Pre-Type 1 (insulin-dependent) diabetes: common endocrinological course despite immunological and immunogenetic heterogeneity

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Summary. In an ongoing prospective study 32 individuals have been evaluated for insulin secretory dynamics, islet cell antibodies and HLA antigens, during the preclinical phase of Type 1 diabetes mellitus. Twenty-four out of the 32 subjects were islet cell antibody-positive. To date, 14 subjects (10 islet cell antibody-positive, four islet cell antibody-negative) have progressed to develop overt diabetes. Several patterns of HLA-DR expression were noted (DR3/DR4, DR3/DR3,

DR3/x, DR3/DR1, DR4/x, DR4/DR7, DR5/DR7, DR1/DR7 and DR1/DR2). Irrespective of differences in islet cell antibody status or HLA-DR alleles, pre-diabetic individuals exhibited a similar slow course of progressive β -cell dysfunction.

Key words: Type 1 diabetes, islet cell antibody, HLA antigens, heterogeneity.

There is increasing evidence that Type 1 (insulin-dependent) diabetes is a slow autoimmune disease [1, 2]. During the preclinical phase, we have described progressive loss of first phase insulin release in response to intravenous glucose in five monozygotic twins, one first degree relative and three subjects who presented with transient glycosuria [3–6]. Following these studies, we have screened first degree relatives of Type 1 diabetic patients for cytoplasmic anti-islet antibodies and have studied insulin secretion of the antibody-positive relatives. In addition, we have evaluated patients referred to us with a history of transient glycosuria or asymptomatic mild glucose intolerance. So far, we have identified and studied 32 non-obese individuals; 14 have developed overt diabetes and 18 are non-diabetic, but fall into one or more high risk categories (islet cell antibody-positive and/or Ia T cell-positive and/or progressive and profound abnormality of first phase insulin secretion). In this study, we present evidence that all patients progressively lose their first phase insulin release prior to overt diabetes, despite differences in DR alleles expressed and/or detectable cytoplasmic anti-islet cell antibodies.

Subjects and methods

Subjects

The following groups of subjects at increased risk for the development of Type 1 diabetes were included in this analysis: (1) monozygotic twins, initially discordant for Type 1 diabetes ($n = 24$, maximum follow-up 22 years); (2) first degree relatives of Type 1 diabetic patients ($n = 70$, maximum follow-up 22 years); (3) first degree relatives of Type 1 diabetic patients in whom the follow-up has been of relatively

short duration ($n = 700$, maximum follow-up 18 months), and (4) patients referred for the evaluation of asymptomatic hyperglycaemia or transient glycosuria ($n = 6$).

The proband was classified as having Type 1 diabetes, primarily based on insulin dependency and ketosis-proneness under basal conditions; detection of islet cell antibodies in the index diabetic subject, presence of other autoimmune endocrinopathies and absence of obesity supported this assignment.

Coded sera stored at -20°C were tested for islet cell cytoplasmic antibodies by indirect immunofluorescence using frozen sections of human pancreas. Serum samples were read independently by two observers on a scale from 0 to + + + + without knowing their origin. On average, one serum sample was screened per year of follow up.

Intravenous glucose tolerance tests were performed by infusion dextrose (0.5 g/kg body weight) over a 2–4 min period. For oral glucose tolerance tests, 100 g glucose was administered. Whole venous blood glucose was measured by autoanalyser (Technicon Instruments, Tarrytown, New York, USA). Serum immunoreactive insulin was measured by a double-antibody technique. Early peak insulin response to intravenous glucose was calculated as the sum of the 1 min and 3 min values. Distribution of early peak insulin response (1 min + 3 min) during 407 intravenous glucose tolerance tests in more than 200 normal subjects (aged 8–77 years) was as follows: first percentile 46 mU/l; fifth percentile 67 mU/l; tenth percentile 81 mU/l; fiftieth percentile 169 mU/l.

Results

Of the 32 subjects included in this analysis, 24 were found to have circulating islet cell antibodies (ICA) (five twins, 16 first degree relatives and three subjects without a family history of diabetes). We found that approximately 2% of first degree relatives screened were ICA-positive. Out of the 24 ICA-positive subjects, 10 (four twins, five first degree relatives and one patient with a history of transient glycosuria) have already developed overt diabetes with fasting hyperglycaemia.

