CONNECTION BETWEEN HYPERENZYMEMIA AND AUTOIMMUNIZATION IN DISEASES OF THE PANCREAS

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Experiments on healthy dogs have revealed the presence of autoantibodies against antigens from homologous pancreatic tissue and against trypsin and pancreatin, obtained from pancreas. The titer of autoantibodies is increased if pancreatic function is stimulated, the pancreatic ducts ligated, and the pancreaticojejunostomy is constricted by scar tissue.

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The role of autoantibodies in the pathogenesis of diseases of the pancreas has not yet been explained. Investigations in this direction have been few in number and have mainly been devoted to the detection of autoantibodies against pancreatic tissue in the blood serum [2, 6, 9, 11, 14, 16, 17]. Some investigators regard the presence of pancreatic autoantibodies in the blood as a manifestation of autoaggression, especially in the development of chronic pancreatitis [9, 14]. In the overwhelming majority of cases of pancreatitis, activity of the pancreatic enzymes in the blood is increased to a level at which it must exert some influence on the body, and under these conditions autoantibodies may behave as antienzymes.

In the present investigation the relationship between the blood level of pancreatic enzymes and the intensity of autoimmunization against pancreatic tissue and pancreatic enzymes was studied.

EXPERIMENTAL METHOD

Initial data were obtained on 38 healthy mongrel dogs of both sexes weighing from 6.8 to 15 kg. Later, the pancreatic ducts were ligated in 10 animals, in five animals pancreatic function was stimulated by subcutaneous injection of 1% pilocarpine solution, and in four dogs investigations were carried out at various times after pancreaticojejunostomy.

The titer of autoantibodies was determined by Boyden's passive hemagglutination test (PHT) [12] with slight modifications. Besides antigens of pancreatic origin (saline extract of homologous pancreatic tissue, pancreatin and trypsin manufactured at the Leningrad Meat Combine), antigens from homologous kidney, heart, and spleen tissue were used as controls. From these enzyme preparations a 1% suspension of pancreatin and a solution of trypsin containing 0.25 mg/ml physiological saline were made up. A 10% suspension of the organs was prepared in physiological saline. The suspension of kidney, heart, and spleen tissues was placed in a refrigerator at 4° for 3 h for extraction. The suspension of pancreatic tissue and the pancreatin suspension were incubated at 37° for 3 h for autodigestion. After centrifugation the protein content of the supernatant was determined by the biuret method, and the liquid was diluted with physiological saline to a concentration of 0.25 mg/ml. The solutions of antigens were inactivated for 60 min at 56°.

TABLE 1. Titer of Normal Autoantibodies against Pancreatic Preparations and Homologous Tissues in Blood Serum of Healthy Dogs

Antigen	Titer of autoantibodies (M±σ)
Trypsin	4.0±1.4
Pancreatin	3.7±1.1
Pancreas	3.1±1.9
Kidney	3.0±1.7
Spleen	3.2±1.5
Heart	3.2±1.6

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TABLE 2. Titer of Autoantibodies against Trypsin and Enzyme Activity of Blood Serum in Dogs after Ligation of Pancreatic Ducts

Index studied	Statistical index	Days after ligation of ducts: 3	6	9–10	11–12	14–17	18–19	25–27	28–31	32–36	39–40
Titer of autoantibodies (log_2 n)	M	+3,0	+3,2	+2,6	+2,5	+1,9	+1,6	+3,3	+3,8	+3,0	+1,1
	$\pm m$	0,53	1,00	0,40	0,60	0,35	0,38	0,59	0,72	0,58	0,61
	P	<0,02	<0,05	<0,02	<0,02	<0,05	>0,05	<0,05	<0,05	<0,05	>0,05
Amylase activity (in mg/min)	M	80,5	78,0	24,1	8,7	4,6	3,7	6,2	6,7	3,5	3,8
	$\pm m$	21,6	12,8	6,9	1,1	0,7	0,4	1,2	1,8	0,4	0,7
	P	<0,001	<0,001	<0,01	<0,01	>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
Lipase activity (in units)	M	2,4	2,71	1,80	2,22	1,04	1,20	1,04	1,05	0,98	0,96
	$\pm m$	0,26	0,23	0,22	0,24	0,21	0,23	0,15	0,15	0,16	0,23
	P	<0,001	<0,001	<0,001	<0,001	<0,01	<0,05	>0,05	>0,05	>0,05	>0,05

For the hemagglutination test fresh erythrocytes of a donor dog obtained on the day of the test were used. They were treated with 1 : 100,000 tannin solution in saline buffer, pH 7.2, at 37° for 20 min. Sensitization was carried out by mixing equal volumes of 5% suspension of tanninized erythrocytes and antigen solution in a concentration of 0.25 mg/ml and 4 volumes of saline buffer, pH 6.4. The mixture was incubated in a water bath for 20 min at 37°. Otherwise, the classical method was followed. The following controls were set up: 1) suspension of tanninized erythrocytes + test serum; 2) suspension of sensitized erythrocytes + rabbit serum; 3) control of extinction of titer. The results of the test were read after 3 h, noting the titer at which agglutination took place and also the intensity of the reaction. The value of the titer was expressed as its logarithm to base 2 (log_2 n).

Amylolytic activity was determined by Somogyi's method, and lipolytic activity by a titration method with olive oil emulsion [4]. The results are given as mean values ($M \pm \sigma$) and they were analyzed by the difference method ($M \pm m$).

