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Chitotriosidase as a Marker of Macrophage Stimulation

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Serum chitotriosidase activity was determined in different conditions accompanied by macrophage stimulation. Stimulation of macrophages with zymosan, yeast polysaccharide carboxymethylglucan (fraction II), and lysosomotropic preparation Triton WR-1339 1.5-2.0-fold increased enzyme activity. Chitotriosidase activity in intact Wistar rats was similar to that in humans, while in CBA and A/Sn mice this parameter was 5-fold higher. Sharp increase in chitotriosidase activity in the serum from patients with type I Gaucher's disease was probably related to intense secretion of the enzyme by macrophages. Under experimental conditions, stimulation of rat and mouse macrophages (mainly liver cells) caused no increase in chitotriosidase activity typical of patients with Gaucher's disease.

Key Words: *macrophage stimulation; chitotriosidase; lysosomes; lysosomal storage diseases*

Recent studies showed that serum chitotriosidase (CT) activity in patients with type I Gaucher's disease increased by more than 100-1000 times and now serves as a diagnostic marker of this disorder [2,4]. Enzyme activity markedly varies (9-195 nmol/ml/h) in apparently healthy humans and progressively increases with age. CT activity is very low in 6.2% young individuals and high in 3.1% individuals [4]. CT activity moderately increases (by 2-3 times) in patients with lysosomal storage diseases (Krabbe disease, GMI gangliosidose, and Niemann—Pick A and B disease) [5].

CT is mainly formed in macrophages [8]; stimulated macrophages produce considerable amounts of CT mRNA [7]. In neutrophil precursors CT is synthesized and accumulated in specific granules.

CT cleaves the synthetic substrate 4-methylumbelliferyl (MUF) β-D-N,N',N''-triacetylchitotrioside. Chitin, a component of cell walls and membranes in various microorganisms, is the natural substrate for this enzyme. Chitinase of plants protecting them from various pathogenic fungi is an analogue of mammalian CT. Probably, mammalian CT also has some protective properties [7-9]. Functions of CT are now extensively studied. The role of this enzyme in combating foreign bacteria and fungi, whose membranes include chitin, is of particular interest. Biological functions of CT, as well as its cell origin in Gaucher's disease and other lysosomal storage diseases, are unknown.

Here we studied CT activity in blood serum from experimental animals during macrophage stimulation with zymosan, carboxymethylglucan, and Triton WR-1339 taken up by Kupffer cell lysosomes and promoting accumulation of undegraded lipids.

MATERIALS AND METHODS

We examined 13 patients (aged from 3 months to 30 years) with type I and II mucopolysaccharidoses and 14 apparently healthy individuals (18-20 years). Experiments were also performed on male Wistar rats

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weighing 180-200 g and CBA and A/Sn mice weighing 17-20 g (Institute of Cytology and Genetics).

β-1,3-D-Carboxymethylglucan (CMG, fraction II; Chemical Institute, Slovenian Academy of Sciences) is a chemically modified yeast polysaccharide. Some CBA mice were killed 48 h and 7 days after single intraperitoneal injection of 25 mg/kg CMG. This period corresponded to the highest functional activity of mononuclear phagocytes [11]. Triton WR-1339 (Ruger Chemical) in a dose of 1 g/kg was administered intraperitoneally to other CBA mice, which were killed 48, 72 h, and 7 days postinjection. Wistar rats were killed 48 h after injection of 100 mg/kg zymosan (Sigma).

LS-lymphosarcoma was induced by intravenous injection of methyl nitrosourea and intramuscularly inoculated into the hip of CBA mice (10^6 cells). Cyclophosphamide (25 mg/kg, Sigma) was injected intravenously, when the size of lymphosarcoma reached 1.5-2.0 cm. The mice were decapitated 24, 48, and 72 h postinjection.

The blood was centrifuged and the serum was stored at -20°C.

CT activity was measured as described elsewhere [2,4] using fluorescent substrate 4-MUF β-D-N,N', N''-triacetylchitotrioside (Sigma). Blood serum (10 μl) was incubated with 200 μl substrate (22 μmol/liter) in McIlvain phosphate buffer at pH 5.2 for 30 min. The reaction was stopped by addition of 2 ml 0.15 M glycine buffer (pH 10.6). Fluorescence was measured on a Perkin Elmer 650-10S spectrofluorometer at excitation and emission wavelengths of 360 and 455 nm, respectively. The results were expressed in nmol released MUF per 1 ml for 1 h.

Morphometrical electron microscopy was performed as described previously [6]. The results were analyzed by Student's t test. The differences were significant at $p<0.05$.