Table 1. Pre-Type 1 diabetes study

Patient no	Relation to proband	Proband: age at diagnosis of diabetes (years)	Follow-up of pre-diabetes (years)	Time ICA-positive pre-diabetes (years)	Percentile of normal intravenous glucose tolerance test	Oral glucose tolerance test result	Fasting blood glucose (mmol/l)	HLA-DR	Relation to HLA of proband	Clinical outcome (years) [references]
1	Monozygotic twin	13	9	> 8	27, 33, 27, 1, <1			4, X	Identical	Type 1 diabetes (1974) [3–6]
2	Monozygotic twin	12	11	5	75, 77, 79, 73, 38, 9			3, X	Identical	Type 1 diabetes (1980) [3–6]
3	Monozygotic twin	21	8	> 7	20, 8, 1				Identical	Type 1 diabetes (1981) [3–6]
4	Monozygotic twin	11	3	Negative	1			1, 7	Identical	Type 1 diabetes (1981) [3–6]
5	Monozygotic twin	8	2	> 2	<1, <1			3, 4	Identical	Type 1 diabetes (1983) [3–6]
6	Sibling	6	3	> 3	1					Type 1 diabetes (1977) [3–6]
7	Sibling	11	3	> 3						Type 1 diabetes (1976)
8	Sibling	4	1 month	> 1 month						Type 1 diabetes (1983)
9	Offspring	2	1 month	> 1 month					Haplo-identical*	Type 1 diabetes (1981)
10	Unrelated		7 months	> 7 months						Type 1 diabetes (1983)
11	Offspring	11	2 months	Negative	<1				Haplo-identical*	Type 1 diabetes (1983) [3–6]
12	Unrelated		3 months	Negative	<1					Type 1 diabetes (1983) [3–6]
13	Offspring	16	7 months	> 7 months	<1					Diabetes – diet treatment (1983)
14	Unrelated		1	Negative	<1					Diabetes Orinase (1983)
15	Monozygotic twin	54	> 3	> 3			4.29	3, X	Identical	Non-diabetic [3–6]
16	Sibling	14	> 1	> 1						Non-diabetic
17	Sibling	16	> 1	> 1	57, 79	Normal	4.18	3, 3	Identical	Non-diabetic
18	Sibling	7	> 1	> 1	41, 67, 77	Normal	3.69			Non-diabetic
19	Sibling	7	> 1	> 1			3.19			Non-diabetic
20	Sibling	11	> 6 months	> 6 months	<1, <1	Normal	4.24	1, 2	Identical	Non-diabetic
21	Parent	11	> 6 months	> 6 months	46, 54	Normal	4.51	1, 2	Haplo-identical*	Non-diabetic
22	Unrelated		> 6 months	> 6 months	9, 5, 3	Normal	3.85			Non-diabetic
23	Unrelated/Poly-glandular failure		> 1 month	> 1 month	69		2.97			Non-diabetic
24	Monozygotic twin	19	> 3	Negative	10	Impaired	5.23	3, X	Identical	Non-diabetic
25	Sibling	15	> 2 months	> 2 months	3		3.30			Non-diabetic
26	Offspring	13	> 1	> 6 months	9		3.69		Haplo-identical*	Non-diabetic
27	Offspring	45	> 3	> 2 months	<1	Impaired	6.11		Haplo-identical*	Non-diabetic
28	Offspring	35	> 5	> 1 month	<1, <1, <1	Indeterminate	5.17	4, 7	Haplo-identical*	Non-diabetic
29	Sibling	10	> 3 months	Negative	<1	Indeterminate	4.51	1, 3	Identical	Non-diabetic
30	Unrelated		> 10 months	Negative	<1, <1	Impaired	4.90			Non-diabetic
31	Monozygotic twin	37	17	Negative	91, 64, 45, 29	Normal	3.91	5, 7	Identical	Non-diabetic
32	Sibling	4	> 1 month	> 1 month	24			4, 4	Identical	Non-diabetic

* One haplotype in common; fasting blood glucose normal range: 3.9–5.6 mmol/l

In these subjects, ICA were detected between 8 years and 1 month before the diagnosis of clinical diabetes (Table 1). It is important to note that all except one of these patients (subject 3) were ICA-positive at the initial determination. There was no evidence of transient ICA positivity (ICA persisted until and beyond the onset of overt diabetes). Among the ICA-negative subjects, who have also been tested for β -cell function with long-term follow-up (19 twins, 65 first degree relatives), only one twin (subject 4) developed overt diabetes (one other twin, subject 24, has demonstrated impaired glucose tolerance).

