Pancreatic Islet-cell Antibodies in Diabetes Mellitus Correlated with the Duration and Type of Diabetes, Coexistent Autoimmune Disease, and HLA Type

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SUMMARY

In a study of 972 patients with diabetes mellitus, humoral pancreatic islet-cell antibodies (I.C.Ab.) were detected in highest prevalence in insulin-treated diabetics with (38 per cent) and without (22 per cent) associated overt organ-specific autoimmune disease (A.I.D.) where consideration was not given to the duration of diabetes. They were also detected in 8 per cent of diabetics treated with oral hypoglycemic agents (O.H.A.), but not in diabetics requiring diet alone and in only 0.5 per cent of 434 control subjects. Six per cent of 522 patients with overt organ-specific A.I.D. but not diagnosed to be diabetic had I.C.Ab.s. I.C.Ab.s were present in the sera of 2 per cent of 157 first-degree relatives of I.C.Ab.-positive subjects.

In insulin-treated diabetics and, to a lesser extent, in diabetics not requiring insulin, the prevalence of humoral I.C.Ab. was strongly dependent on the duration of the diabetes, being 60 per cent during the first year from diagnosis in the insulin-treated group and falling to 20 per cent at two to five years and to 5 per cent at 10-20 years. The prevalence of I.C.Ab. in insulin-treated diabetics showed no correlation with the patient's age at the time of testing when the duration of diabetes was taken into account.

Diabetics who did not require insulin for treatment but who were I.C.Ab.-positive showed a significant tendency to subsequently require insulin and to have a higher prevalence of other autoantibodies than insulin-independent diabetics who were I.C.Ab.-negative.

Persistence of I.C.Ab. for more than five years from diagnosis of diabetes was associated with coexistent overt organ-specific A.I.D. and with HLA-B8, A1, and A1 + B8.

DIABETES *26*:138-47, February, 1977.

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Accepted for publication November 2, 1976.

Pancreatic islet-cell antibodies (I.C.Ab.) were first described in the sera of patients with polyendocrine disease associated in the majority with insulin-treated diabetes determined by the indirect immunofluorescence test and cryostat sections of human pancreas.[1,2] Subsequently, serum I.C.Abs have been detected in about 50 per cent of young recently diagnosed

insulin-treated diabetics by the same technique.[3] Antibodies reactive with live tissue-cultured human insulinoma cells have been described in the sera of 34 out of 39 insulin-treated diabetics.[4]

An increased prevalence of the serologically defined histocompatibility antigens B8 and W15 have been reported in insulin-treated or juvenile-onset diabetes.[5-8] The prevalence of CW3 has also been reported to be increased in juvenile-onset diabetes.[9]

The purpose of the present study was to determine more precisely what correlations might exist between the prevalence of I.C.Ab. and the age of onset, the duration and the type of diabetes, sex, and coexistence of other evidence for organ-specific autoimmunity, and the HLA type.

PATIENTS AND METHODS

Patients

Diabetics—Autoantibody studies

Sera or plasma from 952 diabetic patients attending the Diabetic Out-patient Departments of the Royal Infirmary and the Royal Hospital for Sick Children, Edinburgh, were tested for gastric, thyroid, and pancreatic autoantibodies. Diabetics were classified according to the treatment they required for diabetic control. Control in this context signifies absence of thirst or polyuria, no ketonuria or heavy glycosuria, steady weight in nonobese subjects, and midmorning glucose less than 14 mmol/L. Patients were not treated with insulin unless it was thought that there was no reasonable chance that control might be achieved by diet with or without oral hypoglycemic agents.

The patients comprised:

(a) 628 insulin-treated diabetics with a female-to-male ratio of 1.0:1.0 and age distribution as shown in figure 1A. These included 40 patients with overt organ-specific autoimmune disease (A.I.D.). A.I.D. is defined as one or more of the following: pernicious anemia, thyrotoxicosis, primary hypothyroidism, and idiopathic Addison's disease. In all cases, clinical diagnosis was confirmed by such laboratory studies as were available at the time of diagnosis.

(b) 217 diabetics treated by diet and oral hypoglycemic agents (O.H.A.) with a female-to-male ratio of 1.2:1.0 and age distribution as shown in figure 1B. These included 17 patients with A.I.D.

(c) 107 diabetics treated by diet alone, with a female-to-male ratio of 1.0:1.0 and age distribution as shown in figure 1C. These included two patients with overt A.I.D.

Sera or plasma from the above were collected over a 12-year period and stored at −20° until tested. In 43 patients whose serum was positive for I.C.Ab., an interval of five years or more (range 5-12, mean 8 years) had elapsed, so these patients were recalled and retested. These sera were titrated for I.C.Ab. by use of doubling dilutions from neat strength.

Where I.C.Ab. prevalence has been specifically compared between diabetics with and without A.I.D. (tables 1 and 3), the group of insulin-treated diabetics with A.I.D. has been expanded by the addition of 20 diabetics with A.I.D. whose sera were referred to our laboratory for autoimmune studies.

Diabetics—HLA studies

Those I.C.Ab.-positive diabetics, and insulin-treated diabetics who were I.C.Ab.-negative within three months of diagnosis, who agreed to further study, were HLA-typed. They comprised 100 I.C.Ab.-positive (see table 6) and 22 I.C.Ab.-negative subjects.