TABLE 1. Effects of Zymosan, CMG, and Triton WR-1339 on Serum CT Activity (nmol MUF/ml/h, $M\pm m$)

Series	CT activity
Intact mice	
CBA (n=11)	387.0±30.4
A/Sn (n=8)	389.0±50.6
CMG	
48 h (n=9)	738.7±67.7*
7 days (n=6)	833.5±103.6*
Triton WR-1339	
48 h (n=4)	279.1±28.3
72 h (n=4)	250.8±13.3
7 days (n=4)	794.5±84.9*
12 days (n=5)	493.4±73.0
Wistar rats	
intact (n=9)	29.5±3.5
zymosan (n=9)	43.2±4.1*

Note. *$p<0.05$ compared to intact animals.

RESULTS

CT activity in the serum and saliva of healthy individuals was 73.6±13.3 and 161.3±55.8 nmol MUF/ml/h, respectively. In patients with type I Gaucher's disease, serum CT activity 500-fold surpassed that in healthy individuals (36,243 nmol MUF/ml/h).

In intact Wistar rats serum CT activity was similar to that in humans, while in intact CBA and A/Sn mice this parameter was 5-fold higher (Table 1).

Macrophage stimulator zymosan increased CT activity in rat serum (Table 1). Other macrophage stimulator CMG also increased CT activity in CBA mice (Table 1). Stimulation of macrophages with zymosan and CMG was accompanied by an increase in the num-

TABLE 2. Morphometry of Liver Macrophages after Injection of 25 mg/kg CMG and Zymosan ($M\pm m$)

Parameters	Control	CMG		Zymosan, 2 days
		2 days	7 days	
Numerical density of macrophages per 1 mm²	924.5±38.0	1412.5±92.0*	1782.80±57.35*	1856.8±99.8*
Relative volume of lysosomes, %				
primary	3.8±0.5	1.05±0.14*	1.20±0.08*	11.50±11.47*
secondary	6.1±1.3	19.50±2.72*	16.40±1.66*	8.97±1.16*
Numerical density of lysosomes per 1 μm²				
primary	18.8±0.7	5.4±0.6*	5.80±0.48*	9.6±0.3*
secondary	1.7±0.3	9.70±1.13*	7.20±0.74*	11.40±1.14*

Note. *$p<0.05$ compared to intact animals.

TABLE 3. Effect of Cyclophosphamide on Serum CT Activity (nmol MUF/ml/h) in CBA Mice ($M \pm m$)

Series	CT activity
Intact (n=11)	387.0±30.4
LS-lymphosarcoma (n=13)	238.7±20.8*
LS-lymphosarcoma+CPA	
24 h (n=6)	259.1±30.1*
48 h (n=7)	281.0±66.5
72 h (n=8)	331.9±33.8*

Note. $p<0.05$: *compared to intact mice, *compared to mice with LS-lymphosarcoma.

ber of nonparenchymal liver cells (mainly Kupffer cells) and secondary lysosomes. These changes are morphological signs of macrophage stimulation (Table 2).

Lysosomotropic compound Triton WR-1339 increasing the number of liver macrophages and simulating lysosomal storage diseases [10] also elevated enzyme activity in the serum (Table 1).

Thus, macrophage stimulation 1.5-2.0 times increased CT activity in experimental animals. However, under experimental conditions stimulation of macrophages in rats and mice did not considerably increase CT activity as in type I Gaucher's disease (*i.e.* more than by 100 times).

In mice with LS-lymphosarcoma, serum CT activity decreased (Table 3) probably due to suppression of macrophages and inhibition of nonspecific protective mechanisms [7,10]. Treatment with 25 mg/kg cyclophosphamide did increase CT activity to normal (Table 3). This preparation inhibited tumor growth by inducing apoptosis of tumor cells, but then tumor recurrence was observed in 50% animals.

CT activity is an important diagnostic criterion for type I Gaucher's disease. This parameter also reflects the efficiency of enzyme replacement therapy [2,4,5]. At the same time, measurements of CT activity can be used in evaluating the degree of macrophage stimulation probably reflecting the severity of inflammation [2]. The increase in enzyme activity is important in differential diagnosis between inflammatory and non-inflammatory neurological disorders [4]. In addition, atherosclerosis is accompanied by induction of chitinases in atheromatous plaques and the appearance of CT-secreting macrophages [1,3]. This opens new avenues for evaluating the role of CT in the pathogenesis of various diseases.

Hence, the cause of high serum CT activity in patients with type I Gaucher's disease remains unknown. Under experimental conditions stimulation of rat and mouse macrophages (mainly in the liver) did not cause a considerable increase in CT activity typical of patients with Gaucher's disease.

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