PAPERS AND SHORT REPORTS

Immune changes associated with insulin dependent diabetes may remit without causing the disease: a study in identical twins

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Abstract

Activation of T lymphocytes and islet cell antibodies were studied in two groups of insulin dependent diabetics and their non-diabetic identical cotwins. Group 1 comprised 12 "short term" twin pairs (diabetic twin diagnosed less than five years previously) in whom only a third of the cotwins were likely to develop diabetes; 10 of the 12 non-diabetic cotwins showed increased values of activated T lymphocytes, islet cell antibodies, or both. Group 2 comprised 10 "long term" twin pairs (diabetic twin diagnosed more than 11 years previously) in whom none of the non-diabetic cotwins was likely to develop diabetes; these pairs were selected because all the non-diabetic cotwins had shown islet cell antibodies at some time in the past, but only two still did so (one with an increased value of activated T cells). There was relative glucose intolerance in the cotwins of the short term group but not in those of the long term group.

Non-diabetic cotwins of diabetics may show the immune changes associated with insulin dependent diabetes and relative glucose intolerance, but these changes may remit without leading to diabetes.

Introduction

Insulin dependent diabetes is due to destruction of the insulin secreting islet cells of the pancreas, probably mediated by an

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immune process.¹ At the time of diagnosis of insulin dependent diabetes some 85% of patients have islet cell antibodies and increased concentrations of activated T lymphocytes-that is, T lymphocytes expressing the HLA-DR antigen on their surface.² ³ The HLA-DR antigen is coded by genes in the HLA region of the sixth chromosome. Genes in this region confer genetic susceptibility to insulin dependent diabetes.4 Genetic factors, however, cannot alone cause insulin dependent diabetes, as only a minority of identical cotwins of insulin dependent diabetics develop the disease.5

We speculated whether islet cell antibodies and activation of T lymphocytes could be found not only in insulin dependent diabetics but in their identical cotwins also, and if so whether these immune changes were confined to those cotwins who were likely to develop the disease. We therefore studied activated T cells and islet cell antibodies in two groups of identical twin pairs discordant for insulin dependent diabetes-namely, (a) a group of short term diabetics and their cotwins, of whom we calculated that no more than one third would develop the disease; and (b) a group of long term diabetics, none of whose cotwins were likely to become diabetic.

Patients and methods

We studied identical twin pairs discordant for insulin dependent diabetes. Monozygosity was established by clinical history and testing for 12 blood groups, as described.5

We calculate from our series of 250 pairs of identical twins that the overall concordance rate for insulin dependent diabetes is about 35%.5 The chance of the second twin becoming diabetic is greatest within five years of the diagnosis of diabetes in the first twin and falls to less than 3% after 11 years. Thus if a pair is discordant after this time it is highly unlikely that the nondiabetic twin will develop the disease.

We chose two groups of twin pairs.

"Short term" group-In 12 twin pairs the diabetic twin had been diagnosed within five years previously (mean 1.6 (SEM 0.4) years), so that no more than about four of the non-diabetic cotwins were expected to develop diabetes.

"Long term group"-There were 10 pairs in whom the diabetic twin had been diagnosed more than 11 years previously (mean 16.1 (1.4) years) and in whom none of the non-diabetic cotwins were expected to develop diabetes. These pairs were chosen from 50 long term discordant pairs in our series

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because the non-diabetic cotwins were known to have had islet cell antibodies. Actually there were 13 such pairs, but in three pairs one of the twins was unavailable for study. None of the 50 pairs was tested within four years of the diagnosis of diabetes in the affected twin so we cannot be certain that the non-diabetic cotwins in the remaining pairs had never had islet cell antibodies.

For this study each subject had immunological and glucose tolerance tests performed on a single occasion.

Of the 12 cotwins in the short term group, six were male, the mean age was 18.4 (SEM 2.2) years, and the mean body mass index (weight (kg)/height (m)²) 19.7 (SEM 0.7) (see table). Of the 10 cotwins in the long term group, five were male, the mean age was 28.2 (3.4) years, and the mean body mass index 21.9 (1.0).

None of the subjects was ill or receiving drugs at the time of study, apart from one short term diabetic twin (case 4; table) who needed maintenance steroid inhalers for asthma. The percentage of activated T cells from this asthmatic diabetic are not included in the results.

Two groups of healthy subjects served as controls for the glucose tolerance studies: nine had a similar age (mean 17·3 (1·5) years), sex distribution (three male), and body mass index (19·7 (1·3)) to the non-diabetic cotwins in the short term group, and eight had a similar age ($29\cdot9(1\cdot4)$) to the non-diabetic cotwins in the long term group. A control group of 22 normal healthy subjects (mean age 22·9(2·8) years; 12 male; body mass index 20·8(1·4)) was studied for levels of activated T lymphocytes.