EXPERIMENTAL RESULTS

The level of the amylolytic activity in the blood serum of the healthy animals was 3.37±0.72 mg/min, and that of the lipolytic activity 0.32±0.13 unit. The titer of autoantibodies in the blood serum of healthy animals is given in Table 1. The intensity of the immunologic reaction in the healthy animals was assessed at not more than ++ in dilutions with a value of $log_2 = 1$-2.

After ligation of the pancreatic ducts, a sharp increase in activity of the pancreatic enzymes in the blood serum was observed, with a maximum on the 3rd-6th day after the operation. The titer of autoantibodies against antigens of pancreatic origin was considerably increased. The titer of autoantibodies against trypsin was higher than that against pancreatin and pancreatic tissue extract. The intensity of the immunologic reaction also was considerably increased, and in dilutions of $log_2 = 4$-5 it was estimated as +++ or ++++. Changes in the titer of autoantibodies were fluctuating in character. The change in titer of autoantibodies against trypsin (Table 2) shows that the initial wave of increases in titer of autoantibodies was observed in the first two weeks after ligation of the ducts, and coincided with the maximum of enzyme activity in the blood serum. Later, by the 17th-19th day after operation, the titer was reduced. The second wave of increase in titer of autoantibodies reached a maximum by the 27th-31st day after operation, when the increase in titer of autoantibodies against antigens of pancreatic origin took place against the background of normalization of the serum enzyme activity. In parallel tests using antigens from kidney and spleen tissue, no significant or regular changes in titer were detected.

Immunologic tests were carried out on four dogs with pancreaticojejunostomy 6-12 months after the operation. In two of these

TABLE 3. Effect of Stimulation of Pancreatic Function on Level of Enzyme Activity and Titer of Autoantibodies in Serum of Dogs

Index studied	Statistical index	Time after stimulation		
		3 h	6 h	24 h
Amylase activity (in mg/min)	M	34,7	32,3	5,8
	$\pm m$	5,4	5,2	0,5
	P	<0,001	<0,001	<0,01
Lipase activity (in units)	M	3,49	2,78	1,18
	$\pm m$	0,30	0,36	0,17
	P	<0,01	<0,01	>0,05
Titer of autoantibodies against trypsin ($\log_2$ n)	M	+1,4	+1,6	+1,6
	$\pm m$	0,52	0,51	0,50
	P	<0,05	<0,05	<0,05
Titer of autoantibodies against pancreatin ($\log_2$ n)	M	+1,40	+1,60	+1,00
	$\pm m$	0,51	0,25	0,39
	P	<0,05	<0,01	<0,05
Titer of autoantibodies against pancreatic tissue ($\log_2$ n)	M	+0,80	+2,00	+1,60
	$\pm m$	0,02	0,44	0,25
	P	<0,02	<0,02	<0,01

dogs the blood level of pancreatic enzymes and the titer of autoantibodies against antigens of pancreatic origin were increased, the latter to $\log_2 = 6$-7. At necropsy on these animals cicatricial stenosis of the anastomosis and indurative pancreatitis were discovered. In the remaining animals, no increase was observed in the enzyme activity and titer of autoantibodies, and at necropsy the state of the pancreas and of the anastomosis was good.

To examine the relationship between the titer of autoantibodies and the blood level of pancreatic enzymes, tests with stimulation of pancreatic function were used. In this series of experiments also, after stimulation of pancreatic function the increase in enzyme activity was accompanied by an increase in the titer of autoantibodies against antigens of pancreatic origin (Table 3). The highest titer of autoantibodies was observed 6 h after stimulation, but it remained high even 24 h after the beginning of the tests. In parallel tests, just as in previous investigations, no substantial changes were found in the content of autoantibodies against antigens from the kidney and heart tissues.

The rapid increase in titer of autoantibodies after ligation of the pancreatic ducts and stimulation of pancreatic function cannot be explained in terms of classical immunology, from the standpoint of which the increase in titer of autoantibodies should have taken place much later in the course of the pathological conditions created in the experiments.

Evidently, the autoimmune reaction is related to physiological processes and may take part in their regulation. This point of view is confirmed by the presence of normal autoantibodies in the blood of healthy animals. If a role of regulators of physiological functions is ascribed to autoantibodies, the character of the change in titer of autoantibodies against antigens of pancreatic origin in the present experiments begins to become clear to some extent. The pancreas constantly secretes into the blood stream a small quantity of pancreatic enzymes [5, 15], the level of which rises considerably during an increase in functional activity of the gland and in pancreatic pathology [5, 10], so that the existence of normal autoantibodies can be assumed as a unique physiological protective mechanism against pancreatic enzymes. This hypothesis is confirmed by the rapid increase in titer of autoantibodies in the present investigation.

A factor of importance to the explanation of these results is the hypothesis put forward by Grabar on the basis of a series of investigations, concerning the physiological nature of antibody production in general and of autoantibody production in particular [13]. According to this hypothesis, autoantibody formation is

a special case of the physiological mechanism of formation of globulins adapted to fixation and to transfer of particular substances. The sharp increase in content of autoantibodies can also be regarded as "a unique adaptation of physiological functions of the body to particular conditions of existence" [1]. The early increase in content of autoantibodies, which is fluctuating in character, is not specific for pancreatic pathology. A similar increase in titer of autoantibodies also takes place in experimental pathological conditions of the lungs and heart [3, 7, 8]. The writer regards this early increase in titer of autoantibodies during stimulation of pancreatic function, after ligation of the pancreatic ducts, and also after pancreaticojejunostomy as a physiological mechanism of protection of the organism against pancreatic enzymes, and not as a state of autoaggression.

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