Gaucher Cells Demonstrate a Distinct Macrophage Phenotype and Resemble Alternatively Activated Macrophages

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Abstract

Although the existence of anti-inflammatory alternatively activated macrophages (aamφ) has been accepted widely based on in vitro studies, their in vivo location, phenotype, and function still are debated. Gaucher disease (GD) is caused by a genetic deficiency in the lysosomal enzyme glucocerebrosidase and is characterized by accumulation of glycosphingolipids in so-called Gaucher cells (GCs). By using immunohistochemical analysis, we investigated whether this results in an aamφ phenotype. GCs are macrophage-like cells, expressing acid phosphatase, CD68, CD14, and HLA class II, but not CD11b, CD40, or dendritic cell markers. GCs show infrequent immunoreactivity for mannose receptor. GCs did not express proinflammatory cytokines such as tumor necrosis factor α and monocyte chemoattractant protein 1, but did express the aamφ markers CD163, CCL18, and interleukin-1 receptor antagonist. Furthermore, CD36 and signal receptor protein α, involved in lipid uptake, also were observed on GCs. Thus, GCs represent a distinctive population of myeloid cells that resemble aamφ but differ from previously described in vitro aamφ.

It has been long recognized that macrophages are highly versatile cells that can respond to stimuli in very diverse ways. Two main types of macrophages can be distinguished based on their phenotype and immune actions: classically and alternatively activated macrophages (camφ and aamφ, respectively).1,2 In contrast with camφ, which promote inflammation and T-helper 1–mediated responses, aamφ exert anti-inflammatory functions. In vitro, aamφ are induced by interleukin (IL)-13 and IL-4, express receptors of innate immunity, and produce anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist (IL-1Ra).1,2 These phenotypes probably represent 2 in vitro extremes of many possibilities that might be dictated by a plethora of factors, such as genetic makeup of the host, tissue microenvironment, and the nature of an infection or insult.

Although heterogeneity in macrophage phenotypes has been observed in vitro, there is not much data on heterogeneity in situ. Therefore, there is an urgent need for additional quantitative and qualitative in vivo markers.1 Recently, an important role for lipid metabolites has been established in inducing anti-inflammatory responses by macrophages.3,4 This provided the rationale for the current analysis in which we took advantage of a natural genetic disease that permits unprecedented insight into in situ macrophage biology in relation to lipid metabolism, Gaucher disease (GD).

GD is a lysosomal storage disorder that is caused by a deficiency of the lysosomal enzyme glucocerebrosidase, which is involved in the degradation of glucocerebroside. People with GD demonstrate a very heterogeneous disease pattern. Disease symptoms may occur early in life, but also might never manifest. Clinical symptoms include hepatosplenomegaly, skeletal disease, anemia, increased
metabolic rate, and loss of fat tissue.\(^5\)\(^6\) GD is a rare disease, and the incidence in the Netherlands is as low as 100 to 200 cases in a population of 16 million.\(^5\) Type I GD is the nonneuronopathic and most prevalent form of disease, and it can be treated effectively with enzyme replacement therapy, in which the mannose-terminated native enzyme is administered intravenously.\(^7\)\(^8\) It is thought that tissue macrophages expressing the mannose receptor (MR) take up the enzyme and transport it to the lysosome, thereby restoring their capacity to degrade glucocerebroside.\(^9\)

The pathologic hallmark of GD is the presence of lipid-laden Gaucher cells (GCs) in organs such as spleen, bone marrow, and liver.\(^5\)\(^10\) Despite the fact that these cells seem unique in morphologic features and metabolism, their exact function in the pathology of GD remains largely unclear. In addition, although expression of a few macrophage markers has been described,\(^11\)\(^12\) GCs have been characterized to a limited extent. Thus far, few studies have addressed immunologic competence of GCs, because these cells are regarded mostly as functionally inert cells, filled with glucocerebroside to an extreme level. This lack of immunologic insight is explained partly by the biochemical nature of previous studies, the lack of in vitro culture systems for GCs, and a paucity of in situ studies of GCs related to the scarcity of tissues. This immunologic lacuna is surprising considering the increased serum levels of proinflammatory and anti-inflammatory cytokines\(^13\)\(^16\) in people with GD.