Subjects or parents gave informed consent and the study was approved by the King's College Hospital ethical committee.

IMMUNOLOGICAL TESTS

Activated T lymphocytes—T Lymphocytes were purified from peripheral venous blood and the HLA-DR antigen (evidence of activation) detected with a fluorescein labelled anti-HLA-DR antibody. Peripheral blood mononuclear cells were obtained from heparinised venous blood by Ficoll-Triosil density gradient centrifugation. Rosetting T lymphocytes were isolated by incubation of the mononuclear cells with neuraminidase treated sheep red blood cells. The rosetted T lymphocytes were separated by centrifugation and freed from sheep red blood cells by hypotonic lysis with trometamol (TRIS) buffered ammonium chloride solution. This procedure yields a population of >95% T cells as determined by a pan T cell monoclonal antibody (Leu 4, Becton Dickinson). Contamination by cells

Details of both study groups

positive for surface immunoglobulins (B lymphocytes) and monocytes, detected by the monoclonal antibody MO2 (Coulterclone), accounted for less than 3% of the cells. Purified T lymphocytes were stained by direct immunofluorescence with the fluorescein labelled monoclonal antibody anti-HLA-DR (Becton Dickinson). This antibody obtained from clone L 243 recognises a non-polymorphic HLA-DR determinant.⁶ The purified T cells were incubated with saturating amounts of the antibody for 30 minutes at 4°C. After washing, the percentage of positive cells was determined using a Polyvar Reichert-Jung incident light microscope equipped for epifluorescence. At least 400 cells were counted and the results expressed as the percentage of fluorescein stained T cells minus 2% to allow for contamination by B lymphocytes and monocytes, which normally bear HLA-DR determinants.

Islet cell antibodies—Serum was tested by indirect immunofluorescence on a fresh group O pancreas for the presence of both cytoplasmic and complement fixing islet cell antibodies. We have recorded the results simply as positive or negative.

GLUCOSE TOLERANCE TESTS

After an overnight fast the non-diabetic cotwins and controls were studied at least 15 minutes after an intravenous cannula was inserted into the antecubital fossa under local anaesthesia. Basal blood samples were taken at -10 minutes and zero time, followed by a 75 g (or 1.75 g/kg, whichever was the less) oral glucose challenge. The glucose was dissolved in 300 ml water and drunk over four minutes. Further blood samples were taken at 10, 30, 60, 90, and 120 minutes after the glucose load. Whole blood glucose was measured by the glucose oxidase method (Yellow Springs analyser). Glycosylated haemoglobin (HbA_{1c}) was measured by electroendosmosis (Corning Instruments). The glucose response was calculated as the area under the glucose curve from basal to 180 minutes using a method of least squares.

STATISTICAL ANALYSIS

Results, expressed as mean and standard error of the mean (SEM), were compared by Student's *t* test (two tailed) for paired or unpaired observations. Results were considered significant at p < 0.05. Levels of significance with Wilcoxon's rank sum test and Student's *t* test were similar.

Twin pair No		Duration of disease (years)	Sex	Islet cell antibodies				%Activated T lymphocytes	
	Age (years)			Diabetics		Non-diabetics		Diabetics	Non-diabetics
				Cytoplasmic antibodies	Complement fixing antibodies	Cytoplasmic antibodies	Complement fixing antibodies		
				Short u	erm group				
1	23.70	0.22	F	+	+	-	-	5.72	4.10
2	12.80	0.21	м	+	+	+	+	8.32	9.90
3	18.90	1.54	м	+	-	+	-	7.21	6.33
4*	10.80	2.17	F	+	+	+	+	1.78*	4.14
5	20.20	1.20	м	+	-	-	-	5-47	2.34
6	11.75	0.67	м	+	+	+	+	13.90	10.00
7	11.25	0.33	M	+	+	+	+	12.35	9.88
8	19.00	0.20	F	+	+	+	+	9.00	7.63
9	15.40	1.83	M	+	_	+	+	5.56	2.71
10	14.20	3.17	F	+	+	_	_	6.86	9.91
ĩĩ	25.90	2.25	F	-	_	-		8.30	7.75
12	36.20	5.00	F	-	-	-	-	5.60	4.60
Mean/total SEM	18·37 2·16	1·62 0·41	6 F, 6 M	10	7	7	6	7·83† 0·87	6·60 0·85
				Long te	erm grout				
13	46.25	22.00	м	+		-	-	5.03	2.90
14	15.20	13.00	м	Ś	>	_	-	0	1.60
15	24.70	13.00	F	_	-	+	+	4.27	7.57
16	44.75	23.25	F	-	-	_	-	4.89	2.80
17	20.75	14.40	M	-	-	_	-	4.40	3.04
18	37.70	20.75	M		_		-	1.75	0.89
19	28.20	15.00	F	-		-	-	3.25	1.73
20	19.70	11.10	F	+	+			5.31	3.36
21	20.30	11.75	Ň	+	_	-	-	2.51	2.39
22	30.20	17.00	F	+	-	+	-	5.63	3.73
Mean/total SEM	28·20 3·40	16·10 1·40	5 F, 5 M	4	1	2	1	3·70 0·57	3·00 0·58

*Diabetic twin taking steroid inhalers for asthma.

