ANTIBODY FORMATION TO CELL MEMBRANES OF ADIPOSE TISSUE IN HUMAN BLOOD SERUM

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Adipose tissue is one of the principal components of the body. In man, with an increase in age of the adult as a rule there is an increase in the fraction of fat in the body and, under certain conditions, the normal value is exceeded, a state described as obesity [8]. Meanwhile, obesity can be observed at any stage of ontogeny starting with the newborn infant, due to the effect of many endogenous and exogenous factors. Obesity and the hormonal and metabolic disturbances accompanying it are among factors giving rise to metabolic immunodepression, i.e., inhibition of cellular immunity and of macrophagal function [2, 9]. On the other hand, metabolic immunodepression, characteristic of aging and of age-dependent obesity, is coupled with certain features of stimulation of humoral immunity, including intensification of autoantibody production to antigens of various tissues and organs [2, 6]. However, the search for antibodies to components of adipose tissue in human blood has not hitherto been undertaken, and the main purpose of the present study was to remedy this omission.

EXPERIMENTAL METHOD

Blood was taken for analysis from healthy individuals and patients with fibroadenomatosis of the breast aged from 25 to 66 years, 12-14 h after the last meal. More than 80% of the subjects were over 50 years old, and their body weight exceeded the ideal value (according to Brock's formula) on average by 29.7 ± 3.2%. During the 30 min after blood was taken, the serum was separated by centrifugation and kept at −20°C. Subcutaneous adipose tissue was obtained from the anterior abdominal wall during operations on the abdominal organs. Two preparations of plasma-cell membranes, conventionally described as crude and pure, were isolated from the adipose tissue. The first was obtained by the method described by Egutkin [3], the second (for which collagenase was used, and ultracentrifugation carried out in a Percoll gradient) by the method of Belsham and co-workers [7]. The yield of pure antigen, expressed as protein, did not exceed 500-1000 μg/10 g adipose tissue, whereas the yield of crude antigen was 4-5 times greater. On immunization of rabbits with the crude preparation, the first time it was injected intradermally into the footpads (1.5 mg as protein), and 3 weeks later into the lymph nodes (7 mg as protein). Blood samples were taken from the immunized rabbits on the 8th day after injection of the antigen into the lymph nodes, The titer of antibodies to the crude and pure preparations of adipose tissue membranes in human and rabbit blood was determined by indirect ELISA, using secondary antibodies containing a peroxidase label (Leningrad Research Institute of Vaccines and Sera) or by the passive hemagglutination test (PHT) with fresh sheep's red blood cells, loaded with $CrCl_3$. A search also was made for the antigen in human adipose tissue and blood by means of an alternative form of the last test. The level of immune complexes circulating in the blood was determined by the method in

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TABLE 1. Data on Discovery of Circulating Antibodies and Antigen in Subjects

Subjects tested	Age	Group	Antibody titers to adipose tissue cellmembrane			Quantity of adipose tissure antigen, titer
			ELISA	PHT		
				with "crude" antigen	with "pure" antigen	
K. A.	63	FAD	+	—	—	—
Kon.A.	66	FAD	+	—	—	1/160
K. V.	61	FAD	+	—	—	—
A. N.	54	FAD	1/640	1/80	1/40	1/80
O.N.	63	FAD	1/640	1/20	1/20	1/40
B.G.	50	H	+	—	—	—
P.G.	48	H	1/640	1/40	1/20	1/40
S. A.	54	FAD	+	—	—	—
D.G.	31	H	+	—	—	1/20
M.I..	25	H	+	—	—	—
G. E.	64	H	+	1/20	1/10	1/40
V.N.	58	H	1/640	1/640	1/160	—
P T.	53	H	+	1/10	—	—
K.Z,	59	H	+	1/20	1/10	—
K.I..	51	H	+	1/20	1/40	—
I.V.	50	H	+	1/40	1/20	—

Legend. FAD) Fibroadenoma, H) healthy subjects. +) Antibodies found in titer under 1/640, —) neither antibodies nor antigen were found.

[4], concentrations of sugar, lipids, insulin, and growth hormone by methods adopted in the Laboratory of Endocrinology [2], and protein in the preparations of adipose tissue by Lowry's method in the modification of Belsham and co-workers [7].

EXPERIMENTAL RESULTS

In the preliminary stage of the investigation, using ELISA and the "pure" membrane preparation from adipose tissue, antibodies to adipose tissue were found in three of the 15 healthy individuals tested, who were aged, 30, 52, and 55 years and had an excess body weight of 28.1, 1.3, and 41.0% respectively. Later, to obtain a positive control (sera with antibodies to membrane components of adipose tissue) rabbits were immunized with the crude membrane preparation from adipose tissue. The immune serum obtained was used subsequently in immunologic tests with the blood serum of 16 women (10 healthy and six with fibroadenos tosis of the breast).