TABLE 1

I.C.Ab. prevalence in different clinical conditions, irrespective of the duration of the disease in question

Clinical Condition			
Diabetes treated with insulin			
With Autoimmune Disease	23/60	38%	
with thyrotoxicosis	5/24		21%
with primary hypothyroidism	4/7		57%
with pernicious anaemia	3/6		50%
with thyrotoxicosis + PA	1/2		50%
with Addison's disease	7/15		47%
with Addison's disease and other A.I.D.	3/6		50%
Without Autoimmune Disease	127/588	22%	
Diabetes treated with OHA's			
With Autoimmune Disease	2/17	12%	
Without Autoimmune Disease	14/200	7%	
Diabetes treated with diet			
With Autoimmune Disease	0/2	0%	
Without Autoimmune Disease	0/105	0%	
Autoimmune disease without diabetes			
Thyrotoxicosis	5/204		2%
Primary hypothyroidism	2/43		5%
Pernicious anemia	3/28		11%
Thyrotoxicosis + PA	2/18		11%
Primary hypothyroidism + PA	3/16		19%
Addison's disease	7/169		4%
Addison's disease and other A.I.D.	7/44		16%
	29/522	5.6%	
Tuberculous Addison's disease	0/63	0%	
First-degree nondiabetic relatives of 60 I.C.Ab.- +ve subjects (156 diabetics, 4 nondiabetics)	4/157	2.5%	
Control population	2/434	0.5%	

Nondiabetics—Autoantibody studies

Sera or plasma from the following comparison groups were tested for I.C.Ab.:

434 control subjects with a female-to-male ratio of 1.1:1.0 and age distribution as shown in figure 1D. They consisted of 260 blood donors, 100 unrelated healthy friends of patients, and 74 hospital controls. None of these subjects were known to have endocrine disease.

157 first-degree nondiabetic relatives of I.C.Ab.-positive subjects.

522 patients with A.I.D. and 63 patients with tuberculous Addison's disease whose sera were referred to our laboratories for autoantibody studies.

Nondiabetics—HLA studies

300 healthy Caucasians from the Oxford area served as HLA-typing controls.

METHODS

Autoantibody Tests

I.C.Ab.s were detected by the indirect immunofluorescence test as previously described.[2] Fresh postmortem snap-frozen pancreatic tissue of blood group 0 and antihuman IgG-FITC (Wellcome) were used. The results were read in a Leitz orthoplan microscope fitted with a mercury-vapor lamp and Pleom illuminator with KG1, BG38, KP490 × 2, GT 475, TK510 dichroic mirrors, and K515 filters. Humoral antibodies to thyroid-cell cytoplasm, to parietal cell cytoplasm, and to thyroglobulin were tested for as previously described.[10] Patients with an antithyroglobulin titer of 25 or more or with antibodies to thyroid cell or gastric parietal-cell cytoplasm were denoted thyrogastric antibody-positive.

HLA Typing

The standard NIH-Terasaki microlymphocytotoxicity test and the same antisera were used throughout to determine the following antigens: HLA-A1, 2, 3, 9, 10, 11, 28, 29 -AW 23, 24, 25, 26, 30, 31, 32, 33 of the A series; HLA-B5, 7, 8, 12, 13, 14, 18, 27-BW 35, 40, 15, 16, 17, 21, 22, 37, 38, 39, 41 of the B series; and HLA-CW1, 2, 3, 4, 5 of the C series. The antigens of particular interest in this study were HLA-A1, HLA-B7, HLA-B8, and HLA-B15. A1, B7, and B8 were determined by three and BW15 by four well-defined antisera, respectively.

Data Analysis

All probability values were derived by use of the chi-squared test with Yates' correction unless otherwise stated.

RESULTS

The prevalence of humoral I.C.Ab. in different clinical groups with and without organ-specific A.I.D. is shown in table 1, irrespective of the duration of the diseases in question. The difference in prevalence of I.C.Ab. in insulin-treated diabetics with (38 per cent) and without (22 per cent) A.I.D. is statistically significant ($P < 0.01$). Only 8 per cent of diabetics treated with oral agents and no diabetic treated by diet alone had the antibody. Nondiabetics with A.I.D. and nondiabetic relatives of I.C.Ab.-positive subjects had higher prevalence of I.C.Ab. (5.6 per cent and 2.5 per cent, respectively) than the control population (0.5 per cent).

Twenty-four of the 324 diabetics in groups b and c required insulin treatment for diabetic control before the end of the study. These comprised seven out of 16 I.C.Ab.-positive and only 17 out of 308 I.C.Ab.-negative diabetics. Mean age at diagnosis and duration of follow-up were comparable in I.C.Ab.-positive and -negative groups. The correlation between I.C.Ab. positivity in diabetics initially treated without insulin and the subsequent requirement for insulin is statistically significant ($P < 10^{-6}$). Morover, as the mean period between diagnosis and the commencement of insulin therapy in the seven I.C.Ab.-positive patients was three years, four months (range 10 months-eight years, one month), and as six of the remaining nine I.C.Ab.-positive patients were within this period from diagnosis, it may be anticipated that more of these will eventually require treatment with insulin.