†Mean excludes case 4.

Results

IMMUNOLOGICAL TESTS

Activated T lymphocytes

Figure 1 shows the concentrations of activated T lymphocytes detected in all subjects (except case 4).

Non-diabetic cotwins—Percentages of activated T cells higher than control values (mean and 2 SD $4 \cdot 1\%$) were found in nine of the 12 non-diabetic cotwins in the short term group and one of the 10 in the long term group.

Diabetic twins—Increased percentages of activated T cells were found in 11 of the 12 short term and six of the 10 long term diabetic twins. Mean values in the short term and long term diabetic twins were significantly higher than in their non-diabetic cotwins.



FIG 1—Percentage of activated T lymphocytes in insulin dependent diabetic twins and their non-diabetic cotwins in both short term and long term groups and in normal controls (stippled area). Bars are means.

Islet cell antibodies

Non-diabetic cotwins (table)—Islet cell antibodies were found in seven of the 12 non-diabetic cotwins in the short term group (six with complement fixing antibodies). All 10 cotwins in the long term group had had islet cell antibodies detected at some time after diagnosis of the diabetic twin (six with complement fixing antibodies) but in this study they were present in only two (one of whom had complement fixing antibodies) (fig 3).

Diabetic twins (table)—Islet cell antibodies were found in 10 of the 12 short term diabetics (seven of whom had complement fixing antibodies) and four of the 10 long term diabetics (one with complement fixing antibodies).

Activated T lymphocytes and islet cell antibodies

Non-diabetic cotwins—Increased percentages of activated T lymphocytes or islet cell antibodies were present at the time of study in 10 of the 12 non-diabetic cotwins in the short term group and two of the 10 in the long term group (table).

Diabetic twins—All 12 short term and seven of the 10 long term diabetics had either increased percentages of activated T lymphocytes or islet cell antibodies at the time of study (table).

GLUCOSE TOLERANCE TESTS (NON-DIABETIC COTWINS)

Glucose concentrations in the non-diabetic cotwins of the short term group were significantly higher at both 90 minutes $(5\cdot8\ (0\cdot4)\ v\ 4\cdot6\ (0\cdot2)\ mmol/l;\ 104\cdot5\ (7\cdot2)\ v\ 82\cdot9\ (3\cdot6)\ mg/100\ ml)$ and 120 minutes $(5\cdot6\ (0\cdot3)\ v\ 4\cdot5$ (0.3) mmol/l; 100.9 (5.4) v 81.1 (5.4) mg/100 ml) (fig 2). There was no difference between the cotwins in the short term group and their controls in fasting glucose concentrations (4.2 (0.1) v 4.3 (0.1) mmol/l; 75.7 (1.8) v 77.5 (1.8) mg/100 ml) or glycosylated haemoglobin values (6.4 (1.2) % v 6.1 (0.7) %).

In the non-diabetic cotwins of the long term group there were no significant differences in fasting blood glucose concentrations $(4 \cdot 0 (0 \cdot 2) v 4 \cdot 3 (0 \cdot 1) \text{ mmol/l}; 72 \cdot 1 (3 \cdot 6) v 77 \cdot 5 (1 \cdot 8) \text{ mg/100 ml})$, glucose concentrations at any other time point, or glycosylated haemoglobin values $(6 \cdot 4 (0 \cdot 2) \% v 6 \cdot 5 (0 \cdot 2) \%)$.



FIG 2—Mean blood glucose concentrations after oral glucose challenge in 12 nondiabetic cotwins of short term insulin dependent diabetics (\bigcirc — \bigcirc) and nine controls (\bigcirc — \bigcirc). Bars are SEM. *p<0.05.

Conversion: SI to traditional units-Glucose: 1 mmol/l~18 mg/100 ml.



FIG 3—Presence of islet cell antibodies in non-diabetic cotwins in relation to time from diagnosis of diabetic twin (long term group). Black areas: tested and positive for antibodies. Hatched areas: tested and negative for antibodies. White areas: not tested.