The use of the crude preparation of adipose tissue in ELISA revealed a positive reaction in the blood serum of all 16 subjects (evidently due to nonspecific sorption of the components of the reaction in the planchet), but it was found in the highest titer (over 1/640) in four persons. The PHT with the crude and pure antigens revealed antibodies in nine (titer 1/10-1/640) and in eight (titer 1/10-1/160) of 16 cases respectively; the highest titers (1/40-1/160 when the pure antigen was used, 1/80-1/160 with the crude antigen) were found in two subjects (A.N and V.N.). When the blood sera of the subjects were tested with human renal antigen, its titer (if a positive result was obtained) did not exceed 1/10. Antigen of adipose tissue was found in the blood of six of the 16 subjects, by means of the PHT, in titers of between 1/20 and 1/160 (the titer in the control did not exceed 1/10). The highest values of the titer were observed in two patients with fibroadenomatosis (1/160 and 1/180 respectively) (Table 1). Accordingly, the value of certain anthropometric parameters and parameters of lipid and carbohydrate metabolism were compared in groups of individuals in whom antibodies to the "pure" adipose tissue antigen had been found (in whatever titer) or had not been found in the PHT. No difference could be found between the parameters compared in these groups of subjects. It was a different picture when these parameters were assessed in subjects with the highest titers of antibodies and antigen the first group ("antibodies +") was found to have a significant increase in the excess of body weight (39.4 ± 1.5% compared with 28.3 ± 3.3%) and a fall in the cholesterol level in high-density lipoproteins (α-cholesterol) (44.0 ± 1.0 mg % compared with 54.4 ± 2.3 mg %), and also a tendency for the blood growth hormone level to fall. The second group ("antigen +") had a significant increase in the total cholesterol concentration (297.0 ± 24.0 mg % compared with 217.0 ± 9.0 mg %) and a tendency for the basal blood insulin level to be reduced and for age to be increased. No differences between the groups compared likewise could be found with respect to levels of

circulating immune complexes (0.235 ± 0.085 if antibodies were present, 0.219 ± 0.065 without them), although it must be pointed out that unfortunately the value of this parameter was determined after the sera had been kept for some time at –20°C.

In this investigation for the first time antibodies to membrane components of white adipose tissue were found in human blood serum and evidence was obtained in support of the possibility that these components (antigens) are released into the general circulation. The preliminary character of the study did not allow any conclusion to be drawn on whether the results can be connected with a particular period of ontogeny (the overwhelming majority of the subjects tested were over 50 years old), or with the severity of the obesity (the excess body weight of most of the subjects exceeded 20%), but even in the preliminary stage of the work antibodies were found in healthy individuals with excess body weight of only 1.3% (see above), nor can the nature of the antibodies discovered by identified It is important to note that this last problem can be solved with the aid of more sophisticated methods of investigation, for identification of an autoimmune process in adipose tissue (autoimmune adipositis) may prove to be just as important for our understanding of the mechanisms of development of individual forms of emaciation and obesity as for assessment of the prognosis and methods of treatment of such states.

Autoimmune disturbances as a rule are the result of changes in certain properties of tissue antigens or of the state of certain subsystems of immunity [5]. The discovery that persons with the highest titers of adipose tissue antibodies and antigen tend correspondingly to be those with the greatest degree of overweight and of hypercholesterolemia, while in harmony with our ideas on the development and s nifestations of the phenomenon of metabolic immunodepression [2, 9], does not, as we have seen, contradict the view that these abnormalities may be autoimmune in character. On the other hand, antibodies to adipose tissue, which were identified in some subjects, are not necessarily linked with autoimmune changes, but nevertheless, they maintain the pathological process in adipose tissue. In this respect, it will suffice to quote data indicating that antibodies to rat adipose tissue induced in rabbits in experiments in vitro gave rise (depending on their concentration) to an insulinlike effect in relation to glucose utilization in the adipocytes, whereas their injection daily for 4 days in vivo led to lysis of some adipocytes and to their infiltration by lymphocytes [10], and that normal human immunoglobulins of the M and G classes can stimulate lipogenesis in adipose tissue [11]. On the other hand, it is important to note that the adipocytes themselves may be the site of synthesis of factors of the immunity system and, in particular, the component of complement (the so-called adipsin) whose production is depressed in some forms of obesity [12]. Later, after the specificity of the antibodies we found has been confirmed, it would be interesting to estimate the frequency of discovery of antibodies to adipose tissue and its antigens in the general circulation in various forms of obesity (hypertrophic, hyperplastic, android, gynoid, and so on) and in different types of diabetes melitis, during pregnancy the postnatal period (in which differences have been found in the state of the adipose tissue depending on the pattern of infant feeding [1]), and also before and after operations associated with removal or a subsequent change in volume of the mass of body fat In addition, it is worth noting that if an analysis of this kind were undertaken in patients with lipomas, liposarcomas, and neoplasms located outside the adipose tissue (it may be recalled that our observations showed that both antibodies and antigen of adipose tissue were found simultaneously only in the blood of a patient with fibroadenomatosis), and also in certain autoimmune conditions (including autoimmune diseases of the endocrine system).

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