Antipancreatic Cellular Hypersensitivity in Diabetes Mellitus

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SUMMARY

The occurrence of organ-specific, cellular hypersensitivity against pancreatic components was examined in twenty-two diabetics by means of the leucocyte migration test. An extract was prepared from pooled porcine pancreatic glands, in which atrophy of the exocrine tissue had been induced by ligation of the pancreatic duct. A specifically altered in vitro reactivity to the pancreatic preparation, consistent with a state of organ-specific, cellular hypersensitivity, was demonstrated in the diabetic group as compared to a control group. Intracutaneous injection of the same preparation in six diabetics with a positive in vitro reaction induced a typical delayed type reaction in four. DIABETES *20*:424-27, June, 1971.

The literature on diabetes mellitus from early this century contained sporadic descriptions of mononuclear cell infiltration in and around the islets of Langerhans in the pancreas of patients suffering from diabetes. These reports did not attract much attention until it was later established that round cell infiltration of the endocrine pancreas was a frequent feature especially of infantile diabetes mellitus of short duration.[1]

It has been emphasized[2] that the rarity of these histopathological changes is probably more apparent than real, as they are easily overlooked. In a recent, more extensive study, mononuclear cell infiltration, predominantly lymphocytes, could be demonstrated in and around the islets of Langerhans in 68 per cent of patients with juvenile diabetes mellitus of short duration.[3] As a qualitative phenomenon this cellular infiltration and the associated histopathological changes (in juvenile diabetes) cannot be distinguished from the ones found in autoimmune disorders in which organ-specific, humoral and/or cellular hypersensitivity is detectable, e.g. idiopathic Addison's disease.[4,5]

Attempts to demonstrate circulating antibodies specific to tissue components of normal, endocrine pancreas in sera from patients with diabetes mellitus have so far been unsuccessful.[6,7] For these reasons, it was decided to investigate whether organ-specific, cellular hypersensitivity against antigenic components of the islets of Langerhans could be demonstrated in vitro and in vivo in patients with diabetes mellitus.

MATERIAL AND METHODS

Twenty-two patients with diabetes mellitus (table 1) and eighteen normal controls (without known genetic disposition to diabetes) were examined. In five pigs (Danish land-race) a surgical ligation of the pancreatic duct was performed, and the pancreatic glands were removed eight weeks later. Macroscopically the glands were small and atrophic with dilated excretory ducts. In the light microscope a pronounced fibrosis and massive atrophy of the exocrine parenchyme was obvious, whereas the islets of Langerhans were well preserved. In the electron microscope considerable degenerative changes were found in the exocrine cells, but the subcellular structures of the endocrine tissue were normal.

The atrophic glands were cut into minute pieces with scissors, pooled in a single batch and homogenized (Potter-Elvehjelm) in a tenfold volume of 0.25 M sterile sucrose. All procedures were carried out at temperatures between 0 and 4° C. The homogenate was separated in three fractions by differential centrifugation. Fraction I (600 x g for 10 min.) contained fibrous tissue and undisrupted cells and was discarded. Electron microscopic examination showed that fraction II (5,000 x g for 20 min.) contained mitochondria, microsomal vesicles and fragments of collagen fibers. Fraction III (104,000 x g for 60 min.) contained all subcellular organelles or pieces of organelles, but no collagen. Secre-

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TABLE 1

Clinical summary of patients with diabetes mellitus

Case Number	Name	Sex	Age (years)	Interval between Clinical Diagnosis and Immunological Study (years)	Duration of Treatment Insulin (y = years; d = days)	Oral	LMT Migration Index
1	EA	F	65	15	—	15y	0.62
2	EMC	F	58	13	6y	1y	0.97
3	MSH	F	20	0	—	—	0.97
4	KH	F	21	3/12	3d	—	0.67
5	JH	F	11	2/12	7d	—	0.59
6	KMJ	F	25	10-6/12	10-6/12y	—	0.65
7	LL	F	13	1-7/12	1-7/12y	—	0.97
8	AWL	F	75	6	6y	—	0.64
9	MOP	F	45	8/12	4/12y	—	0.92
10	PB	M	23	1/12	1/12y	—	0.48
11	CBC	M	29	1/12	—	—	0.71
12	HHC	M	11	1/12	4y	—	0.85
13	TVD	M	18	11	11y	—	0.74
14	VGF	M	45	3	—	3y	0.45
15	KNH	M	27	19	19y	—	1.00
16	TJ	M	21	8	8y	—	1.01
17	CJ	M	20	2	3/12y	1-9/12y	0.68
18	JBJ	M	15	9/12	9/12y	—	0.52
19	VHJ	M	55	18	18y	—	0.67
20	FK	M	33	3/12	—	—	0.65
21	DN	M	21	7	—	—	0.65
22	SAV	M	44	8/12	—	8/12y	0.76

