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Plasma tumor necrosis factor-a (TNF-a) levels in Gaucher disease

Helen Michelakakis [a,*], Cleopatra Spanou [b], Anastasia Kondyli [b], Evagelia Dimitriou [a], Sonja Van Weely [c], Carla E.M. Hollak [c], Marinus H.J. Van Oers [c], Johannes M.F.G. Aerts [c]

[a] *Department of Enzymology and Cellular Function, Institute of Child Health, 'Ag. Sophia' Children's Hospital, 11527 Athens, Greece*
[b] *Department of Immunology and Histocompatibility, 'Ag. Sophia' Children's Hospital, 11527 Athens, Greece*
[c] *Department of Biochemistry and Hematology, Academic Medical Centre, Amsterdam, The Netherlands*

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Abstract

Tumor necrosis factor-a (TNF-a) levels were measured in the plasma of patients with different types of Gaucher disease (GD) and patients with other lysosomal storage diseases. The highest TNF-a levels were observed in the most severe neuronopathic type of GD, exceeding those found in healthy individuals as well as patients with other lysosomal disorders. Type I GD cases showed a wide range of TNF-a levels ranging from normal to 2.5 × the highest control value. TNF-a is a pleiotropic cytokine produced mainly by activated macrophages. Our data suggest that it may play a role in the pathophysiology of GD disease.

Keywords: Cytokine; TNF; Gaucher disease

1. Introduction

Gaucher disease (GD) is a lipid storage disorder caused by the deficient activity of the lysosomal enzyme β-glucocerebrosidase. This deficiency results in the impaired hydrolysis of glucosylceramide (GC), a glycolipid that is preferentially cleared by macrophages of the monocyte-macrophage system. Lipid-laden macrophages are believed to be the cellular source of secondary abnormalities in Gaucher disease (GD) and are implicated in the pathogenesis of the disorder [1]. Although in vitro studies have shown that glucosylceramide treatment of monocytes stimulates interleukin-1 (IL-1) secretion data regarding cytokine levels in GD patients are scarce [2]. In the present study levels of TNF-a, a pleiotropic cytokine [3], were measured in the plasma of GD patients as well as patients with other lysosomal disorders.

2. Patients and methods

A total of 64 patients were examined. They included 30 patients with Gaucher disease (25 type I, 4 type II and 1 type III) and 34 patients with other lysosomal disorders namely metachromatic leukodystrophy ($n = 5$), Krabbe leukodystrophy ($n = 5$), GM_1-gangliosidosis ($n = 6$), GM_2-gangliosidosis ($n = 3$), mucopolypacharidosis III B ($n = 7$), mucolipidosis II, III ($n = 4$) and Niemann-Pick type C ($n = 3$). Eleven healthy individuals served as controls. GD diagnosis was established by assaying β-glucocerebrosidase in white blood cells and/or fibroblasts cultured from skin biopsies [4]. The diagnosis of the other lysosomal disorders was established by assaying the appropriate lysosomal enzymes as previously described [5].

TNF-a was measured in plasma prepared from heparinised blood using the immunoenzymetric assay kit (TNF-a EASIA) by Medgenix (Belgium). The results were expressed in pg/ml.

The investigation of GD patients also included the assay in plasma of β-hexosaminidase, α-mannosidase [5] and chitotriosidase [6]. Severity score indexes were established according to Zimran [7].

3. Results

The results obtained in our study with regard to TNF-a levels are shown in Fig. 1. On the whole the GD patients

* Corresponding author. Fax: +30 1 7700111.

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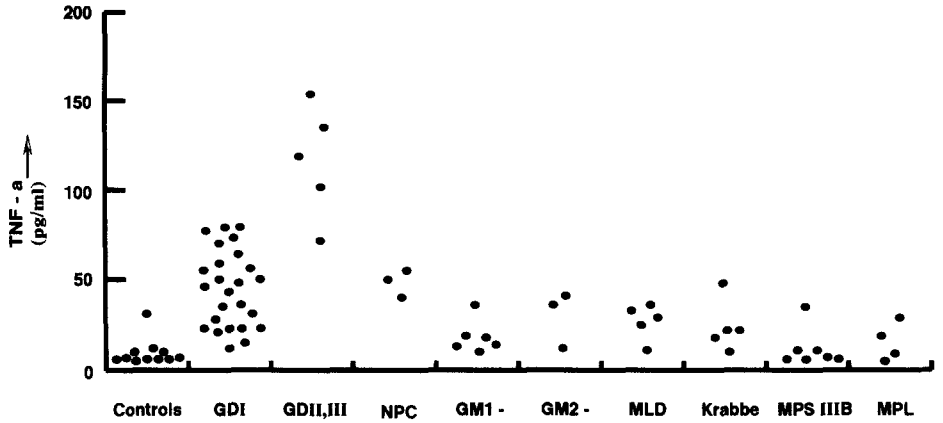

Fig. 1. TNF-a levels in patients with Gaucher disease, cases type I, (GDI), type II, III (GDII, III), GM_1 and GM_2-gangliosidoses (GM_1, GM_2), metachromatic leukodystrophy (MLD), Krabe leukodystrophy, mucopolysaccharidosis III B (MPS III B) and mucolipidoses II, III (MPL).

showed higher TNF-a values than any of the groups examined, followed by Niemann-Pick C patients. The highest TNF-a plasma levels were found in neuronopathic GD cases exceeding by far those found in the control group as well as in patients with other lysosomal storage diseases. Furthermore with one exception there was a clear distinction between them and the type I cases. The latter showed a wide range of values ranging from normal to 2.5 × the highest control value.