Figures 1A and 1B show that I.C.Ab. occurred in all age groups of insulin-treated diabetics and diabetics treated with oral agents. The duration of diabetes profoundly affects I.C.Ab. prevalence in both insulin- and oral-agent-treated diabetics (figures 2A and 2B). The prevalence in newly diagnosed diabetics requiring insulin for treatment was 65 per cent. This value was maintained during the first month from clinical diagnosis but thereafter fell, so that it was about 55 per cent at 1-12 months, 40 per cent at one to two years, 20 per cent at two to five years, 10 per cent at 5-10 years, and 5 per cent at 10-20 years. As duration increased still further, there was some flattening out of the curve, but at over 20 years from diagnosis only 3 per cent of insulin-treated diabetics were I.C.Ab.-positive.

The possible effects of age, sex, and coexistent organ-specific autoimmune disease on the prevalence of I.C.Ab.s in insulin-treated diabetics were analyzed

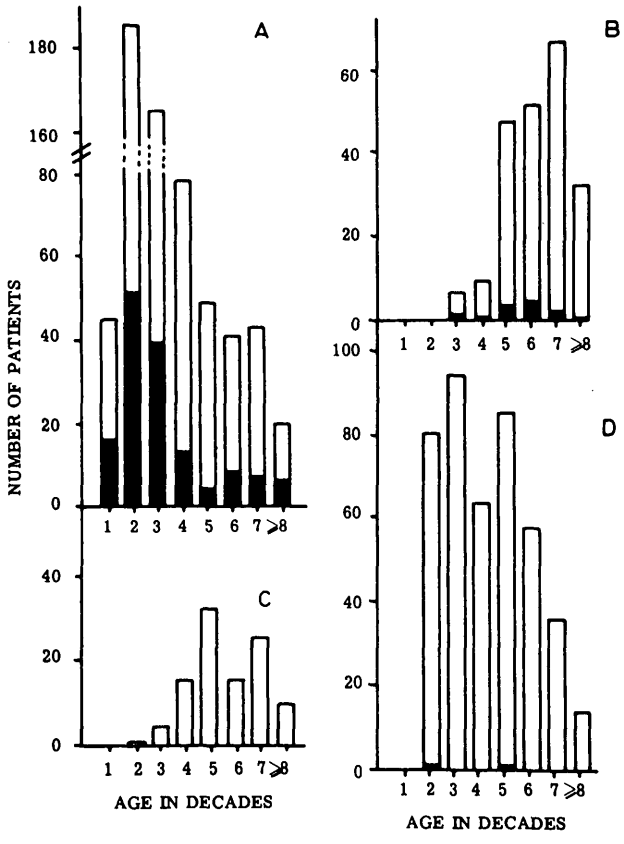

FIG. 1. Prevalence of I.C.Ab. in diabetics and in control subjects according to the patient's age at the time of testing. A. 628 insulin-treated diabetics; B. 217 diabetics requiring oral hypoglycemic agents (OHA); C. 107 diabetics requiring diet alone; D. 434 control subjects. ■ I.C.Ab.-positive □ I.C.Ab.-negative

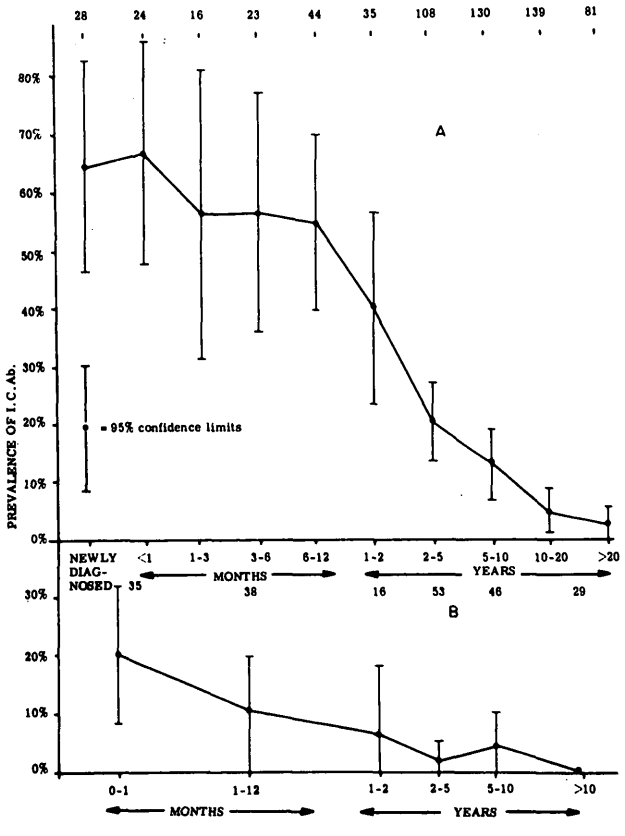

FIG. 2. Prevalence of I.C.Ab. based on the duration of diabetes. A. 628 insulin-treated diabetics; B. 217 diabetics requiring OHA.

TABLE 2

Comparison of the prevalence of I.C.Ab. in male and in female diabetics treated with insulin according to the duration of the diabetes

	Duration from diagnosis of diabetes	
	<1 year	>5 years
Male	38/76 = 50%	11/175 = 6%
Female	42/59 = 71%	16/175 = 9%
	P <0.05	N.S.

in patients who had had diabetes for less than one year or over five years. As shown in figure 3, I.C.Ab. prevalence remains constant at about 60 per cent during the first year of diabetes throughout the first four decades of life. The numbers in the older groups were too small for analysis, but over-all I.C.Ab. prevalence appears to be as high or higher than in younger insulin-treated patients. Over five years from diagnosis, there was no clear effect of age on I.C.Ab. prevalence, though again there might be a slight tendency for increased prevalence in the older groups.