tory granules could not be demonstrated in fractions II and III. Fraction III was selected as standard preparation for evaluation of antigenic activity in the leucocyte migration test and was resuspended in 0.25 M sterile sucrose, the protein concentration being adjusted at 1 mg./ml. The preparation was stored in tubes containing 2 ml. at —20° C. Each portion was thawed only once. The concentration of insulin in fraction III was 25,000 μU./ml. determined by an immunological double-antibody technic.[8]

The leucocyte migration test (LMT)[9] was used as in vitro presumptive assay for detection of cellular hypersensitivity, using white cells from peripheral blood of patients and controls. In pilot experiments the highest nontoxic concentration of the pancreatic preparation (fraction III) was found to be 100 μg. protein/ml. culture medium. Comparable preparations of liver and kidney were added in parallel series to ensure organ-specificity. Furthermore, the LMT was performed in patients and controls with porcine and bovine insulin at various concentrations ranging from 15 to 450 μg./ml. culture medium.

In vivo assay of cellular hypersensitivity was performed by means of intracutaneous testing with porcine insulin and fraction III. Sterility was ensured by aerobic and anaerobic cultivations. In four nondiabetic controls and seven diabetics the following preparations were injected intracutaneously on the volar side of the forearm: (1) 1,200 μUnits porcine insulin, (2) 10 μg. protein of fraction III and (3) 50 μg. protein of fraction III. All injections were given in 100 μL. 0.9 per cent NaCl. The reactions were read after 10 min. and after 24, 48 and 72 hours. Infiltrations exceeding 5 x 5 mm. were considered positive. Plasma samples from the diabetics were examined for circulating insulin-binding antibodies by means of a double-antibody technic.[10]

RESULTS

The results of the leucocyte migration experiments using 100 μg. of fraction III as antigen are shown in figure 1. The migration indices (MI) of each diabetic patient are presented in table 1. The group of twenty-two diabetics is significantly different from the group of normal controls ($p < 0.001$). Twelve of the twenty-two diabetics (55 per cent) had MI's below 0.70.* Only one of the controls had an MI similarly low. Low MI's were found with equal frequency in male and female patients.

In five patients the MIs were found to be below or equal to 0.65 before insulin treatment was started. In none of these patients could insulin-binding antibodies

*Repetitive tests were performed in several cases and gave confirmative results.

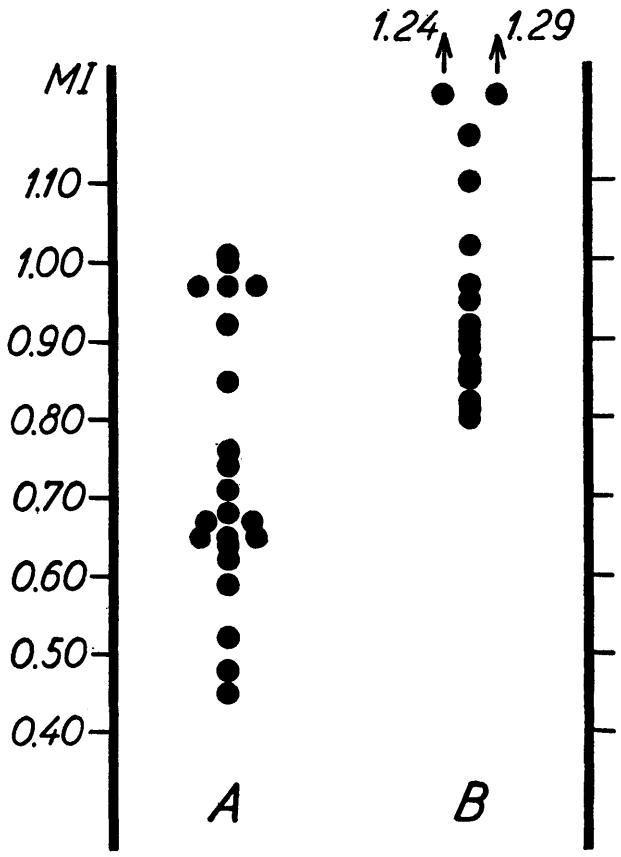

FIG. 1. Leucocyte migration test (LMT) with porcine pancreatic fraction III (see text) in (a) twenty-two patients with diabetes mellitus and (b) eighteen healthy controls.

be demonstrated. Inhibition of the leucocyte migration was not induced by porcine or bovine insulin, nor by the liver or kidney preparations (MI's ranging from 0.91-1.16).

