The Swedish childhood diabetes study III: IgM against coxsackie B viruses in newly diagnosed Type 1 (insulin-dependent) diabetic children – no evidence of increased antibody frequency

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Summary. Sera from essentially all Swedish children aged 0–14 years with Type 1 (insulin-dependent) diabetes mellitus with onset during an autumn period (October–December 1985) and a late spring period (May–June 1986) were selected. In all, 98 patients were analysed for IgM antibodies against coxsackie B virus serotypes 1 through 5 by a μ -antibody capture radio immunoassay technique. Sera from 94 referent children matched for age, sex and residential area, collected during the same period, were also analysed. During the autumn period, 10 out of 67 (15%) diabetic children were IgM positive while 14 out of 75 (19%) of the healthy referent children demonstrated positivity. During the late spring period only one out of 31 (3%) children with diabetes and two out of

19 (10%) referent children were IgM positive. In the diabetic patients, five were coxsackie B2 positive while coxsackie B1, 3, 4 and 5 were represented by one to three patients each. Eight referent children were coxsackie B4 positive, six were B3 positive and two B2 positive, while no referent children were positive against coxsackie B1 and 5. During these two periods in late 1985 and early 1986 these data demonstrate no evidence of increased antibody frequency against coxsackie B virus 1 through 5 at the onset of childhood diabetes in Sweden.

Key words: Type 1 (insulin-dependent) diabetes mellitus, coxsackie B virus, immunoglobulin M, radioimmunoassay.

Since Gamble et al. in 1969 and 1973 recorded higher levels and frequency of neutralising antibodies against coxsackie B (CB) virus in patients with newly diagnosed Type 1 (insulin-dependent) diabetes mellitus than in control subjects, there are now several such reports, most of which have found neutralizing CB antibodies more frequently in newly diagnosed diabetic patients than in healthy control subjects [1–3].

During the last few years, sensitive techniques for measurement of IgM antibodies against CB viruses have been developed. In reports from Europe and Australia, paediatric diabetic patients, newly diagnosed, showed 30–39% CB-IgM positivity compared with about 6% in control subjects using an enzyme-linked immunofluorescence assay (ELISA) [4, 5]. Gamble and Cumming applying a similar ELISA on Type 1 diabetic patient sera and control sera could demonstrate a CB-IgM response more often only among the patients in the age group 0.5–2 years [6]. By the use of an immunofluorescence test CB-IgM was found at similar frequencies in children with recent onset Type 1 diabetes as in control subjects that were siblings of the patients (23 and 24%, respectively) [7].

The aim of this work was to define the frequency of IgM antibodies against CB 1–5 in Swedish children acquiring Type 1 diabetes during two defined periods, and to compare this with the frequency in carefully matched healthy referent children studied during the same periods.

Subjects and methods

Subjects

The present paper is part of the nationwide case referent study in Swedish children aged 0–14 years with Type 1 diabetes mellitus onset. All of the 44 paediatric departments in the 24 Swedish counties collaborated. For each patient seven years old or more, two referent children were selected through the population registry (SPAR-DAFA) matched for sex, age, and county of residency. For ethical reasons only sera from hospital patients were allowed to be taken as reference in children who were younger than 7 years. The total material from the whole year of collection consisted of sera from 389 Type 1 diabetic patients and 321 referent children. Since the epidemiology of enterovirus infections exhibits a marked seasonal variation in temperate climates [8], sera from two different seasons, one autumn period (October–December 1985) and one late spring period (May–June 1986)

Table 1. Outcome of matching Type 1 (insulin-dependent) diabetic patients and referent children for sex and age

Age	Boys		Girls	
	Patients	Referent children	Patients	Referent children
0-4	11	10	10	10
5-9	18	15	14	15
10-14	18	18	27	26

Table 2. Seasonal distribution of coxsackie-B-IgM-positive (CB+) and negative (CB-) patients

	Patients			Referent children		
	CB+	CB-	all	CB+	CB-	all
October	1	19	20	4	20	24
November	5	27	32	5	29	34
December	4	11	15	5	12	17
May/June	1	30	31	2	17	19
All	11	87	98	16	78	94

were selected for this study. The outcome of the matching of patients and referent subjects is given in Tables 1 and 2. Sera were analysed without prior knowledge of the patients' status. The study was approved by the Ethics Committee at the Karolinska Institute.