Discussion

This study shows that an immune process associated with insulin dependent diabetes and characterised by increased concentrations of activated T lymphocytes and production of islet cell antibodies may also occur in the non-diabetic cotwins of insulin dependent diabetics. Ten of the 12 short term non-diabetic cotwins showed increased concentrations of activated T lymphocytes, islet cell antibodies, or both, though no more than four would be expected to develop diabetes. In some cases these immune changes were accompanied by impairment of glucose tolerance indicating pancreatic damage.

Our observations suggest that these immune and metabolic changes associated with diabetes may remit without leading to insulin dependent diabetes because (a) increased concentrations of activated T lymphocytes were less prevalent in cotwins in the long term group than in those in the short term group; (b) islet cell

antibodies previously present in the 10 cotwins in the long term group had disappeared in all but two (fig 3); (c) impairment of glucose tolerance was seen in cotwins in the short term group but not in those in the long term group; and (d) a prospective study of 10 other cotwins of short term diabetics who had been discordant for more than 10 years showed an improvement in glucose tolerance over that time (Heaton et al, paper presented to the British Diabetic Association, Belfast, 1985).

We do not know what determines whether the damage to the islets will lead to complete destruction of the insulin secreting cells or to their recovery. If the destruction is an immune mediated process then it may be a question of the intensity of that process; it can hardly depend on genetic predisposition alone, as our results come from identical twin pairs.

If we could understand what limits the immune mediated damage in those twins in whom it had remitted we might learn how to arrest the process before it leads to diabetes.

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Effect of pregnancy on moderate renal failure in reflux nephropathy

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Abstract

During a 10 year study of women with reflux nephropathy 20 women had plasma creatinine concentrations in the range 0.2-0.4 mmol/l (2.3-4.5 mg/100 ml). Six experienced pregnancies exceeding 12 weeks' gestation. Pregnancy was associated with rapid deterioration in function in all six, resulting in end stage renal failure in four women within two years after delivery despite adequate control of blood pressure. Of the 14 women who did not have a prolonged pregnancy, four had periods of uncontrolled hypertension, all of which were related to non-compliance or loss from follow up, or both. Uncontrolled hypertension was also associated with accelerated renal failure, and all four women progressed quickly to end stage renal failure. The remaining 10 women were observed for from five to 10 years; in all 10 renal function deteriorated slowly, and none reached end stage renal failure within seven years.

It is concluded that pregnancy in patients with reflux nephropathy and moderately severe renal failure has a deleterious effect on renal function.

Introduction

The effect of pregnancy on renal disease is controversial. Some reports suggest that it is deleterious, especially if renal function is already impaired.¹⁵ Others have argued that the rapid decline in renal function seen in some patients reflects the clinical course of the

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underlying disease.⁶⁻¹¹ We have reported a high incidence of complications in gravidas with reflux nephropathy.¹² Since 1973 we have prospectively studied patients presenting with reflux nephropathy to determine the features associated with a poor prognosis. We now present our findings.

Patients and methods

During a prospective 10 year study of patients with reflux nephropathy six women with plasma creatinine concentrations of 0.2-0.4 mmol/l (2.3-4.5 mg/ 100 ml) experienced pregnancies exceeding 12 weeks' gestation. The course of renal disease during and after the pregnancy in these six patients was compared with that in 14 women with the same degree of renal impairment who did not experience prolonged pregnancy.

Reflux nephropathy was considered to be present if the patient had the typical radiological features of clubbed polar renal calices with overlying cortical scars.¹³ In all but one vesicoureteral reflux had been shown at some time on micturating cystography. In 15 from whom renal biopsy tissue was available, including the one in whom reflux had not been documented, histological findings were typical.¹⁴ Plasma creatinine and urea concentrations were determined by autoanalyser (Technikon SMAC).

Results

Twenty women with reflux nephropathy had plasma creatinine concentrations in the range 0.2-0.4 mmol/l (2.3-4.5 mg/100 ml) in the 10 years 1973-83.

PATIENTS WITH PROLONGED PREGNANCIES

Six patients (mean age 26.0 (range 21-39) years) experienced pregnancies exceeding 12 weeks (table). All had plasma creatinine concentrations exceeding 0.2 mmol/l (2.3 mg/100 ml) before 20 weeks' gestation. In one case the fetus died in utero at 22 weeks, and one woman was delivered at 30 weeks of a baby that died of hyaline membrane disease. In the remaining four cases the babies were delivered at 30-38 weeks' gestation; all four babies survived. Four patients were treated during pregnancy with plasma exchange, in two cases after intravenous heparin treatment.

Renal biopsy was performed during pregnancy in two cases and within one month after delivery in one. All specimens showed considerable focal