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ANTI-PANCREATIC, CELLULAR
HYPERSENSITIVITY IN DIABETES MELLITUS.
ANTIGENIC ACTIVITY OF FETAL CALF
PANCREAS AND CORRELATION WITH
CLINICAL TYPE OF DIABETES

By

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Lymphocytic infiltration in and around the islets of Langerhans is a characteristic feature of juvenile diabetes mellitus of short duration (5, 6). The significance of this phenomenon still remains to be elucidated, but the observation is from the theoretic point easy to associate with the finding of anti-pancreatic, cellular hypersensitivity in diabetes mellitus (8), and both features suggest that cell-mediated immunity may play a role during the initial pathogenic phase of juvenile diabetes.

In our preliminary work the anti-pancreatic, cellular hypersensitivity was demonstrated in patients with diabetes mellitus by means of the leucocyte migration test (LMT) (2) with a preparation of duct-ligated, porcine pancreas as antigen. The number of patients with diabetes examined, however, was small.

The purpose of the present study has been:

1) By means of the LMT to assess the occurrence of anti-pancreatic, cellular hypersensitivity in diabetes, using a pancreatic antigen prepared from another species.

TABLE 1

Patients with Diabetes Mellitus and Control Persons classified according to Sex and Age.

Groups of patients	A	B	C	D
Number of patients	37	36	24	45
Number of males	22	17	10	24
Number of females	15	19	14	21
Age in years (mean)	7-44 (26)	11-57 (30)	43-78 (59)	18-87 (31)

Group A: Juvenile diabetes mellitus (diagnosis before 40 years of age) untreated or treated with insulin for less than one year.

Group B: Juvenile diabetes mellitus treated with insulin for one year or more.

Group C: Maturity onset diabetes (diagnosis after 40 years of age) untreated or treated with insulin.

Group D: Healthy control persons without family history of diabetes mellitus.

2) In a larger patient material to examine whether anti-pancreatic, cellular hypersensitivity is correlated to any particular clinical type of diabetes mellitus.

MATERIAL AND METHODS

The material comprises 97 patients with diabetes mellitus and 45 healthy control persons without a family history of diabetes (Table 1).

Pancreatic extract. The pancreas (Fig. 1) from 7 calf embryos of 16-20 weeks of gestation was aseptically removed and frozen at -30°C immediately after slaughtering. The glands were minced with forceps and scissors and homogenized in a Warren blender at 15000 r.p.m. for two min. The material was pooled in one batch and extracted in a tenfold volume of Hank's balanced salt solution for 24 hours at 4°C . After centrifugation ($6000 \times g$ for 20 min) the supernatant was transferred to ampoules and lyophilized. Before use the extract was redissolved in sterile water, standardized by protein determination a.m. Lowry, and kept in 5 ml ampoules at 4°C until use. New preparations were made every 14th day. The insulin concentration in the final extract was $50 \mu\text{U}$ per ml, as determined radio-immunologically by a double antibody method (4). For comparison, the insulin content of the fetal calf pancreas was 5 mg per g dry weight, as determined by the same double antibody method after acid-alcohol extraction.

Leucocyte migration test (LMT). The LMT was used for in vitro estimation of cellular hypersensitivity (2, 3, 9). In pilot experiments the highest, non-toxic concentration of the fetal calf pancreatic extract was found to be $5 \mu\text{g}$ protein