Therefore, the present study addressed the hypotheses and concepts that glycolipid accumulation will affect the immune phenotype of macrophages in GD and that this is reflected in an aamφ phenotype. We analyzed expression patterns of cellular markers and of proinflammatory and anti-inflammatory mediators characteristic for camφ and aamφ in GD spleens in situ.

**Materials and Methods**

**Clinical Data**

We examined tissue samples from 2 patients with type I GD who underwent splenectomy. Histologic findings for both spleens were comparable, although the spleen of patient 1 contained more GCs.

**Case 1**

The patient was a man (born 1944, Ashkenazi Jewish origin, genotype N370S/N370S) who was examined in 1991 because of massive splenomegaly and thrombocytopenia. Enzyme replacement therapy began in July 1997 (pretreatment platelet count, 40 × 10^9/L; chitotriosidase concentration, 9,414 mol/h–1mL–1), but splenic pain and thrombocytopenia necessitated splenectomy in February 1998. At last follow-up, he was well (hemoglobin concentration, 12.1 g/dL [121 g/L]; platelet count, 360 × 10^9/L [360 × 10^9/L]; chitotriosidase concentration, 1900 nmol/h–1mL–1). His other medical problems include type 2 diabetes mellitus, hyperlipidemia, and hypertension.

**Immunohistochemical Analysis**

Immediately after splenectomy, spleen tissue fragments were frozen in liquid nitrogen, transported on dry ice, and stored at –80°C until use. Immunohistochemical analysis was performed on frozen sections of spleen from patients with GD to assess whether GCs express the markers shown in **Table 1**, which describes the surface markers for cellular subset identification and the effector molecules (enzymes, cytokines), along with antibodies used for detection. Representative data are shown in **Image 1**, **Image 2**, **Image 3**, and **Image 4**.

Sections of human tonsil were included in each staining procedure as positive control tissue samples for expression of all markers evaluated. The immunohistochemical procedures used have been described in detail.\(^17\)\(^18\) In brief, frozen sections of 6 μm were cut and thaw-mounted on glass slides. Slides were kept overnight at room temperature in a humidified atmosphere. After air drying the slides for 1 hour, slides were fixed in fresh acetone containing 0.02% (vol/vol) hydrogen peroxide to reduce endogenous peroxidase activity. Slides then were air dried for 10 minutes.

To visualize residual endogenous peroxidase activity before antibody staining, sections were incubated for 10 minutes at room temperature with 4-chloro-naphthol (Sigma-Aldrich, St Louis, MO), resulting in a black precipitate that can be distinguished easily from specific antibody staining. Slides were incubated with primary antibody overnight at 4°C. Incubations with secondary rabbit antimouse-Ig-biotin (DAKO, Glostrup, Denmark) and tertiary horseradish peroxidase (HRP)-labeled avidin-biotin-complex (ABC/HRP, DAKO) were performed for 1 hour at room temperature.
HRP activity was revealed by incubation for 10 minutes at room temperature with 3-amino-9-ethyl-carbazole (AEC, Sigma-Aldrich), leading to a bright red precipitate. Sections were counterstained with hematoxylin and embedded with glycerol-gelatin. Primary antibody reagent omission control staining was performed in all procedures. Double-labeling of cells was performed as described previously, with alkaline phosphatase–conjugated secondary antibodies and a substrate solution containing Fast Blue BB Base (blue reaction product; Sigma-Aldrich) and naphthol AS-MX phosphate (Sigma-Aldrich).

### Results

**GCs Express a Specific Set of Myeloid Markers**

To determine whether GCs are a homogeneous myeloid population, spleens from patients with GD were analyzed for markers that identify different myeloid subsets (Table I summarizes marker functions). GCs can be identified easily with certainty, even in sections counterstained only with hematoxylin, owing to their extreme size.

