Pro-inflammatory cytokines and the pathogenesis of Gaucher’s disease: increased release of interleukin-6 and interleukin-10

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Summary

Gaucher’s disease is characterized by hepatosplenomegaly, bone-marrow infiltration, osteonecrosis and bone thinning, associated with the presence of pathological macrophages that contain undegraded glycosphingolipids. To investigate the possible role of cytokines in the systemic and local manifestations of established Gaucher’s disease, interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNFα) and interleukin-10 (IL-10) were measured in freshly-separated serum. Samples from eight male and 14 female patients with type 1 Gaucher’s disease were compared with sera from 22 healthy age- and sex-matched controls. Concentrations of IL-6 and IL-10 were significantly elevated in sera from patients with Gaucher’s disease (11.9 ± 1.8 (SEM) pg/ml and 5.4 ± 0.5 (SEM) pg/ml, respectively) compared with those of controls (4.1 ± 0.9 (SEM) and 0.8 ± 0.3 (SEM) pg/ml, p < 0.0001). No significant differences in concentrations of TNFα or IL-1β were identified. IL-6 has been implicated in the development of localized osteolysis in multiple myeloma and in the development of post-menopausal osteoporosis. High concentrations of IL-6 in the serum of patients with Gaucher’s disease may thus reflect the development of the bone lesions commonly associated with this disorder. Since IL-6 and IL-10 are important regulators of lymphocyte growth and differentiation, and IL-6 concentrations were significantly raised in patients with oligo- or polyclonal increases in serum immunoglobulins, enhanced release of these cytokines from pathological macrophages provides a pathological link between Gaucher’s disease and associated lymphoproliferative disorders.

Introduction

Gaucher’s disease is an inherited disorder in which deficiency of the lysosomal enzyme, acid β-glucocerebrosidase, leads to progressive accumulation of undegraded glucocerebroside within cells of the mononuclear phagocytic series.¹ The disease is inherited as an autosomal recessive condition due to defects in the glucocerebrosidase gene that maps to human chromosome 1q.² A hallmark of Gaucher’s disease is the Gaucher’s cell—a pathological macrophage that occurs typically in the bone marrow, spleen and liver. The disorder is associated with massive visceral enlargement, skeletal disease and in the rare neuronopathic variants Types II and III, with neuronophagia and gliosis.² There is, however, no strict relationship between the severity of the enzyme deficiency and the development of clinical disease; indeed some individuals with homozygous deficiency of acid β-
glucocerebrosidase may never require treatment. In other patients, partial deficiency of the enzyme is associated with bone-marrow failure, hepatic cirrhosis, pulmonary infiltration and gross hypersplenism. The most common clinical features of the disorder (hepatosplenomegaly and marrow disorder) appear to result directly from the local effects of Gaucher’s cell infiltration accompanied by parenchymal injury.3

Osseous manifestations are common in patients with type 1 (non-neuronopathic) Gaucher’s disease.4-6 Many skeletal lesions have been identified in Gaucher’s disease, including localized osteolysis, avascular necrosis, and diffuse osteopenia.7-10 Avascular necrosis (AVN) resembles the manifestations of Caisson disease and sickle-cell anemia; infiltration of Gaucher’s cells in the bone marrow appears to compromise effective blood flow at the epiphysis where localized ischaemia leads to bone infarction and collapse in the subchondral bone and, ultimately, osteoarthritis. The diffuse osteopenia seen in Gaucher’s disease has not been explained, although it probably represents a systemic disorder affecting bone metabolism.