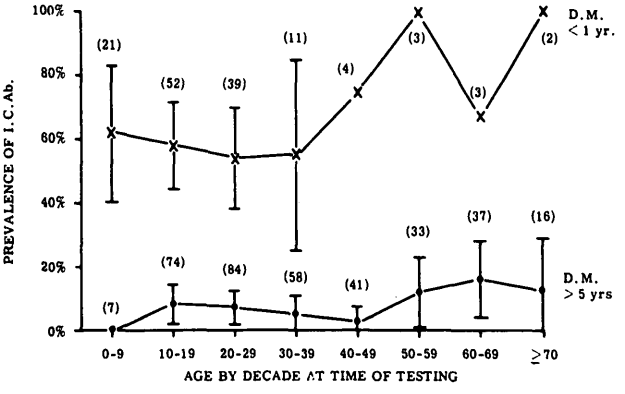

FIG. 3. Prevalence of I.C.Ab. in insulin-treated diabetics of short (<1 year) and of long (>5 years') duration according to the patient's age at the time of testing.

The higher prevalence of I.C.Ab. in female than in male insulin-treated diabetics was statistically significant only within a year of diagnosis (table 2).

The prevalence of I.C.Ab. in insulin-treated diabetics with and without coexistent overt A.I.D. is com-

TABLE 3

Prevalence of I.C.Ab. in insulin-treated diabetics with and without associated overt organ-specific autoimmune disease and according to the duration of the diabetes

	Duration from diagnosis of diabetes	
	<1 year	>5 years
With other autoimmune disease	8/13 = 62%	9/34 = 26%
Without other autoimmune disease	71/126 = 56%	22/318 = 7%
	N.S.	P <0.001

pared in table 3. The prevalence of I.C.Ab. within a year of diagnosis is apparently the same in both groups (about 60 per cent), but over five years from diagnosis patients with coexistent A.I.D. have a significantly higher antibody prevalence. However, even in this group, the prevalence of I.C.Ab. is less than half the value of a similar group within a year of diagnosis.

The prevalence of thyroglobulin antibodies and of gastric cytoplasmic antibodies increases in both the normal population and in insulin-treated diabetics with age and is higher in females than in males.[10] Table 4 shows the prevalence of thyroid and/or gastric antibodies in male and female insulin-treated diabetics of different ages according to I.C.Ab. status. Positive associations between the presence of I.C.Ab. and thyrogastric antibodies that reached statistical significance were observed only in male, not in female, patients.

Among diabetics treated without insulin (groups b and c), 11 out of 16 I.C.Ab.-positive patients (mean age 50 years range 18-82) as opposed to only 73 out of 308 I.C.Ab.-negative patients had thyrogastric antibodies (P <0.0005). The prevalence of thyrogastric antibodies in I.C.Ab.-positive diabetics treated without insulin is comparable to that in insulin-treated diabetics of similar age irrespective of their I.C.Ab. status.

The findings from the follow-up study of 43 I.C.Ab.-positive diabetics whose serum was retested after a mean of eight years are shown in table 5. The patients have been grouped according to the duration of diabetes when they were initially tested. Sixteen out of 27 diabetics I.C.Ab.-positive within a year of diagnosis, as against only one out of 10 diabetics I.C.Ab.-positive over five years from diagnosis, were negative on retesting. Furthermore, eight out of 10 in the latter group had maintained their titers, as against only five out of 27 in the former.

Of the 122 diabetics who were HLA-typed, 100 were I.C.Ab.-positive. Their clinical features are summarized in table 6. Within the I.C.Ab.-positive group, the frequencies of A1, B8, and BW15 were 52, 61, and 11 per cent, respectively (table 7). A1 and B8 are significantly increased from controls, but not BW15. The comparison with I.C.Ab.-negative insulin-treated patients is an interesting one as it sug-

TABLE 4

Prevalence of antibodies to thyroglobulin, thyroid cytoplasm, and/or gastric cytoplasm in 628 insulin-treated diabetics according to the patient's age at testing, sex, and the presence or absence of humoral I.C.Ab.

	Prevalence of thyrogastric antibodies					
	Male			Female		
	<40 yrs	>40 yrs	Total	<40 yrs	>40 yrs	Total
I.C.Ab.-positive	10/55	9/12	19/67	22/64	9/13	31/77
I.C.Ab.-negative	17/190	25/59	42/249	44/166	45/69	89/235
	P <0.05	P <0.05	P <0.01	N.S.	N.S.	N.S.

TABLE 5

Longitudinal study of 43 I.C.Ab.-positive diabetics followed up after an interval of 5-12 years (mean eight years)

		I.C.Ab. status when retested 5-12 years later					
					Average titer score‡		
Duration from diagnosis at 1st testing	No. I.C.Ab.-+ve at 1st testing	No. with same titer*	No. with reduced titer†	No. negative	1st testing	Retesting	Fall in score
≤ 1 year	27	5	6	16	4.0	1.3	2.7
1-5 years	6	1	4	1	4.5	2.0	2.5
≥ 5 years	10	8	1	1	4.6	4.6	0

*Same titer = ± one dilution.

†Reduced titer = change by more than a single dilution.