With specific antibodies, GCs were negative for the dendritic cell markers CD1a and dendritic cell–specific intercellular adhesion molecule–grabbing nonintegrin (data not shown), clearly demonstrating that they are not mature or immature dendritic cells or follicular dendritic cells. Image 1 shows expression of various macrophage markers on GCs and surrounding spleen cells. GCs were localized in the red pulp and strongly expressed lysosomal acid phosphatase (Images 1A and 1B, arrows), HLA class II (Image 1C, arrows), and CD68 (Image 1D, arrows). CD14 expression by GCs generally was membrane-associated (Image 1E, arrows) but occasionally also was found intracellularly (Image 1E, arrowheads). Sporadically, CD14– GCs were observed (Image 1E, asterisks). GCs did not express CD11b (Image 1F, arrows). In contrast, surrounding cells in the red pulp expressed all markers as expected for red pulp macrophages (Images 1A-1E; Image 1F, arrowheads). Thus, GCs represent
Gaucher cells (GCs) express many but not all macrophage markers. 

A. Intracellular acid phosphatase activity of GCs (arrows) (×100). 

B. GCs (asterisks) and smaller splenic red pulp macrophages (arrows) are both positive for acid phosphatase (×200). 

C. HLA class II is expressed intracellularly and on the membrane of GCs (arrows) (×100). 

D. GCs express high intracellular levels of CD68 (arrows) (×100). 

E. CD14 is expressed on the membrane of GCs (arrows) and occasionally is found intracellularly (arrowhead) (×100). CD14– cells also are observed (asterisks) (×100). 

F. CD11b is not expressed on GCs (arrows) but is observed on surrounding spleen cells (arrowheads) (×100).
Low expression of effector molecules by Gaucher cells (GCs). **A**, Interleukin (IL)-6 expression is membrane-associated (arrows), but IL6– cells also are found (arrowheads) (×200). **B**, GCs are weakly positive for IL-1β (arrows), whereas surrounding spleen cells demonstrate much stronger staining (arrowheads) (×200). **C**, Inducible nitric oxide synthase is observed very weakly in GCs (arrows) and not in surrounding cells (×200). **D**, Although GCs are negative for monocyte chemoattractant protein 1 (arrows), juxtaposed cells show strong immunoreactivity (arrowheads) (×200). **E**, IL-10 is expressed in GCs (arrows) and surrounding cells (arrowheads) (×200).
a distinct macrophage subpopulation, compared with red pulp macrophages, not only in sheer size and glycolipid accumulation, but also in terms of their immunologic phenotype.

GCs Do Not Produce Typical Proinflammatory Molecules

Because levels of several cytokines in the plasma of people with GD are elevated, including proinflammatory cytokines tumor necrosis factor α (TNF-α), IL-6, IL-8, and IL-1β, spleens were analyzed to establish whether GCs are the source of these cytokines and other activation markers. IL-6 was observed associated with the GC membrane (Image 2A, arrows), but many GCs were only weakly positive or even negative for IL-6 (Image 2A, arrowheads). Although many red pulp cells contained high levels of intracellular IL-1β (Image 2B, arrowheads), GCs were negative (Image 2B, arrows). GCs also were weakly positive for inducible nitric oxide synthase (Image 2C, arrows). Monocyte chemoattractant protein 1 (MCP-1) was not observed in GCs (Image 2D, arrows), although strongly MCP-1+ cells were found juxtaposed to GCs (Image 2D, arrowheads), suggesting interaction with CCR2 on GCs. IL-10 was expressed weakly in GCs (Image 2E, arrows) and in surrounding spleen cells (Image 2E, arrowheads).
Furthermore, GCs did not express other proinflammatory mediators such as IL-1α, IL-12p40, TNF-α, and interferon-γ (data not shown), suggesting that spleen GCs are unlikely to function as a major source of these cytokines.

**GCs Resemble Alternatively Activated Macrophages**

Because GCs did not phenotypically resemble camφ, the expression of markers characteristic for aamφ was determined. To conveniently identify GCs and macrophages, sections were costained for acid phosphatase activity, with the exception of the marker chitotriosidase. Chitotriosidase and IL-1Ra were expressed frequently and strongly by GCs (Images 3A and 3B, respectively, arrows). IL-1Ra was associated with the cell membrane, and both IL-1Ra+ and IL-1Ra− GCs were observed (Image 3B, arrows and arrowheads, respectively). No other positive spleen cells were observed. CD163 was expressed on a variety of acid phosphatase–positive cells. Large GCs were negative for CD163 (Image 3C, arrowheads), and smaller GCs demonstrated intracellular staining (Image 3C, arrows). Surrounding cells
also expressed CD163, and these cells often were juxtaposed to GCs (Image 3D, arrows). MR was expressed only infrequently by GCs (Image 3E, arrows), and, surprisingly, most cells were negative (Image 3F, arrowheads). Double labeling for the aamφ markers CCL18 and MR showed that CCL18 was expressed by all GCs (Image 3F, asterisks), whereas MR expression was expressed by endothelium (Image 3F, arrowheads). A double-positive cell was observed only occasionally (Image 3F, arrows). Thus, GCs express many but not all markers that are characteristic for aamφ.