Table 2 shows the results of the intracutaneous testing in seven diabetics and in four nondiabetic controls. In all persons examined, a typical wheal-and-flare reaction developed 10 min. after the injection of 10 μg. as well as 50 μg. of fraction III. 10 μg. of fraction III did not induce a cutaneous delayed type reaction, and neither did 50 μg. in the four controls nor in the one diabetic with an MI about 1.00 (case number 7, MI: 0.97). On the other hand a typical delayed type intracutaneous reaction developed after twenty-four hours in four of the six diabetics with low MIs.* Insulin, in a dose corresponding to the amount contained in 50 μg. of fraction III, did not induce delayed type reactions either in controls or patients.

DISCUSSION

Specific, antigen-induced inhibition of the migration of leucocytes is used as a presumptive in vitro correlate to cellular hypersensitivity.[11,12] We have used it previously in Addison's disease and have interpreted it to show organ-specific, species-nonspecific, cellular hypersensitivity in that disease.[4-5] Therefore the specifically altered in vitro reactivity of leucocytes from diabetics demonstrated in this study might reflect a cellular hypersensitivity against some antigenic component(s) present in fraction III. This fraction was prepared from porcine pancreas, which was made exocrine atrophic through ligation of the pancreatic duct.

The reaction must be considered organ-specific, since comparable preparations of liver and kidney did not induce specific inhibition of the leucocyte migration. Furthermore the insulin content of fraction III cannot be held responsible for the reaction, since neither porcine nor bovine insulin caused inhibition of the leuco-

*The intracutaneous reactions were still present after forty-eight hours and declining after seventy-two hours.

TABLE 2

Leucocyte migration test (LMT) and twenty-four hour intracutaneous reactions in seven patients and four controls

Case Number	Name	Sex	LMT Migration Index	Intracutaneous Test with (All Injections in 100 μL. 0.9 per cent NaCl) Fraction III (50 μg.)	Fraction III (10 μg.)	Porcine Insulin (1,200 μUnits)
1	EA	F	0.62	5 × 5 mm.	negative	negative
7	LL	F	0.97	negative	negative	negative
8	AWL	F	0.64	7 × 8 mm.	negative	negative
10	PB	M	0.48	6 × 8 mm.	negative	negative
17	CJ	M	0.68	7 × 9 mm.	negative	negative
19	VHJ	M	0.67	negative	negative	negative
21	DN	M	0.65	negative	negative	negative
Four controls		M	normal	negative	negative	negative

cyte migration in parallel experiments.

Thus the results suggest to us that an organ-specific, species-nonspecific, antipancreatic hypersensitivity of the cellular type is actually demonstrable in patients with diabetes mellitus. The reactivity appears to be directed against some cytoplasmic, antigenic component(s) of the atrophic pancreatic gland, but the present study does not make possible a conclusion as to whether the antigenic activity is associated exclusively with the endocrine pancreas.

In this connection it is of interest to observe that five out of six diabetics, not yet treated with insulin at the time of investigation, had low MI values, i.e. displayed signs of antipancreatic hypersensitivity. Seven patients had had their diabetes for less than six months at the time of study, and four of these presented MIs below 0.70. Thus the antipancreatic hypersensitivity seems to occur independently of previous insulin treatment and at a very early stage of the disease.

Intracutaneous testing with antigen is the classical method for demonstrating cellular (delayed type) hypersensitivity. The fact that fraction III induced a typical delayed type intracutaneous reaction in a majority of the patients in which an inhibition of the leucocyte migration was detected, supports the supposition that the specifically altered reactivity demonstrated in vitro actually reflects a state of organ-specific, antipancreatic cellular hypersensitivity.

The lymphocytic infiltration of the islets of Langerhans in juvenile diabetes of short duration and the antipancreatic hypersensitivity demonstrated in the present study are easily associable from the theoretic point of view[13] and draw attention to a possible role of organ-specific cellular hypersensitivity reactions in the islets of Langerhans during the initial pathogenetic stages of juvenile diabetes mellitus.

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