‡Scoring system: I.C.Ab. negative = 0. Positive at neat strength = 1. Positive 1 in 2 = 2. Positive 1 in 4 = 3 . . . Positive 1 in 128 = 8.

TABLE 6

Clinical features of the 100 I.C.Ab.-positive diabetics who were HLA-typed

Type of diabetes	No. HLA typed	Number with				
		Autoimmune disease	Thyro-toxicosis	Pernicious anemia	Primary hypothyroidism	Addison's disease
Insulin-treated	90	14	7	4	5	1
Diet ± OHA, but subsequently requiring insulin	3	0	0	0	0	0
Maintained throughout on diet ± OHA	7	2	1	1	0	0

TABLE 7

Prevalence of HLA antigens A1, B8, and BW15 in diabetics and in controls

	No. HLA-typed	% Prevalence of						
		A1	AW24	B7	B8	BW15	CW3	CW5
I.C.Ab.-positive diabetes	100	52**	13	13‡‡	61†§§	11	26	22
insulin-treated diabetics ± A.I.D.	93	51‡‡	14	12	61†§§	11	27	23
insulin-treated diabetics + A.I.D.	16	63‡‡	19	19	75	19	19	6
insulin-treated diabetics − A.I.D.	77	48‡‡	13	10	58§	9	29	26
diabetics not requiring insulin ± A.I.D.	7	71	0	28	57	14	14	14
<40 years at onset of diabetes	74	50‡‡	14	14	59‡	9	28	26
>40 years at onset of diabetes	26	58‡‡	12	12	65//	15	19	12
I.C.Ab.-negative* insulin-treated diabetics	22	32	36††	23	32	18	36	14
Oxford normal population	300	35	13	23	28	14	30	14

*within three months of diagnosis.
Uncorrected P-values for comparison with normal population: †<10^{-7} ‡<10^{-6} §<10^{-5} //<0.001 **<0.005 ††<0.01 ‡‡<0.05
Uncorrected P-value for comparison with I.C.Ab.-negative insulin-treated diabetics: §§<0.05

gests a higher frequency of A1 and B8 in the I.C.Ab.-positive diabetics, but the differences were significant only for B8. A larger group of insulin-treated patients who are negative for I.C.Ab. within three months of diagnosis needs to be studied. The prevalence of A1, B8, and BW15 in I.C.Ab.-positive diabetics, regardless of their treatment, was at least as high in those diagnosed after 40 years of age as in those diagnosed before that age.

On the other hand, B7 was significantly less prevalent in I.C.Ab.-positive diabetics than in the normal population. There was a tendency for AW24, BW15, and CW3 to be more prevalent in I.C.Ab.-negative insulin-treated diabetics.

There appears to be an association between B8, A1, and B8 + A1 and persistence of I.C.Ab., while there is a trend for BW15 to have a lower prevalence in diabetics who have been I.C.Ab.-positive for a long time (figure 4). The over-all pattern shown in figure 4 was similar when the 16 patients with associated overt autoimmune disease (see table 6) were excluded.

Twenty-nine diabetics whose serum was retested 5-12 years after being found I.C.Ab.-positive were among those HLA-typed. Again it was noted that the frequency of A1, B8, and A1 + B8 was increased in those patients in whom the titer of I.C.Ab. had not decreased with time compared with their frequency in those in whom the titer fell or became negative (table 8). Again, no correlation was seen between BW15 and the persistence of I.C.Ab. A negative correlation be-

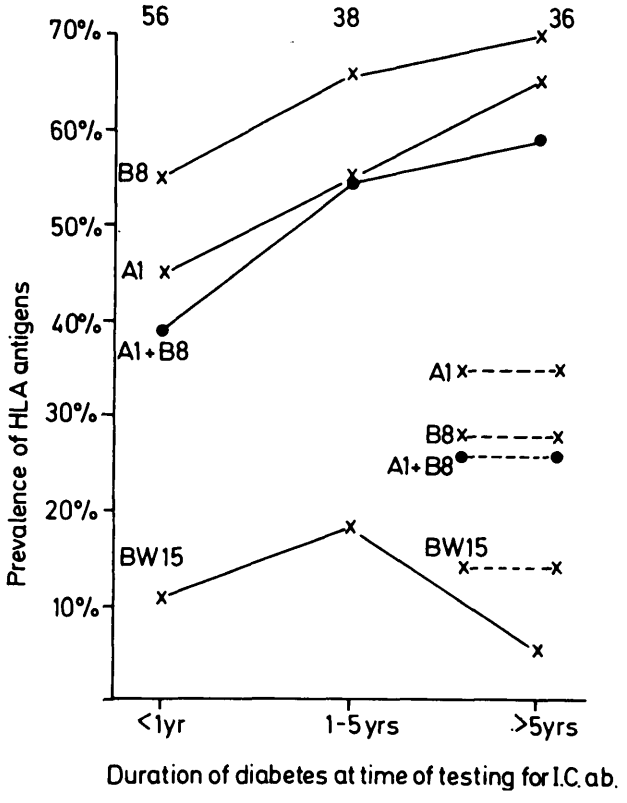

FIG. 4. Prevalence of HLA antigens A1, B8, and BW15 in 100 I.C.Ab.-positive diabetics (see table 6). Patients are entered at more than one point if they were shown to be I.C.Ab.-positive at more than one time interval from the diagnosis of the diabetes.

tween the persistence of the antibody and CW5 was noted.

