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Proinflammatory and proosteoclastogenic potential of peripheral blood mononuclear cells from Gaucher patients: Implication for bone pathology

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ABSTRACT

Gaucher disease (GD) is caused by mutations in the GBA gene that confer a deficient level of activity of glucocerebrosidase (GCase). This deficiency leads to the accumulation of the glycolipid glucocerebroside in the lysosomes of cells of monocyte/macrophage system. Bone compromise in Gaucher disease patients is the most disabling aspect of the disease. However, pathophysiological aspects of skeletal alterations are still poorly understood. On the other hand it is well known that inflammation is a key player in GD pathology. In this work, we revealed increased levels of the proinflammatory $CD14^{+}CD16^{+}$ monocyte subset and increased inflammatory cytokine production by monocytes and T cells in the circulation of GD patients. We showed increased levels of osteoclast precursors in PBMC from patients and a higher expression of RANKL in the surface of T cells. PBMC from patients presented higher osteoclast differentiation compared to healthy controls when cultured in the presence of M-CSF alone or in combination with RANKL. In vitro treatment with Velaglucerase reduced osteoclast levels to control levels. On the other hand THP-1 derived osteoclast precursors cultured in the presence of conditioned media from PBMC of GD patients presented higher differentiation to active osteoclasts. This induction involved TNF-α and RANKL.

1. Introduction

Gaucher disease (GD) (OMIM ID: 230800) is an autosomal recessive lysosomal storage disorder caused by mutations in the gene encoding β-glucocerebrosidase (GCase) (E.C. 3.2.1.45), causing a deficiency in GCase enzymatic activity. This alteration leads to the accumulation of its substrate glucocerebrosidase mainly in macrophages [1], resulting in lipid-laden activated macrophages referred as "Gaucher cells". Phenotypes can be classified depending on the presence or not of neuronopathic manifestations in types II/III or I, respectively. Type I GD is the most frequent form, and is characterized by hepatosplenomegaly, anemia, thrombocytopenia and skeletal alterations. After more than 20 years of the introduction of enzyme replacement therapy for Gaucher disease, experts view this disorder as a principally skeletal disorder with high morbidity, and with some of the effects refractory to therapy [2]. Bone manifestations include bone pain, bone crises, osteopenia, osteoporosis, osteolytic lesions and osteonecrosis [3]. The pathological mechanisms of bone alterations in Gaucher disease are still not completely understood [4].

The bone is a dynamic tissue undergoing continuous remodeling. Skeletal disease results from a disruption of the fine balance between osteoblastic bone formation and osteoclastic bone resorption. Osteoclasts originate from the fusion of osteoclast precursors belonging to the monocyte/macrophage lineage, originating multinucleated cells. M-CSF is a crucial cytokine for the proliferation and survival of osteoclastic precursor cells. Osteoblasts express RANKL at cell surface and binding to its receptor RANK results in the activation of signaling cascades controlling lineage commitment and activation of osteoclasts [5]. This process is inhibited by the presence of osteoprotegerin (OPG), the RANKL neutralizing soluble decoy receptor. RANKL could also be expressed on dendritic cells, T and B cells, suggesting that osteoclastic bone resorption could be influenced by these cells. Osteoclastogenesis was reported to occur in cocultures of monocytes and T cells [6]. Activated T cells in inflammatory conditions lead to T cell production of osteoclastogenic cytokines such as RANKL and TNF-α. TNF-α was also reported to induce the formation of osteoclastic cells independently of the RANKL/RANK/OPG axis as it could support osteoclastogenesis in

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the presence of OPG or when bone marrow hematopoietic precursors from RANK null mice where cultured in vitro with this cytokine [7–9]. Alternatively, in the presence of TNF-α, osteoclast formation is induced by low concentrations of RANKL, and TNF-α increases the effects of RANKL [10]. Most of the T-cell cytokines, including interferon (IFN)-γ, IL-4, and IL-10, inhibit osteoclastogenesis [11]. However, among T cell subsets, Th17 cells have been suggested to be the osteoclastogenic T cell subset. Recently, IL-17 has been suggested to be involved in the upregulation of osteoclast precursor formation in inflammation by increasing the release of RANKL, which may synergize with IL-1β and TNF-α [12]. On the other hand, Treg inhibits osteoclast differentiation and function [13]. Treg subset is involved in tolerance maintenance and immunological homeostasis countering the inflammatory effects of the Th1/Th17 cells [14]. This subset has been shown to be altered in pathologies with an inflammatory involvement.

Higher numbers of circulating osteoclast precursors have been reported in several bone diseases associated with bone loss [15]. In bone loss-associated diseases, those osteoclast precursors are recruited from peripheral blood mononuclear cells (PBMCs) [16] and osteoclast formation occurs through stimulation by RANKL and macrophage colony-stimulating factor (M-CSF) [17–19]. In diseases with inflammatory conditions, osteoclastogenic cytokines necessary for osteoclast formation are produced by PBMCs themselves [16]. In a previous work it was shown that monocytes from GD patients cultured in the presence of M-CSF and RANKL gave rise to an increased level of mature and active osteoclasts which was reverted by treatment with Imiglucerase [20].