In GD patients no correlation was found between TNF-a levels, residual white blood cell or fibroblast β-glucocerebrosidase activity, plasma α-mannosidase and chitotriosidase activities. On the other hand a positive correlation that was statistically significant ($P < 0.05$) was found between TNF-a and plasma β-hexosaminidase activity.

Severity scoring indexes (SSI) were available in 10 type I patients. In general patients with a higher SSI had a higher β-hexosaminidase activity and a higher TNF-a

Table 1
Severity score indexes and plasma levels of β-hexosaminidase, TNF-a and chitotriosidase at different time points of ceredase treatment in Gaucher disease type I cases. Control TNF-a values: 5–31 pg/ml

Patients	Time (mth)	SSI	β-Hexosaminidase (nmol/ml per h)	TNF-a (pg/ml)	Chitotriosidase (nmol/ml per h)
1	0	19	3127	70	12729
	3	–	2869	64	13929
	6	–	2528	82	6918
	12	–	2773	56	9412
2	0	14	2607	35	15081
	3	–	2420	22	19200
	6	–	2233	18	17400
	12	–	1987	16	16769
3	0	3	2155	23	–
4	0	11	3715	77	39992
	3	–	2458	60	24631
	6	–	3008	82	–
	12	–	2266	52	27589
5	0	14	2822	59	11180
	3	–	1979	50	8318
	7	–	2296	33	9670
	11.5	–	2109	50	
6	0	3	1107	15	–
7	0	9	3101	23	–
8	0	8	1138	23	–
9	0	4	1701	21	–
10	0	17	3265	23	22522
	0.5	–	3653	45	22913
	3	–	2608	38	14167
	6	–	1240	22	12845
	12	–	1113	8	

value. However the relation was not always that clear e.g. patient 10 with a high SSI value had high β-hexosaminidase but low TNF-a levels whereas high TNF-a levels were observed in patient 4 who had a lower SSI value (Table 1).

TNF-a was measured in 5 type I patients on Ceredase at different time points after the initiation of treatment. It was observed that similarly to chitotriosidase and β-hexosaminidase TNF-a tended to decrease over the period studied. In fact 12 months after the initiation of Ceredase treatment all five patients had lower TNF-a levels than before therapy (Table 1).

4. Discussion

The impaired activity of the lysosomal enzyme β-glucocerebrosidase in Gaucher disease prevents the catabolism of glucosylceramide (GC) that preferentially accumulates in macrophages. These lipid — laden cells accumulate mainly in spleen, liver and bone and are believed to be responsible for the pathologic processes characterising the disorder [1]. Altered monocyte-macrophage function due to the loading of the cells with undigested GC is suggested by the work of several authors [2,8–10]. Cytokines are emerging as molecules of key importance in homeostasis, development and pathophysiology. Alterations in their production and/or secretion may have important implications in the pathophysiology of GD. However although in vitro studies have shown that GC stimulated the secretion of interleukin-1 by macrophages [2], data concerning cytokine levels in GD patients are scarce [10]. TNF-a is a pleiotropic cytokine that has been implicated in several physiological and pathological processes [11–14]. Activated macrophages and T-lymphocytes are the principal source of the cytokine [3] that can nonetheless be produced by other cells such as astrocytes and microglia [15,16].

In our study increased TNF-a levels were observed in GD patients in a manner apparently related to the severity of the disorder, the highest levels being found in the most severe neuronopathic form of the disorder. TNF-a has been reported to change ionic channel expression and membrane potential of oligodendrocytes in vitro, to disrupt myelin, to cause necrosis of oligodendrocytes and to induce gliotic proliferation [17,18].

Plasma levels do not necessarily reflect the brain situation, furthermore studies concerning the behaviour of TNF-a in the brain of GD patients are not available. However increased circulating levels of TNF-a could modulate the transmigration of lymphocytes across cerebral endothelial cells [19] that could in turn secret cytokines in the brain as well as induce the secretion of TNF-a by microglia and astrocytes [16,20].

Type I cases showed a wide range of TNF-a levels. The range of values is compatible both with the behaviour of other secondary serum abnormalities in Gaucher disease and the range of the clinical manifestations of the disorder [1].

The observed changes in the TNF-a levels during therapy and in particular in the patients showing high levels before its initiation further supports the concept of a causal relation between the disorder and cytokine secretion.

In conclusion then our results show for the first time that TNF-a mainly produced by activated macrophages is elevated in the plasma of GD patients. The elevation is not paralled by any other lysosomal storage disorder. However before any definite conclusions are reached regarding the origin and mechanism of secretion of TNF-a as well as its implications in the pathology of the disorder more studies are required.

Thus it will be important to evaluate TNF-a levels in large numbers of GD type I patients in relation not only to SSI but also to individual manifestations e.g. bone involvement, since TNF-a and other cytokines have been implicated as potential inducers of osteoporosis [21,22] both before and during therapy.

Furthermore analysis of several cytokines in plasma of GD patients, studies involving cytokine expression in vitro by peripheral monocytes of GD patients as well as in situ analysis of affected organs for expression of cytokines or cytokine mRNA, will be very important in understanding the basis of altered cytokine production and/or secretion and its contribution to the pathogenesis of Gaucher disease.

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