### GCs Have Ambiguous Lipid and Antigen Recognition Characteristics

In general, aamφ are well equipped for phagocytosis of apoptotic cells and lipids. To determine whether GCs are potentially still capable of phagocytosis, despite their lack of MR, immunoreactivity for CD36 (scavenger/lipid receptor) and signal-regulatory protein α (SIRPα; down-regulatory receptor) was assessed. CD36 was expressed by GCs (Image 4A), suggesting their active participation in lipid phagocytosis. SIRPα showed a similar expression pattern. Most GCs were strongly positive (Image 4B, arrows), whereas others seemed to be negative (Image 4B, arrowheads). Surrounding cells were negative for SIRPα.

Because GCs potentially are capable of phagocytosis, we determined whether they express key molecules for recognition and presentation of antigen. Although GCs expressed CD14, they did not express toll-like receptor 2 or 4, indicating they are unable to recognize and respond to lipopolysaccharide and peptidoglycan. In addition, because they did not express MR, they also are unable to recognize microbial carbohydrates. Finally, although GCs expressed high levels of HLA class II, they were negative for CD40, which is an important costimulatory signal for T lymphocytes (data not shown).

### Discussion

The simple dichotomy of aamφ and camφ as defined in vitro is unlikely to fully describe the pleiotropic functions that macrophages have during immune responses and in disease. GD provides an extremely interesting and valuable opportunity to study the dynamic nature of macrophages in vivo under pathophysiologic conditions. As summarized in Table 2, we demonstrated that spleen GCs have a unique phenotype mostly resembling aamφ, do not express MR, are a major source of chitotriosidase, which we propose to be a marker for aamφ, and are unlikely to contribute to the systemic inflammation observed in patients with GD. In contrast, GCs express anti-inflammatory mediators and, therefore, might compensate for the inflammatory compounds in GD, which are produced by macrophages surrounding the GCs.

GCs expressed SIRPα and scavenger receptors such as CD36 and CD163. SIRPα is a myeloid-specific, membrane-bound protein that is associated with phagocytosis. Its ligand, CD47, functions as a marker of self-recognition on erythrocytes, and CD47-deficient erythrocytes are phagocyted by splenic red pulp macrophages. Because we found strong immunoreactivity of SIRPα on spleen GCs, the local uptake of erythrocytes could be hypothesized to be a main source of the accumulated lipids. In addition, SIRPα negatively regulates nuclear factor κB, down-regulates T-cell proliferation and activation, and down-regulates maturation of and cytokine production by dendritic cells. Thus, the presence of SIRPα on GCs might indicate anti-inflammatory actions. In addition to SIRPα, the lipid receptor CD36 was expressed on GCs, also indicating an ability of GCs to scavenge and accumulate lipids. These data confirm previous findings and are especially interesting in the context of SIRPα expression and in the light of recent reports that describe anti-inflammatory actions of lipid mediators. Whether glycolipid accumulation in GCs directly results in such mechanisms remains to be studied.

GCs demonstrated a highly heterogeneous expression pattern for CD163, which is characteristic for aamφ.
Interestingly, intermediate-sized GCs mainly expressed CD163 intracellularly. We hypothesize that these cells might reflect a transitional state from macrophages to GCs and might be a main source of soluble CD163. Soluble CD163 has been shown to act as an anti-inflammatory mediator. It is thought to be secreted by chronically activated macrophages. It has been shown to act as an anti-inflammatory mediator.26 Interestingly, plasma levels of soluble CD163 are increased in people with GD.27