The following is a summary of the features of the 'pre-diabetic' phase of Type 1 diabetes as shown by current studies of these 32 patients (Table 1):

- (1) ICA preceded the clinical diagnosis of overt insulin-dependent diabetes in the majority, but not all subjects studied (subjects 4, 11, 12).
- (2) During the 'pre-diabetic' phase, the presence of ICA was temporally correlated with a progressive linear decline in β -cell function (subjects 1, 2, 3, 5, 20, 22, 31).
- (3) Progressive loss of first phase insulin response to intravenous glucose was a good endocrine marker of early progressive β -cell dysfunction, with a virtual absence of this insulin release in the year preceding the development of overt Type 1 diabetes (subjects 1, 3, 4, 5, 11, 12).
- (4) Elevations in fasting blood glucose levels and gross abnormalities in oral glucose tolerance appeared only later during the prediabetic phase; a delayed and subsequently diminished insulin response to oral glucose preceded elevations of blood glucose during tolerance testing (subjects 1, 3, 4, 5, 6, 11, 12).
- (5) During the prediabetic period in all patients, there was a selectively greater decrement in insulin response to intravenous glucose, with a relatively preserved insulin release following intravenous tolbutamide, intravenous arginine, oral glucose and intravenous glucagon (subjects 1, 2, 3, 4, 5, 11, 12, 13, 14, 20, 30).
- (6) Some ICA-positive subjects had stable insulin release (subjects 1, 18, 21) of variable duration (subject 1).
- (7) HLA-DR3 and -DR4 alleles are not essential for the phenomenon of progressive β -cell dysfunction (subjects 4, 20, 21, 31). One patient (No.20) with HLA-DR2/DR1 (extremely rare in Type 1 diabetes) is an ICA-positive sibling, HLA-identical to two brothers with Type 1 diabetes and has progressive and profound loss of first phase insulin release to intravenous glucose.
- (8) Despite complete loss of first phase insulin release to intravenous glucose, some individuals still had normal oral glucose tolerance (subjects 20, 28). Following this, oral glucose tolerance and insulin response have been noted to deteriorate over a period of between 7 months (subject 10) to more than 5 years (subject 28).

Discussion

These and other studies indicate that the development of Type 1 diabetes can be subdivided into several stages, including (1) genetic predisposition without evidence of

active autoimmunity; (2) evidence of autoimmunity with normal insulin release; (3) progressive decline of first phase insulin release to intravenous glucose with normal blood glucose; (4) overt hyperglycaemia with some residual C-peptide secretion, and (5) complete β -cell destruction with no C-peptide secretion. The progression of autoimmune β -cell destruction can be monitored by serial evaluation of insulin secretory dynamics. First phase insulin release stimulated by intravenous glucose is lost at a time when significant insulin secretion can be obtained following oral glucose, intravenous glucose, intravenous tolbutamide and intravenous glucagon. We believe that this linear loss of glucose-stimulated insulin secretion is secondary to progressive β -cell destruction, similar to that found in studies of partially pancreatectomized rats [7].

Independent of specific HLA-DR alleles and ICA status, all the prediabetic patients seen so far have followed a similar endocrine course. Within families, sharing an HLA haplotype with the diabetic proband, but not the specific DR-type, seems to be important for the development of immunologically-mediated β -cell damage.

A screening programme for immunological markers in combination with serial measurements of β -cell function should permit the prediction of the onset of overt Type 1 diabetes in the majority of individuals destined to develop this disease. A similar strategy should facilitate the evaluation of immunotherapy initiated before the occurrence of overt Type 1 diabetes.

References

1. Irvine WJ, Gray RS, Steel JM (1980) Islet-cell antibody as a marker for early Type 1 diabetes mellitus. In: Irvine WJ (ed) *Immunology of Diabetes*. Teviot Scientific Publications, Edinburgh, pp 117-154
2. Gorsuch AN, Spencer KM, Lister J, McNally JM, Dean BM, Bottazzo GF, Cudworth AG (1981) Evidence for long prediabetic period in Type I (insulin-dependent) diabetes mellitus. *Lancet* 2: 1363-1365
3. Srikanta S, Ganda OP, Jackson RA, Gleason RE, Kalcany A, Garovoy MR, Milford EL, Carpenter CB, Soeldner JS, Eisenbarth GS (1983) Type I diabetes mellitus in monozygotic twins: chronic progressive beta cell dysfunction. *Ann Int Med* 99: 320-326
4. Srikanta S, Ganda OP, Eisenbarth GS, Soeldner JS (1983) Islet cell antibodies and beta cell function in monozygotic triplets and twins initially discordant for Type I diabetes mellitus. *N Engl J Med* 308: 322-325
5. Ganda OP, Srikanta S, Brink SJ, Morris MA, Gleason RE, Soeldner JS, Eisenbarth GS (1984) Differential sensitivity to beta cell secretagogues in early Type I diabetes mellitus. *Diabetes* (in press)
6. Srikanta S, Ganda OP, Gleason RE, Jackson RA, Soeldner JS, Eisenbarth GS (1984) Pre-Type I diabetes: linear loss of beta cell response to intravenous glucose. *Diabetes* (in press)
7. Bonner-Weir S, Trent DF, Weir GC (1983) Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71: 1544-1553

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