March.

Though the numbers were small the age of onset of diabetes in these twins shows some clustering at 4-6 years and 10-15 years. This age distribution resembles that found in juvenile diabetes in the population as a whole, in which peaks have been reported at 5 and 12 years.³ This again suggests that environmental factors may play a role in both identical twins and non-twins who develop diabetes.

The epidemiological aspects of this study support the view that environmental factors are important in the aetiology of many cases of juvenile diabetes. Though we have not shown virus antibody differences between the affected and unaffected twins of the discordant pairs the differing prevalence of certain virus antibodies in the concordant and discordant twins may be a reflection of different immune response genes. In view of the probable association of these genes with the 2nd HL-A locus,²² this would be consistent with our previous finding of a difference in the frequency of HL-A8 between the concordant and discordant pairs.²³

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