An important but unexpected finding was the lack of detectable expression of MR on GCs. Because treatment of GD is based on MR-mediated uptake of the mannose-terminated enzyme, this finding has major implications for the understanding of GD. Interestingly, strong MR immunoreactivity was observed on many surrounding myeloid cells. Although the patients whose tissue samples we studied had to undergo splenectomy, they subsequently responded well to enzyme replacement therapy. This suggests that not only mature GCs contribute to disease but also their precursors, which can be corrected by treatment. This assumption is in agreement with animal models for GD in which inflammation is observed in the absence of mature GCs.28

GCs did not produce the typical proinflammatory molecules TNF-α, IL-1β, or MCP-1, but showed prominent expression of the anti-inflammatory molecule IL-1Ra. In contrast, surrounding spleen cells demonstrated strong and frequent immunoreactivity for camφ markers such as IL-1β and MCP-1 but did not express IL-1Ra. Part of the GCs expressed IL-6, which is an elusive cytokine, and its role during inflammation remains unclear. It has been suggested to be produced with camφ, but also with aamφ.29 Recently, IL-6 has been described as having an important role in MCP-1 induction and monocyte recruitment.30 Interestingly, MCP-1+ cells were found juxtaposed to IL-6+ GCs. Therefore, during GD, IL-6 might be involved in the migration of newly formed monocytes into the spleen and toward GCs. Thus, GCs might not participate in proinflammatory mechanisms themselves, but might indirectly induce an inflammatory response by activation of surrounding cells, possibly owing to physical disruption of tissue architecture and function.

Two important serum markers for GD are chitotriosidase and the chemokine CCL18 (PARC/AMAC-1 [pulmonary and activation-regulated chemokine/alternative macrophage activation-associated CC chemokine]). Both molecules are correlated strongly with disease symptoms and can be used to monitor the efficacy of treatment.

Chitotriosidase is a relatively newly described molecule that is thought to be secreted by chronically activated macrophages.33 It is involved in the degradation of chitin substrates, but its role in human innate immunity is largely unknown. Ym1 is a chitinase-like protein that is produced by alternatively activated mouse macrophages.34 In parallel with Ym1, chitotriosidase could be a novel human marker for aamφ, and strong intracellular chitotriosidase immunoreactivity indeed is observed in GCs.

The newly identified chemokine, CCL18, also has been shown to be a highly specific marker for aamφ and high CCL18 expression is found in GCs. Interestingly, CCL18+ cells were MR−, which argues against the prevailing idea that both might be specific markers for aamφ and emphasizes the need for new, adequate in vivo markers for aamφ. Although in vitro, aamφ are generated by a specific cytokine milieu in which IL-4 has an important role, we could not detect IL-4 in spleen tissue samples from patients with GD (data not shown). However, disturbed lipid metabolism is known to affect cell signaling and cytokine production, and we hypothesize that this might result in the observed phenotype.36

Although markers such as HLA class II, chitotriosidase, and acid phosphatase were expressed on virtually all GCs, expression levels of markers such as IL-1Ra, CD163, and IL-6 were heterogeneous, with some GCs strongly positive and others completely negative. This might reflect different stages in GC formation and underlines the variety of phenotypes that can be displayed by macrophages in vivo. In future studies, it will be important to include more cases to confirm our findings and, in addition, to determine whether the observed GC phenotype also is found in different types of GD and in other tissues.

It will be imperative to develop and use in vitro models for GC to determine the effect of glucocerebrosidase deficiency and subsequent lipid accumulation on macrophage function and which cellular mechanisms are involved. Unfortunately, there is no proper in vitro cell system available. Although there are ample data about the effect of lipid accumulation on macrophage function in general, these systems do not completely mimic the unique lipid accumulation that GCs demonstrate. The knockout mouse for GD has not proved highly useful for detailed pathophysiologic or functional studies, because these mice demonstrate rapid neonatal death. However, recently, viable mouse models were developed using the introduction of point mutations in the glucocerebrosidase gene, which resulted in the formation of storage cells in various tissues.41 This model possibly will provide an important tool for future functional studies.

Immunophenotypic analysis of GCs contributes to our understanding of macrophage biology in general. GCs not only emphasize the in vivo heterogeneity of macrophage populations but they also demonstrate the importance of constantly redefining and fine-tuning their phenotypic limits.
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