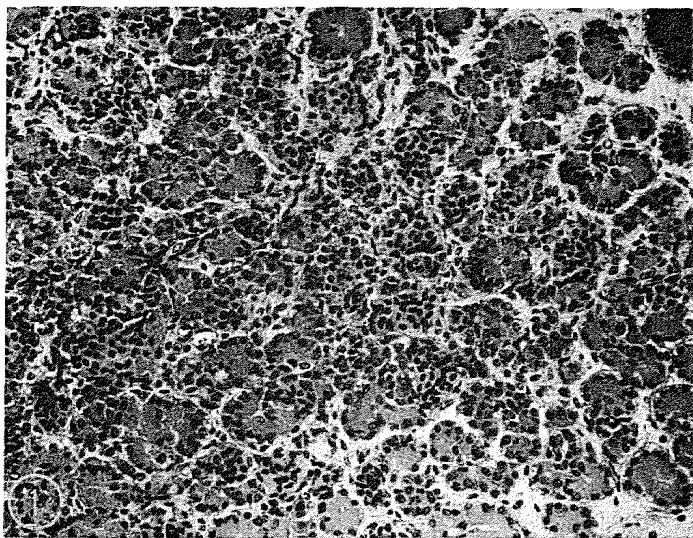


Fig. 1.

Fetal calf pancreas of 18 weeks of gestation. Hematoxylin-eosin. Magnitude: 500 $\times$. The clusters of dark and dense cells represent the endocrine part of the gland.

per ml culture medium, and this dose was used as routine. Comparable extracts of fetal calf liver and thymus were added in parallel cultures to ensure organ-specificity. A migration index (MI) indicates stimulation if above, and inhibition if below unity.

Insulin antibodies. The occurrence of insulin antibodies in serum from the patients and controls was examined by means of a double antibody technique (1).

RESULTS

With the fetal calf pancreatic extract as antigen, 26 of 97 patients with diabetes mellitus had MI-values beyond the 95 per cent range of 45 healthy controls. Calculations by use of Wilcoxon's test showed that the diabetic group differed significantly from the control group ($0.02 > p > 0.01$). The extracts of fetal calf liver and thymus did not induce migration

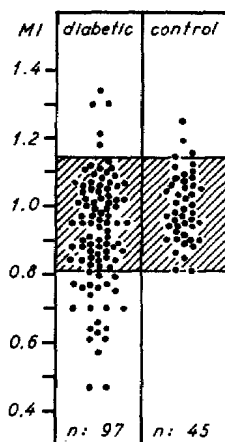


Fig. 2.

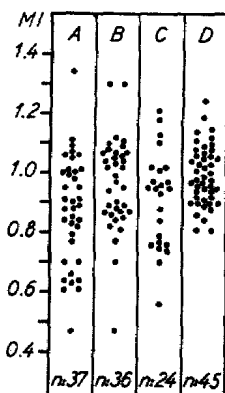
Leucocyte migration test in 97 diabetics and 45 controls. Hatched area: Mean MI $\pm$ 2 SD.

MI: Migration index =
area of antigen-containing cultures
area of antigen-free cultures.

inhibition (migration indices 0.80–1.13). In the control group (group D) the MI-values were uncorrelated to age and sex. The finding is interpreted as an indication of anti-pancreatic, cellular hypersensitivity in 27 per cent of the diabetics (Fig. 2).

Fig. 3 presents the results of the LMT in the patients ac-

Fig. 3.



Leucocyte migration test in 97 diabetics and 45 controls.

A: 37 juvenile diabetics untreated or treated with insulin for less than one year.

B: 36 juvenile diabetics treated with insulin for one year or more.

C: 24 maturity onset diabetics.

D: 45 healthy control persons without a family history of diabetes.

Statistical evaluation by use of Wilcoxon's test:

Group A ~ group D: $p < 0.01$

group B ~ group D: $0.1 < p$

group C ~ group D: $0.1 > p > 0.05$

group A ~ group B: $0.05 > p > 0.02$

group A ~ group C: $0.1 < p$

group B ~ group C: $0.1 < p$

TABLE 2

Some clinical Features of Diabetic Patients in Groups A, B, and C correlated to Leucocyte Migration Test with Fetal Calf Pancreatic Extract.