With the identification of serum factors which influence the action of osteoblasts and osteoclasts, there is now an opportunity to investigate their role in the pathogenesis of skeletal disorders. Several pro-inflammatory mediators including IL-1β, IL-6, TNF-α and prostaglandin-E2 have been implicated in diverse skeletal disorders such as osteoporosis,12,13 Paget’s disease,14 and rheumatoid arthritis.15,16 The macrophage is an important source of many of these mediators, and may initiate auto-regulatory pathways for the activation of lymphocytes and other cells that originate from the bone marrow. We postulated that the pathological storage of lipid within Gaucher’s cells acts as a potent stimulus for macrophage activation and the release of serum factors that exert localized and systemic effects on the skeleton and cells of the lymphoid system. Monoclonal and polyclonal gammapathies have been frequently reported in patients with Gaucher’s disease,17-19 which may be complicated by the development of amyloidosis,20 leukaemia,21,22 Hodgkin’s disease23 and multiple myeloma.24,25 Since elevated plasma immunoglobulins,26 angiotensin-converting enzyme27 and lysosomal hydrolases28 have been reported previously, we determined the concentrations of macrophage-derived pro-inflammatory cytokines in the serum of patients with type 1 Gaucher’s disease. We have examined these in relation to prior treatment for the disorder and possible associations with B-cell abnormalities and skeletal disease.

Methods
Twenty-two patients with type 1 Gaucher’s disease were enrolled in this study. There were 14 women (mean age 38 years, range 23–59) and eight men (mean age 46 years, range 27–65 years). The experimental protocol was reviewed and approved by the Local Research Ethics Committee of the East Anglian Health Authority at Addenbrooke’s Hospital. The clinical details of these patients are shown in Table 1.

Serum immunoglobulin profiles were abnormal in 19/22 patients with type 1 Gaucher’s disease (see Table 1). The most common finding was a polyclonal

Results
Table 1  Clinical data for 22 patients with type 1 Gaucher’s disease

<table>
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<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
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<th>Intact spleen</th>
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<th>Bone disease</th>
<th>IL-1β (pg/ml)</th>
<th>TNF (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
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Oligo, oligoclonal gammapathy; Bi, biclonal gammapathy; Poly, polyclonal gammapathy; Panhypo, panhypoglobulinaemia; ND, not detected; –, not tested.

increase in serum IgG concentrations with an overall IgG concentration of over 13 g/l in 13 patients. Two patients had a polyclonal increase in IgM (>1.8 g/l) and three patients had raised concentrations of IgA (>4 g/l). One patient had a biclonal IgA species, one patient had oligoclonal IgA species. One patient had an IgA myeloma M species 15–25 g/l; this last patient had presented with a solitary plasmacytoma of the tibia 5 years previously and Gaucher’s disease was diagnosed on bone-marrow examination during the course of further investigations for splenomegaly and vertebral fractures (Figure 1).

Interleukin 1β was not detected in samples from either Gaucher’s patients nor in the reference population. There were no significant differences between the concentrations of TNF-α in sera from the two groups: the mean (±SEM) concentration of TNF-α was 5.8 ± 1.4 pg/ml in serum samples from patients with Gaucher’s disease and 6.2 (±1.2) pg/ml in serum samples obtained from the healthy controls.

The mean (±SEM) concentration of IL-10 was 5.4 ± 0.8 pg/ml in serum from 13 patients with Gaucher’s disease and 0.8 (±0.3) pg/ml in serum from matched healthy control subjects (p < 0.0001).

No significant differences were observed between male and female subjects for either IL-6 or IL-10. This was true for both Gaucher’s patients and the controls.

Serum IL-6 or IL-10 concentrations in Gaucher’s patients who had undergone splenectomy and those with intact spleens were similar (p = 0.45 for IL-6 and p = 0.39 for IL-10). Similar comparisons between patients on enzyme replacement therapy and patients receiving no form of enzyme replacement revealed a significant difference for IL-10 (p < 0.05) but not for IL-6 (p = 0.82).

Although concentrations of IL-6 were somewhat higher in Gaucher’s patients with bone disease (12.4 ± 3.7 (SEM) pg/ml) compared with patients without radiological evidence of bone disease (9.2 ± 1.7 (SEM) pg/ml), the changes were not statistically significant (p > 0.1). Serum concentrations of IL-10 were similar in Gaucher’s patients with and without signs of bone disease (p > 0.3).

To determine if IL-6 concentrations correlated with evidence of clonal expansion of B cells, IL-6 concentrations in the serum of patients showing monoclonal or biclonal gammapathy were compared with those in patients with no evidence of discrete clonal expansion. Serum IL-6 concentrations in the group...
Figure 1. Micrograph of Leishman-stained bone-marrow aspirate from a patient with multiple myeloma complicating Gaucher’s disease. Note the presence of the large lipid-laden macrophages (Gaucher’s cells) and infiltration by abnormal plasma cells. Serum electrophoresis demonstrated an IgA monoclonal paraprotein and paresis of IgG and IgA, which were undetectable.