DISCUSSION

In the initial papers, the occurrence of I.C.Ab. was largely confined to insulin-treated diabetics with polyendocrinopathy.[1,2] The present paper confirms, in a much larger series, that when diabetics are considered over all and without consideration of the duration of the disease, this group does have the highest prevalence of I.C.Ab. (38 per cent). One of the most striking features of the I.C.Ab. studies in insulin-treated diabetics is, however, the inverse relationship between the prevalence of these antibodies and the duration of the diabetes. The prevalence of I.C.Ab. in insulin-treated diabetes of more than five years' duration was higher in those patients with associated overt organ-specific A.I.D. (26 per cent) than in those without (7 per cent), indicating that I.C.Ab. persists longer in patients with a trait towards A.I.D. However, when insulin-treated diabetics were tested for I.C.Ab. within a year of the diagnosis of diabetes, there was no difference between those who had associated A.I.D. and those who had not (62 and 56 per cent, respectively). The prevalence of I.C.Ab. in newly diagnosed diabetics in whom insulin was necessary for control may be higher than the 65 per cent detected by immunofluorescence reported here, and the findings of Maclaren et al.[4] on insulinoma cells in culture as antigen suggest this. Also, it is not clearly known what the prevalence of I.C.Ab. is in the prediabetic state, although it is apparent that I.C.Ab. occurs in patients with A.I.D. without clinical diabetes (6 per cent) and in the nondiabetic first-degree relatives of I.C.Ab.-positive subjects (3 per cent). It is known that 39 per cent of these I.C.Ab.-positive subjects have diabetes (28 per cent) or lag-storage (11 per cent) glucose tolerance tests but that in the others the glucose tolerance test may remain normal for many years even in the presence of high titers of I.C.Ab. or become abnormal only during periods of metabolic stress.[11] The prevalence of I.C.Ab. in the control population was low, 0.5 per cent. Thus, the majority of diabetics who require insulin for therapy have I.C.Ab. at the beginning of their illness, but I.C.Ab. persists over a prolonged period only in those patients who have a particular genetic trait.

An indication of what that genetic trait might be comes from HLA studies. The present paper shows that the persistence of I.C.Ab. appears to be correlated in a Caucasian population with the presence of B8, so that I.C.Ab.-positive diabetics within a year of diagnosis have a B8 prevalence of 55 per cent, while those in whom I.C.Ab. was present more than five years after diagnosis have a B8 prevalence of 70 per cent. This correlation has also been shown for individual diabetic patients in whom I.C.Ab. titers were tested at intervals of 5-12 years (mean eight years).

TABLE 8

Correlation between the persistence of I.C.Ab. in 29 diabetics retested after 5-12 years and HLA type

I.C.Ab. status at retesting	Total number	Number with				
		A1	B8	A1+B8	BW15	CW5
Same titer*	11	9	9	9	0	0
Decline in titer†	18	6	6	4	1	8
P (Fisher exact test × 2)		<0.03	<0.03	<0.01	N.S.	<0.03

*± 1 dilution
†> 1 dilution

Although the data are preliminary, there is a trend for insulin-treated diabetics who are I.C.Ab.-negative within three months of diagnosis not to show any increase in the prevalence of B8, but possibly to have an increase in BW15, CW3, and especially AW24. Neither BW15 nor CW3 showed a positive correlation with the persistence of I.C.Ab. It is to be remembered, however, that most of the patients selected for HLA studies were I.C.Ab.-positive, and this is the probable reason why BW15 and CW3 were not shown to be increased in the insulin-treated diabetics reported in this paper. The increased frequency of A1 with persistent I.C.Ab. can be attributed to the known linkage-disequilibrium between A1 and B8 rather than to the location of a genetic locus determining I.C.Ab. formation between the A and B loci. CW5 shows a correlation with a fall in I.C.Ab. titer during follow-up of individual patients. B7 occurs with less-than-normal frequency in I.C.Ab.-positive diabetics and may be protective against the formation of this antibody, as suggested by others.[12,13] Thomsen et al.[14] have reported that the association between DW3 and juvenile diabetes is stronger than that with B8, but it remains to be seen if this also holds for I.C.Ab. and its persistence.

The studies reported here have been concerned with I.C.Ab. Cell-mediated autoimmunity to antigenic determinants of the endocrine pancreas and to insulin is a well-established feature of juvenile-onset or insulin-treated diabetes.[15-17] There is no correlation between the occurrence of I.C.Ab. and cell-mediated immunity in insulin-treated diabetics, at least when the duration of the diabetes is not taken into account.[18] It is not, therefore, established whether B8 or DW3 is linked with an immune-response gene concerned solely with I.C.Ab. or with cell-mediated immunity to pancreatic antigens as well.

The findings in relation to B8 and I.C.Ab. reported in the present paper contradict those by Lendrum et al.[19] in a small series of 139 insulin-treated diabetics aged 30 years or under at the onset of the disease. They found no association between the presence of I.C.Ab. and any particular HLA phenotype. However, only 33 of their patients had I.C.Ab. With such numbers it is not possible to make a correlation between the HLA phenotype and persistence of the antibody.