	MI within 95% normal range			MI beyond 95% normal range		
	A	B	C	A	B	C
Patient group						
Number of patients	27	31	13	10	5	11
Mean age in years	26	30	60	26	31	55
Distribution of:						
patients without insulin treatment	9	0	6	5	0	7
patients treated with insulin						
for less than 14 days	6	0	5	2	0	1
patients with predisposition	10	13	3	6	1	2

MI = Migration index.

95 % range (mean $\pm$ 2 SD) of 45 healthy control persons = 0.81-1.17 (0.99 $\pm$ 0.18).

cording to type of diabetes. Comparison between these groups by means of Wilcoxon's test reveals that group A (juvenile diabetes untreated or treated with insulin for less than one year) is significantly different from the control group. There is no significant difference between the control group and groups B (juvenile diabetics treated with insulin for one year or more) and C (maturity onset diabetes). The group of juvenile diabetics untreated or treated with insulin for less than one year (group A), however, is significantly different from the group of patients with juvenile diabetes treated with insulin for one year or more (group B), but not from the maturity onset diabetics (group C).

Table 2 shows some clinical details of the patients in groups A, B and C correlated to the MI-values. Abnormal MI-values were found in several patients untreated or treated with insulin for less than 14 days. Circulating insulin antibodies could not be demonstrated in sera from controls or diabetics untreated with insulin, and no correlation existed between insulin antibody titers and MI-values.

DISCUSSION

In a previous study a preparation of duct-ligated porcine pancreas was used as antigen in the LMT (8). In the present investigation the LMT with an extract of fetal calf pancreas shows abnormal migration indices in 26 of 97 patients with diabetes. The results confirm that diabetes mellitus is associated with anti-pancreatic, cellular hypersensitivity, and that fetal calf pancreas as well as porcine pancreas contain the antigenic component(s).

The precise nature of the antigen(s) is so far unknown, but since extracts neither of fetal calf liver and fetal calf thymus, nor porcine liver and porcine kidney (8) induce migration inhibition, the antigen(s) is presumably organ-specific and species-non-specific. Furthermore, since neither porcine nor bovine insulin induce migration inhibition (8), the immunological specificity is not directed against antigenic determinants on insulin.

In the present study fetal calf pancreas of 16–20 weeks of gestation was used for preparation of the antigen extract. This material was chosen, because the endocrine tissue of fetal calf pancreas at this stage of development constitutes one third of the total parenchyme and is capable of synthesizing insulin, immunologically identical to bovine insulin (10). As the fetal calf pancreas contains exocrine tissue as well, it is not possible from the present data to conclude that the antigenic activity is selectively associated with the endocrine pancreas.

Abnormal MI-values were found in patients not yet treated with insulin and were uncorrelated to the presence of insulin antibodies in sera of insulin-treated patients confirming the findings in our previous report (8).

Although abnormal migration indices were demonstrated in some patients with maturity onset diabetes, anti-pancreatic, cellular hypersensitivity was correlated preferably to the group of patients with juvenile diabetes, untreated or treated with insulin for less than one year. In this context it is interesting to note, that lymphocytic infiltration in the islets of Langerhans which may be considered an *in vivo* correlate to organ-

specific, anti-pancreatic, cellular hypersensitivity, has been described in maturity onset diabetes (7), but is preferably occurring in juvenile diabetes of short duration.

SUMMARY

By means of the leucocyte migration test the occurrence of anti-pancreatic, cellular hypersensitivity was examined in 97 diabetics and 45 healthy controls.

A fetal calf pancreatic extract was used as antigen with appropriate control preparations. A specifically altered in vitro reactivity consistent with a state of organ-specific, anti-pancreatic, cellular hypersensitivity, was demonstrable in 25 per cent of the patients. The anti-pancreatic, cellular hypersensitivity was predominantly found in patients with juvenile diabetes mellitus of short duration.

ACKNOWLEDGEMENTS

Supported by grants from The Danish Hospital Foundation for Medical Research, Region of Copenhagen, Greenland and the Faroe Islands and The Danish Medical Research Council.

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