Inflammation is a key factor in the pathogenesis of GD and increased bone resorption markers have been described in patients. Moreover, we had previously shown that cellular alteration in GD, as modeled by in vitro GCase inhibition, produces bone destruction through the enhancement of macrophages differentiation to osteoclast and osteoclast resorption activity [18–20]. The aim of this study is to evaluate the presence and number of circulating proinflammatory cells and osteoclast precursors from Gaucher patients. Moreover, we aimed to analyze the spontaneous osteoclast formation by PBMC from Gaucher patients and to analyze the factors involved in this mechanism.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Ethical Committee of IBYME (Instituto de Biología y Medicina Experimental, CONICET, Argentina) according to provisions of the Declaration of Helsinki in 1995. Human PBMCs were isolated from patients and healthy blood donors in accordance with the guidelines provided by the ethical committee. The nature and purpose of the study were explained to all volunteers and all patients gave their written informed consent prior to participation in this study. In the case of minors/children written informed consent was obtained from the next of kin, caretakers, or guardians.

2.2. Patient demographics

Twenty four Gaucher patients (11 females and 13 males, median age: 32.16 ± 19.66 years old) were included in the present study. All the patients were under enzyme replacement therapy (ERT) with either Imiglucerase (I) (Cerezyme®, Genzyme, a Sanofi Company, Cambridge, USA) or Velaglucerase (V) (VPRIV®, Shire, Lexington, USA). Patient demographic and clinical data are summarized in Table 1.

Anemia was considered when hemoglobin concentration was below 1 g/dl below lower limit of normal for age and gender at the local laboratory. Thrombocytopenia was assigned positive when platelet count was below 90,000. The presence of splenomegaly or hepatomegaly was evaluated by quantitative abdominal magnetic resonance imaging. Bone involvement was defined positive when the patient presented at least one of the following manifestations: bone pain, bone crisis, osteopenia/osteoporosis, and osteonecrosis. Clinical data is presented as a percentage calculated as the number of affected patients over the total number of patients evaluated for each clinical parameter. The parameter improvement percentage was calculated as the number of patients that presented an improvement after ERT treatment over the number of patients that presented the clinical parameter before ERT treatment for each parameter evaluated. As shown in Table 1 bone involvement was the clinical parameter that presented the lowest improvement after ERT. Twenty four healthy individuals (mean age: 31.93 ± 21.10; range: 15–65 years old) matched for age and sex served as controls.

Table 1
Demographic and clinical data of patients included. Clinical parameters are presented as percentage of the number of patients that presented the symptom over the total of patients tested for that symptom.

Age range (years old)	32,16 ± 19,66	(10–77)		
Female (n)	11			
Male (n)	13			
Prior splenectomy (n)	4			
Genotype (n)				
c.1448 T > C (L444P)/c.1226A > G (N370S)	2			
c.1226A > G/c.481C > T	1			
c.1226A > G/RecNciI	4			
c.245C > T/unknown	2			
c.1226A>G/c.1226A>G	4			
RecNciI/c.1348T>A	1			
c.1226A > G/c.703 T > C	1			
c.1342G > C/c.1348 T > A	2			
RecNciI/c.1226A > G	1			
RecNciI/c.1504C > T	1			
ERT	Velaglucerase		Imiglucerase	
(n)	5		19	
Range (years)	10,79 ± 5,89	(1–20)		
Clinical parameters (%)	At diagnosis		Present	
Anemia	70,83	(17/24)	8,70	(2/23)
Thrombocytopenia	62,50	(15/24)	26,09	(6/23)
Splenomegaly	94,44	(17/18)	27,78	(5/18)
Hepatomegaly	91,30	(21/23)	27,27	(6/22)
Chitotriosidase elevated	80,00	(12/15)	25,00	(4/16)
Bone involvement	79,17	(19/24)	61,90	(13/21)
Parameter improvement (%)				
Anemia	93,75	(15/16)		
Thrombocytopenia	64,28	(9/14)		
Splenomegaly	68,75	(11/16)		
Hepatomegaly	70,00	(14/20)		
Chitotriosidase elevated	90,00	(9/10)		
Bone involvement	25,00	(4/16)		

2.3. Cell isolation

Peripheral blood samples from patients or healthy controls were collected 24 h before ERT infusion by venipuncture in heparin as anticoagulant and immediately processed. Mononuclear cells from whole blood (PBMC) were isolated by Ficoll Hypaque (Sigma, St Louis, MO, USA) gradient separation.

2.4. Flow cytometry

PBMCs from patients with Gaucher disease and healthy controls were incubated in PBS containing 10% of normal human serum for 20 min at 4 °C after which were centrifuged and resuspended in 50 μl of PBS and 10% normal human serum containing different conjugated antibodies. In all cases cells were incubated for 25 min at 4 °C washed with PBS and analyzed on a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). The following antibodies were used: CD51-FITC, CD16-PE and CD14-PECy5.5 to define osteoclast precursors as $CD51^+CD16^+CD14^+$ cells. CD3-FITC, RANKL-PE and CD20-PerCP to assess the levels of RANKL in T cells ($CD3^+RANKL^+CD20^-$) and B cells