The association of diabetes and HLA can be attributed to any of the usual explanations for an association between HLA and a disease,[20] but the most attractive explanation in this particular instance, where the association appears to be linked to the presence of I.C.Ab., is provided by a postulated linkage between HLA and an immune-response gene. This immune-response gene might determine the response of this group of diabetics to an undefined pancreatic islet-cell antigen.

An important observation in the present study is that the prevalence of I.C.Ab. in subjects who develop diabetes that requires insulin for treatment after the age of 40 years (maturity-onset insulin-dependent diabetes) is essentially the same as that in juvenile-onset diabetics who require insulin for treatment, and so is the increased prevalence of B8 and A1 in these patients. Lendrum et al.[19] deny this, but their study of 18 late-onset insulin-treated diabetics of whom only four were I.C.Ab.-positive (because only a total of three out of 18 were studied for antibody at one year or less from diagnosis) does not contain sufficient evidence to make such a conclusion.

The transitory presence of I.C.Ab. in the majority of insulin-treated diabetics is in contrast to the time course of adrenal antibodies in idiopathic Addison's disease[21] or gastric antibodies in pernicious anemia or thyroid antibodies in Hashimoto thyroiditis or primary atrophic hypothyroidism, where the antibodies generally persist for many years. It suggests that some exogenous agent, such as a virus infection of the pancreatic islets, may be an important initial stage in the pathogenesis of insulin-dependent diabetes, giving rise to autoantibodies to the islets in the majority of such patients unless their genetic constitution is such as to prevent this (e.g., B7). In most cases, the antibodies are transient over a period of months to a few years, while in the minority the antibodies persist for many years, and these are the ones who have a particularly strong association with A.I.D. and B8. It is to be noted that the link with persistence of I.C.Ab. and B8 holds when patients with other overt autoimmune diseases are excluded. Among the diseases are primary hypothyroidism and pernicious anemia, neither of which has so far been associated with B8.[22-24] Whether or not humoral I.C.Ab. contributes to pancreatic islet-cell damage or is simply an epiphenomenon is not known; presumably the different immune mechanisms that have been described as possibly effective in relation to chronic autoimmune thyroiditis, including T-cell cytotoxicity and antibody-dependent cell-mediated cytotoxicity, as well as humoral antibodies, may all participate.[25]

The I.C.Ab. would appear to react with all the cells of the pancreatic islets—alpha, beta, and delta—thus

involving the glucagon- as well as the somatostatin-secreting cells. Studies are in progress to determine whether I.C.Ab.-positive diabetics have a different pattern of secretion of glucagon than do I.C.Ab.-negative diabetics.

The lack of clear association between the presence of I.C.Ab. and thyrogastric autoantibodies in insulin-treated diabetics correlates with the observation that thyrogastric antibodies are not, apparently, linked to B8.

The occurrence of I.C.Ab. in diabetics not requiring insulin treatment is correlated with a strong tendency for these patients to subsequently require insulin for metabolic control. Apart from its possible clinical usefulness in predicting the probability of a patient initially controlled on OHA subsequently requiring insulin, it does suggest that the concept of autoimmunity may provide a better way of classifying the different conditions that may make up the spectrum of diabetes, instead of the age of onset of diabetes and the type of treatment they require. From the evidence presented in this paper, it appears that diabetics requiring insulin treatment, whatever the age of onset, may have the same pathogenesis; and so may I.C.Ab.-positive diabetics not requiring insulin treatment (whatever the age of onset) but in a milder form. It is clear that the pathogenesis is linked with autoimmunity to pancreatic islet cells and with HLA-B8 and probably DW3. On the other hand, I.C.Ab.-negative diabetics not requiring insulin treatment (the majority of diabetics controlled on diet with or without oral hypoglycemic agents irrespective of the age of onset) has a different pathogenesis that is unrelated to autoimmunity or to any HLA phenotype so far studied. This concept of an autoimmune form of diabetes (largely synonymous with the need for insulin treatment irrespective of the age of onset) in contrast to other nonimmunologic forms of the disease (I.C.Ab.-negative insulin-independent diabetes) is supported by studies on the clinical association of insulin-treated diabetes with organ-specific autoimmune disease,[19] the higher prevalence of thyrogastric antibodies in insulin-treated diabetics,[10] and the evidence for cell-mediated immunity to the endocrine pancreas in insulin-dependent but not in insulin-independent diabetics.[13,14]

ACKNOWLEDGMENTS

This work was supported by grants from the Scottish Home and Health Department to Dr. W. J. Irvine and from the M.R.C. to Professor P. J. Morris and Dr. W. J. Irvine.

C. J. McCallum is a University of Edinburgh research scholar.

Mrs. Lesley McGrath and Miss Lesley Graham gave excellent technical assistance. We are grateful to the Blood Transfusion Service of the South Eastern Region of Scotland and to Mr. Henry, of the Princess Margaret Rose Orthopaedic Hospital, for control sera.

REFERENCES

[1]Bottazzo, G. F., Florin-Christensen, A., and Doniach, D.: Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet *2*:1279-83, 1974.

[2]MacCuish, A. C., Jordan, J., Campbell, C. J., Duncan, L. J. P., and Irvine, W. J.: Antibodies to pancreatic islet-cells in insulin-dependent diabetics with coexistent autoimmune disease. Lancet *2*:1529-33, 1974.

[3]Lendrum, R., Walker, G., and Gamble, D. R.: Islet-cell antibodies in juvenile diabetes mellitus of recent onset. Lancet *1*:880-83, 1975.