Figure 2. Serum concentrations of a IL-6 and b IL-10 in patients with type 1 Gaucher’s disease. The control population consisted of age- and sex-matched healthy individuals.

Discussion

Although genetically-determined defects in the activity of \( \beta \)-glucocerebrosidase and the concomitant accumulation of glucosylcerebroside appear to be the primary cause of Gaucher’s disease, analysis of
glycosphingolipid content of affected tissues indicates that <2% of the visceral weight is accounted for by the storage material. In established type 1 Gaucher’s disease, the massive enlargement of the spleen and liver cannot therefore be explained by the storage products, and it is clear that the condition results from the indirect effects of lysosomal storage within the Gaucher’s cells. Resident macrophages are widely distributed and are a rich source of secretory products which mediate and control immune and inflammatory activity. Accordingly, we have investigated the possible involvement of cytokines of the interleukin series in the clinical expression of Gaucher’s disease.

Patients with Gaucher’s disease experience malaise, have been shown to have an increased metabolic rate, and may develop intermittent pyrexia in the absence of demonstrable infection. Plasma immunoglobulins and other proteins such as the type 5 isozyme acid phosphatase, angiotensin-converting enzyme, and several lysosomal hydrolases, including chitotriosidase and lysozyme, are frequently elevated. The functional significance of these abnormalities remains unclear, although it is notable that the improvements in haematological parameters that are seen with enzyme replacement therapy are accompanied by reduced plasma chitotriosidase activity.

Monoclonal and polyclonal gammopathies are well-recognized in patients with Gaucher’s disease. Gaucher’s disease has also been associated with an increased risk frequency of lymphoid malignancies including leukaemias, and Hodgkin’s disease.

Hodgkin’s disease and multiple myeloma are protected from bone loss after oophorectomy. IL-6 may mediate the osteoporosis of oestrogen deficiency, since mice lacking IL-6 as a result of targeted gene disruption are protected from bone loss after oophorectomy.

In addition, the secretion of IL-6 by mononuclear cells increases after oophorectomy and overstimulation of IL-6 can trigger the development of monoclonal gammopathy in mice. With these observations in mind, the increased concentrations of IL-6 in the serum of Gaucher’s patients strongly argues for a role for this cytokine as an important mediator of the persistent acute-phase response, immunoglobulin abnormalities and disturbances in bone remodelling that are seen in type 1 Gaucher’s disease. It also seems probable that the greatly increased IL-6 concentration plays a particular role in maintaining a persistent cytokine signal for clonal expansion of plasmacytoma cells in patients with monoclonal gammopathy. Thus IL-6 may be the critical triggering factor for the development of plasmacytoma and multiple myeloma in patients with Gaucher’s disease. The same cytokine may also stimulate the proliferation and activation of osteoclast precursors on the surface of bone adjacent to infiltrating Gaucher’s cells, leading to a net increase in bone resorption in the proximity of a diseased marrow cavity.

The role of IL-10, which was also significantly elevated in the serum of Gaucher’s patients, appears to be less clear. Although cytokines participate in inflammatory and immunological reactions, most have multiple biological targets and activities. The particular role of any given cytokine in a disease state is therefore highly dependent on the spectrum of polypeptides that are co-secreted. Although a solvent-solubilized preparation of glucosylceramide stimulated secretory cytokine activity in cultured macrophages, the role of cytokines in Gaucher’s disease in situ is less clear.

Acute leukaemias, chronic lymphocytic leukaemia, Hodgkin’s lymphoma and acute myelogenous-myelomonocytic leukaemias have all been recorded sporadically in patients with Gaucher’s disease. However, the most common neoplastic disorder in Gaucher’s patients is multiple myeloma, a condition that had developed in one patient described here. Interleukin-6 is known to act as a paracrine regulator of the growth of myeloma cells and serum IL-6 concentrations are frequently elevated in patients with multiple myeloma.

