Congenital rubella syndrome as a model for Type 1 (insulin-dependent) diabetes mellitus: increased prevalence of islet cell surface antibodies

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Summary. An increased prevalence of Type 1 (insulin-dependent) diabetes has been reported in patients with congenital rubella. Rubella virus multiplies in the pancreas, and we have hypothesized that studies of children with congenital rubella would be of great importance in following the development of Type 1 diabetes in a defined, susceptible population. Two hundred and forty-one children with congenital rubella (mean age 17.4 ± 0.3 years; 65% black and hispanic) have been evaluated, 30 of whom already have diabetes and 17 of whom have borderline glucose tolerance. In these latter two groups, HLA-DR3 is significantly increased and HLA-DR2 significantly decreased. Pancreatic islet cell cytotoxic surface antibodies are found in 20% of the total congenital rubella population, including in more than 50% in the time period before they develop diabetes and are not related to any specific

HLA type. In addition, anti-microsomal and anti-thyroglobulin antibodies are found in 34% of this population. The data demonstrate that Type 1 diabetes developing in congenital rubella patients has the genetic and immunological features of classical Type 1 diabetes, namely the presence of HLA-DR3, the absence of HLA-DR2, islet cell surface antibodies before decompensation and an increased prevalence of anti-thyroid antibodies. Patients with non-diabetic congenital rubella represent an easily identifiable group in whom other immunological factors associated with Type 1 diabetes can be elucidated and possibly modified.

Key words: Type 1 diabetes, congenital rubella, islet cell surface antibodies, viral trigger.

In 1949 Hay first reported the association of congenital rubella and diabetes mellitus [1]. Subsequently Menser et al. have reported a 40% prevalence of diabetes in a large group of patients with congenital rubella born in New South Wales and followed since 1942 [2]. Nance's group [3] found a 13% prevalence of abnormalities in glucose and insulin responses in 101 students at the Maryland and Virginia Schools for the deaf, a large proportion of whom were thought to be deaf secondary to congenital rubella. Since rubella virus multiplies and can cause lesions in the pancreas [4], the subsequent development of diabetes is not surprising. In addition, since it has been hypothesized that viruses can infect pancreatic β cells in genetically susceptible individuals leading to the production of antibodies against the β cells [5], we designed our protocol to study longitudinally the genetic, immunological and metabolic features of a large cohort of patients known to have had congenital rubella. Such studies could help define the natural history and pathogenesis of diabetes mellitus if similar genetic and immunological features could be demon-

strated in this group as in classical Type 1 diabetic patients.

Subjects and methods

Patient population

All 242 patients (mean age 17.4 ± 0.3 years; 65% black and hispanic; 52% males) studied to date are either being followed at the Developmental Disabilities Center, St Luke's Roosevelt Hospital or at the Pediatric Diabetes Clinic, Mount Sinai Hospital, New York. They all have stigmata of the congenital rubella syndrome, including different combinations of mental retardation and microcephaly, deafness, cataracts, congenital heart disease and chorioretinopathy. The diagnosis of congenital rubella was, however, based on one or more of three criteria: (1) isolation of rubella virus in the throat, urine and/or cerebral spinal fluid of the newborn; (2) confirmation by detection of rubella specific IgM haemagglutination inhibition (HI antibody) in sera from newborns or persistence of rubella specific IgG haemagglutination antibody on serial specimens beyond the first year of life; (3) presence of clinical stigmata and detection of rubella on specific-HI antibody levels, compatible with congenital rubella specimens obtained at times which do not permit us to rule out post-natal infection. In more than half of all cases the diagnosis was established on the basis of cri-

Table 1. Prevalence of ICSA according to HLA-DR type in congenital rubella

HLA-DR Antigen	ICSA-positive patients
2	6 out of 19
3	8 out of 30
4	6 out of 28
other	8 out of 44
Unknown	7 out of 52
Total	35 out of 173

teria (1) or (2), 16% and 41% respectively. No significant differences in the frequency of these diagnostic criteria were observed for children who are currently diabetic. Thirty children are clinically diabetic according to the criteria of the National Diabetes Data Group [6], 15 of whom are constantly insulin-dependent (eight boys and seven girls), and have had at least one episode of ketoacidosis; one girl has received insulin intermittently and the other 14 (five boys and nine girls) have grossly abnormal oral glucose tolerance with 10 out of 14 having total insulin levels during a 3-h oral glucose tolerance test at least two standard errors below the mean for normal children of the same age (161 ± 9 mU/l) [7].

Typing of HLA antigens

HLA-A, -B and -C antigens were tested with the contrast fluorescence test using 180 mono- or oligo-specific reagents able to recognize 65 HLA antigens [8]. The two-colour fluorescence test described by Van Rood et al. was used for the testing of HLA-DR antigens [9].