[4]Maclaren, N. K., Huang, S. W., and Fogh, J.: Antibody to cultured human insulinoma cells in insulin-dependent diabetes. Lancet *1*:997-1000, 1975.

[5]Singel, D. P., and Blajchman, M. A.: Histocompatibility (HLA) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. Diabetes *22*:429-32, 1973.

[6]Nerup, J., Platz, P., Andersen, O. O., Christy, M., Lynsgoe, J., Poulsen, J. E., Ryder, L. P., Thomsen, M., Staub Nielsen, L., and Svejgaard, A.: HLA antigens and diabetes mellitus. Lancet *2*:864-66, 1974.

[7]Cudworth, A. G., and Woodrow, J. C.: Evidence for HLA linked genes in "juvenile" diabetes mellitus. Br. Med. J. *3*:133-35, 1975.

[8]Nelson, P. G., Pyke, D. A., Cudworth, A. G., Woodrow, J. C., and Bachelor, J. R.: Histocompatibility antigens in diabetic identical twins. Lancet *2*:193-94, 1975.

[9]Schernthaner, G., Mayr, W. R., Pacher, M., Ludwig, H., Erd, W., and Eibl, M.: HLA-A8, W15 and T3 in juvenile onset diabetes mellitus. Horm. Metab. Res. *7*:521-22, 1975.

[10]Irvine, W. J., Clark, B. F., Scarth, L., Cullen, D. R., and Duncan, L. J. P.: Thyroid and gastric autoimmunity in patients with diabetes mellitus. Lancet *2*:163-68, 1970.

[11]Irvine, W. J., Gray, R. S., and McCallum, C. J.: Pancreatic islet-cell antibody as a marker for asymptomatic and latent diabetes and prediabetes. Lancet *2*:1097, 1976.

[12]Van de Putte, I., Vermylen, C., Decraene, P., Vlietinck, R., and van den Berghe, H.: Segregation of HLA-B7 in juvenile onset diabetes mellitus. Lancet *2*:251, 1976.

[13]Ludwig, H., Schernthaner, G., and Mayr, W. R.: Is HLA-B7 a marker associated with a protective gene in juvenile-onset diabetes mellitus? N. Engl. J. Med. *294*:1066, 1976.

[14]Thomsen, M., Platz, P., Andersen, O. O., Christy, M., Lynsgoe, J., Nerup, J., Rasmussen, K., Ryder, L. P., Staub Nielsen, L., and Svejgaard, A.: MLC typing in juvenile diabetes mellitus and idiopathic Addison's disease. Transplant Rev. *22*:125-47, 1975.

[15]Nerup, J., Andersen, O. O., Bendixen, G., Egeburg, J., Gunnarson, R., Kromann, H., and Poulsen, J.: Cell-mediated immunity in diabetes mellitus. Proc. R. Soc. Med. *67*:506-13, 1974.

[16]MacCuish, A. C., Jordan, J., Campbell, C. J., Duncan, L. J. P., and Irvine, W. J.: Cell-mediated immunity to human pancreas in diabetes mellitus. Diabetes *23*:693-97, 1974.

[17]MacCuish, A. C., Jordan, J., Campbell, C. J., Duncan, L. J. P., and Irvine, W. J.: Cell-mediated immunity in diabetes mellitus: lymphocyte transformation by insulin and insulin fragments in insulin-treated and newly diagnosed diabetics. Diabetes *24*:36-43, 1975.

[18]Irvine, W. J., MacCuish, A. C., Campbell, C. J., and Duncan, L. J. P.: Organ-specific cell-mediated autoimmunity in diabetes mellitus. Acta Endocrinol. (Copenhagen) *205* (Suppl.):65, 1976.

[19]Lendrum, R., Walker, G., Cudworth, A. G., Woodrow, J. C., and Gamble, D. R.: HLA-linked genes and islet-cell antibodies in diabetes mellitus. Br. Med. J. *1*:1565-67, 1976.

[20]Morris, P. J.: Histocompatibility systems, immune response, and disease in man. *In* Contemporary Topics in Immunobiology. New York, Plenum Press, Vol. 3, 1974, p. 141.

[21]Irvine, W. J., and Barnes, E. W.: Addison's disease, ovarian failure, and hypoparathyroidism. *In* Autoimmunity in Endocrine Disease, ch. 8, Irvine, W. J., Ed. London, Saunders, Clinics in Endocrinology, Vol. 4, 1975, pp. 379-434.

[22]Whittingham, S., Youngchaiyud, U., Mackay, I. R., Buckley, J. D., and Morris, P. J.: Thyrogastric autoimmune disease. Studies on the cell-mediated immune system and histocompatibility antigens. Clin. Exp. Immunol. *19*:289-99, 1975.

[23]Mawhinney, H., Lawton, J. W. M., White, A. G., and Irvine, W. J.: HL-A3 and HL-A7 in pernicious anaemia and autoimmune atrophic gastritis. Clin. Exp. Immunol. *22*:47-53, 1975.

[24]Goldstone, A. H., Voak, D., Cawley, J. C., and Irvine, W. J.: HLA antigens in pernicious anaemia. Clin. Exp. Immunol. *25*:352-54, 1976.

[25]Irvine, W. J.: Autoimmunity in Endocrine Disease. Proc. R. Soc. Med. 67:548-55, 1974.