Collection of sera

Sera from the patients were taken immediately after the diagnosis of Type 1 diabetes or within the first few days following diagnosis. After centrifugation, sera were sent by express mail to the collecting centre, stored at -20°C for one week and thereafter at -80°C until analysis 1-13 months later. Sera from referent children were sent and stored in a similar way.

Antibody assay

IgM antibodies against CB virus serotypes 1 through 5 were assayed by a μ -antibody capture radioimmunoassay-technique using ^{35}S -labelled CB virus as previously described in detail [9]. The cut-off point used in end-point calculations was three times the cpm value of a buffer blank and a negative serum (both gave the same cpm value), with the provision that the cut-off value should be at least 150 cpm. The specificity and sensitivity of the assay have been presented in a previous study [9].

Statistical analysis

When the statistics were calculated, the matching was dissolved and comparisons were made between patients and referent children as groups, as the preference for matching was given to the time of sampling. To be able to demonstrate a 50% difference between groups in terms of coxsackie IgM antibody frequency at the 5% significance level with a statistical power of 80%, 160-180 samples would have to be studied, depending on the frequency of IgM positivity of the population analysed. As the frequency of CB-IgM positivity has been very different in earlier studies and thus not easily predicted, the higher figure was selected. In all, 192 sera were analysed. The chi square test was used for comparison of frequencies.

Results

Out of 142 sera from children during the autumn period, ten out of 67 (15%) Type 1 diabetic children were CB-IgM positive. During the late spring period only one out of 31 (3%) Type 1 diabetic children were CB-IgM positive (Table 2). In the Type 1 diabetic patients, IgM against CB2 predominated with 5 cases (one of which also showed a response against CB5). Three patients were IgM positive against CB3 (one of which also showed a response against CB4). The remaining three patients were IgM positive against CB1, CB4 and CB5, one case of each.

Fourteen out of 75 (19%) sera from healthy referent children during the autumn period showed CB-IgM positivity, and 2 out of 19 (10%) referent children were CB-IgM positive during the late spring period (Table 2). Eight of the 16 CB positive referent children had IgM to CB4, six to CB3, and two to CB2. None demonstrated IgM to CB1 or CB5.

Discussion

A great number of observations in both animals and man suggest a viral aetiology for at least some cases of Type 1 diabetes mellitus [3, 10, 11]. The evaluation of published seroepidemiological studies is often impaired by insufficient sensitivity and/or specificity in the methods used [12], and whether the control subjects are a representative group may easily be questioned when studying low-incidence diseases like Type 1 diabetes mellitus within large geographical areas [4, 13].

In the present study an apparently type-specific CB-IgM radioimmunoassay [14] has been used. The selection of referent subjects from the population registry matching the index cases which represent consecutive cases in a whole country, makes this study unique. The present finding of a similarly low prevalence of specific CB-IgM in Type 1 diabetic patients as in referent subjects during the autumn period as well as the late spring period probably reflects the seasonal variation of these viral infections in the paediatric population at large. Similar figures have been reported in population studies by other investigators [5, 6]. During the periods studied, these data do not indicate that CB viruses had any relationship to the development of Type 1 diabetes, or, more specifically, the data show that the CB-IgM frequency in the diabetic group was not 50% different from that seen in the referent group. To demonstrate smaller differences the analysis of a larger number of patients would be required.

The present finding was not completely unexpected as in our local (Uppsala county) continuous studies the incidence of CB-IgM among our new Type 1 diabetic patients fell in late 1984 [15] and stayed low during 1985. Gamble and Cumming [6] have shown that among their Type 1 diabetic patients the percentage displaying CB-

IgM was virtually the same regardless of the duration of diabetes. Their results, as well as ours do not indicate a specific role for CB viruses in the pathogenesis of Type 1 diabetes mellitus, but on the other hand, these data do not exclude such a role as there exists a high frequency of antigenic variants of CB viruses attacking humans [16]; possibly with different tissue affinities including the islets of Langerhans [17, 18]. In addition, some workers have suggested, although not proved, that Type 1 diabetic patients may have an "immunological paralysis" [19] to account for the quantitatively lower titres of antibodies to CB viruses found in patients with recent onset Type 1 diabetes than those found in healthy referent subjects [20]. Geographical variations in the epidemiology of diabetogenic variants of CB viruses and of other exogenous diabetogenic factors, possibly including other enteroviruses [21], and differences in the pattern of previous infection of non-diabetogenic CB variant in the population, may explain the variability over time and between areas in the percentage of new Type 1 diabetic patients who display CB-IgM.

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