Pancreatic islet cell antibodies

Pancreatic islet cell surface or cytotoxic antibodies (ICSA) were measured using as targets both the rat insulinoma line RIN5f [10] and neonatal rat islets [11]. In this study both methods for ICSA gave similar results.

Anti-thyroid microsomal and anti-thyroglobulin antibodies were measured by our newly described enzyme-like immuno-sorbant assay (ELISA) technique using human thyroid tissue [12].

Oral glucose tolerance tests

Oral glucose tolerance tests were performed in the standard manner with patients receiving 1.75 g glucose/kg of ideal body weight up to a maximum of 100 g. Blood specimens were obtained at 0, 30, 60, 120 and 180 min and were used for measurement of plasma glucose by the glucose oxidase technique and for determination of serum insulin levels by immunoassay techniques. Total insulin levels are equal to the sum of the values at the five test points. Values > 200 mU/l were designated as hyperinsulinaemia and those < 140 mU/l as hypoinsulinaemia. Borderline and grossly abnormal glucose tolerance tests refer to those where the peak plasma glucose levels were > 8.8 mmol/l and 11.1 mmol/l, respectively.

Results

HLA antigens

Twenty-six out of 30 diabetic children have now had complete HLA typing. HLA-DR3 or DR4 was present in 21 (81%). Four have both HLA-DR3 and DR4 and two are DR3 homozygotes. Of the five not having either DR3 or DR4, two have B8 or B18 in combination with

other DR alleles. Only one has HLA-B7, associated with DR4 and DR5, and DR2 is absent in all 15 totally insulin-dependent patients. If these 26 congenital rubella patients with diabetes are compared with 162 congenital rubella patients without diabetes and 163 control subjects in the New York City area, DR2 is significantly decreased, DR3 is significantly increased and the small increase in DR4 is not statistically significant.

ICSA and anti-thyroid antibodies

The prevalence of ICSA in patients with congenital rubella according to their HLA antigen is summarized in Table 1. There was no significant difference in the prevalence rate according to specific DR antigens and the overall prevalence for ICSA was 20% versus $< 4\%$ in controls.

In the 15 Type 1 diabetic patients, whose mean duration of the disease is 12 years, 37% are still positive for ICSA. In the other 15 diabetic patients, 11 of whom are hypoinsulinaemic, 81% are positive and of the 17 congenital rubella patients with borderline glucose tolerance tests, eight of whom are hypoinsulinaemic, 53% are positive.

ICSA have also been found in 9 out of 13 stored serial specimens obtained in four congenital rubella patients starting at birth; three of these subjects now have diabetes.

Thirty-four percent of the congenital rubella patients also have anti-microsomal or anti-thyroglobulin antibodies and their frequency is 88% (23 out of 26) in congenital rubella patients with positive ICSA.

Discussion

In the present investigations, we have demonstrated that the three major criteria associated with Type 1 diabetes, namely the HLA-DR3, DR4 association, the virtual absence of HLA-DR2, ICSA and hypoinsulinaemia, are also present in patients with the congenital rubella syndrome who develop diabetes. This increased frequency of DR3 and decreased frequency of DR2 have only been reported in Type 1 diabetes [13]. Menser et al. [2] have reported previously that 50% of their patients with both congenital rubella and diabetes had HLA-A8 (now B8), usually found in linkage disequilibrium with HLA-DR3. Nance's group [3] reported an increase in HLA-B15 and B40 in their patients, but our report is the first complete study of the DR antigens [14]. Nance's group [3, 15] also reported that 24.5% of their deaf patients had anti-thyroid microsomal and 7.1% had parietal cell antibodies. They were, however, unable to find cytoplasmic islet cell antibodies and did not look for ICSA. The significance of this failure is not clear since these authors did not study congenital rubella but merely deaf patients. Methodological problems may also have been involved.

Since the prevalence of diabetes is so much higher among our congenital rubella patients than in the general population (at least 12.4% – 30 out of 242 – versus 0.2%) [16], it may be assumed that the difference is caused either by an increase of the penetrance of the susceptibility genotype(s) or of the number of phenocopies or by both [14]. An important increase in the number of phenocopies (i.e. disease without genetic susceptibility) should have resulted in a substantial reduction in the strength of the HLA associations which is not noted. Thus, it is more likely that the severe intra-uterine infection with the virus, rubella, could push the penetrance of the common Type 1 diabetic-susceptibility genotype towards 100% [17]. Thus, our cohort of congenital rubella patients are an identifiable group in whom we can advantageously study immunological mechanisms operative prior to the development of diabetes, including the roles of subsets of T-lymphocytes and of the ICSA. Such studies could then provide a rational basis for intervention protocols aimed at reducing the expression of this severe disease and its host of long-term complications in other, and perhaps all settings.

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