In addition to its effects on plasmacytoma cells, IL-6 induces B-cell maturation, growth of T-cells and acute-phase responses in the liver; these findings are typically observed in patients with Gaucher’s disease. This raises the possibility that IL-6 may play a central role in the development of Gaucher’s disease and its association with multiple myeloma.

IL-6 also has powerful effects on the skeleton: it is a potent stimulator of bone resorption and reduces bone formation in calvarial cultures. More recently it has been suggested that it may mediate the osteoporosis of oestrogen deficiency, since mice lacking IL-6 as a result of targeted gene disruption are protected from bone loss after oophorectomy.

In addition, the secretion of IL-6 by mononuclear cells increases after oophorectomy and overstimulation of IL-6 can trigger the development of monoclonal gammopathy in mice. With these observations in mind, the increased concentrations of IL-6 in the serum of Gaucher’s patients strongly argues for a role for this cytokine as an important mediator of the persistent acute-phase response, immunoglobulin abnormalities and disturbances in bone remodelling that are seen in type 1 Gaucher’s disease. It also seems probable that the greatly increased IL-6 concentration plays a particular role in maintaining a persistent cytokine signal for clonal expansion of plasmacytoma cells in patients with monoclonal gammopathy. Thus IL-6 may be the critical triggering factor for the development of plasmacytoma and multiple myeloma in patients with Gaucher’s disease. The same cytokine may also stimulate the proliferation and activation of osteoclast precursors on the surface of bone adjacent to infiltrating Gaucher’s cells, leading to a net increase in bone resorption in the proximity of a diseased marrow cavity.

The role of IL-10, which was also significantly
increased in the serum from patients with Gaucher's disease, is perhaps less clear. IL-10 appears to be capable of influencing both bone formation and bone resorption; it inhibits osteoblast secretory activity and mineralization, and also reduces the formation of osteoclast-like cells in vitro. IL-10 has no effect on mature macrophages or osteoclasts, and so in a situation where the supply of osteoclast precursors is stimulated by IL-6 (as may be the case in Gaucher's disease), an inhibitory action on osteoblast metabolism may be the predominant effect of local IL-10.

IL-10 contributes to the panel of interleukins that promote the growth of haematopoietic cells, including B- and T-cells, but it may also constitute part of an inflammation-reducing feedback system. As such, IL-10 is considered to be a member of the class of Th2-cytokines that promote IgE production and inhibit cell-mediated immunity. Production of IL-10 may represent an attempt to regulate the inflammatory response which is triggered by the activated Gaucher's cells. Although it stimulates the expansion of B-cells, IL-10 also appears to inhibit the production of pro-inflammatory cytokines by the macrophage. Interleukin-1β and TNF-α, both of which are important mediators of the acute inflammatory response and critical elements in cell-cell signalling in immune cells, are down-regulated by IL-10, and it is not surprising that we failed to detect elevations in either IL-1β or TNF-α in these patients with a longstanding disorder such as Gaucher's disease.

Although the number of Gaucher's patients in this initial study is relatively small, and detailed longitudinal studies were not performed, we found a statistically significant difference in the concentration of IL-10, between patients receiving enzyme replacement therapy and those not so treated. Gaucher's patients treated with therapeutic enzyme show improvements in clinical symptoms and regression of signs of disease, as indicated by improvements in blood counts and reductions in visceral enlargement and circulating markers of macrophage activation. However, at the doses used, complete regression did not occur, since the concentrations of IL-6 remained elevated. Long-term monitoring of therapeutic responses to enzyme replacement therapy, or other treatments for Gaucher's disease, might be more informative if serial cytokine profiles were examined. However, given the daunting complexity and intrinsic redundancy of the cytokine network, more information is required before such a strategy can be adopted for the long-term assessment of this disorder.

We conclude that type 1 Gaucher's disease is associated with greatly increased concentrations of IL-6 and IL-10, cytokines that are known to be important regulators of lymphocyte growth and differentiation, acute-phase responses, and bone resorption. Production of these cytokines probably reflects the response of the pathological macrophage to the glycosphingolipid storage product and provides a mechanistic link between Gaucher's disease and many of its clinical manifestations.

**Acknowledgements**

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**References**


