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Chapter Three

Marked elevation of the chemokine CCL18/PARC in Gaucher disease: a novel surrogate marker for assessing therapeutic intervention

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Abstract

Gaucher disease is characterized by storage of glucosylceramide in lysosomes of tissue macrophages as the result of an autosomal recessively inherited deficiency in glucocerebrosidase. Progressive accumulation of these glycolipid-laden Gaucher cells causes a variety of debilitating symptoms. The disease can be effectively treated by costly intravenous infusions with recombinant glucocerebrosidase. Chitotriosidase is massively secreted by Gaucher cells and its plasma levels are employed to monitor efficacy of enzyme therapy. Broad scale application is hampered by the common genetic defect in this surrogate marker. We report that in plasma of symptomatic Gaucher patients the chemokine CCL18 is on average 29-fold elevated, without overlap between patients' and control values (median control plasma level is 33 ng/mL, range: 10-72 ng/mL, median Gaucher plasma level is 948 ng/mL, range: 237-2285 ng/mL). Plasma CCL18 concentrations decrease during therapy, comparably to chitotriosidase levels. Immunohistochemistry demonstrates that Gaucher cells are the prominent source of CCL18. Plasma CCL18 levels can serve as alternative surrogate marker for storage cells in Gaucher patients and monitoring of plasma CCL18 levels proves to be very useful in determination of therapeutic efficacy, especially in patients that are deficient in chitotriosidase activity. The potential physiological consequences of chronically elevated CCL18 in Gaucher patients are discussed.

Introduction

Gaucher disease is one of the most prevalent lysosomal storage disorders in man. The disease is due to an autosomal recessively inherited deficiency in lysosomal glucocerebrosidase activity (E.C. 3.2.1.45), leading to accumulation of its substrate glucosylceramide in the lysosomes of macrophages [1]. Progressive accumulation of these glycolipid-laden macrophages (Gaucher cells) in various locations in the body causes a spectrum of clinical symptoms. Three different clinical phenotypes are recognized, based on the age of onset of neurological signs. The clinical manifestations of the most common non-neuronopathic variant (type I Gaucher disease) includes anemia and thrombocytopenia, hepatosplenomegaly and skeletal deterioration [1]. A frequent sign is the development of monoclonal or oligoclonal gammopathies [2].

Non-invasive monitoring of Gaucher disease by determination of plasma factors that are exclusively secreted by Gaucher cells is of great importance for various reasons. The glucocerebrosidase genotype of individuals is not always predictive for the phenotypic expression of Gaucher disease [3]. Even among monozygotic twins with abnormal glucocerebrosidase genotypes, a remarkable variability in disease manifestations can occur [4]. The availability of an effective, but very costly therapy has also urged the identification of surrogate markers for Gaucher cells that can guide decision on initiation of therapy and dosing regimens. The identification of factors secreted by Gaucher cells is also of fundamental interest since it may lead to better understanding of the unique pathophysiology of the disorder.

A number of years ago we discovered that Gaucher cells massively secrete a hitherto unknown chitinase. The activity of the enzyme, named chitotriosidase, in plasma of symptomatic Gaucher patients is elevated on average several hundred-fold. For example in the initial chitotriosidase study, plasma activity was found to be elevated on average 641-fold (median control plasma 20 nmol/mL/h, range 4-76 nmol/mL/h, median Gaucher plasma 12824 nmol/mL/h, range 3122-65349 nmol/mL/h) [5]. Plasma chitotriosidase has proven to be a useful surrogate marker for Gaucher disease manifestations and is employed for diagnosis, early determination of onset of disease and monitoring of therapeutic efficacy [5-8]. Plasma chitotriosidase levels do not reflect one particular clinical symptom, but rather are a reflection of the total body burden of Gaucher cells [9]. The use of plasma chitotriosidase as a Gaucher cell marker is hampered by the fact that about 5 to 6% of the population, including Gaucher patients, is deficient in chitotriosidase activity due to a 24-basepair duplication in the chitotriosidase gene. Obviously these individuals can not be monitored by the measurement of plasma chitotriosidase activity [5,10].

To identify novel factors derived from Gaucher cells, we analyzed plasma samples of Gaucher patients before therapeutic intervention and compared that with control plasma using surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF MS) mass spectrometry. In plasma of a symptomatic patient a peptide of 7856 Da was found to be markedly increased in relation to Gaucher disease manifestation. Subtractive hybridization studies previously revealed that RNA encoding for a protein with identical mass is

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upregulated in Gaucher spleen [11]. This protein, named pulmonary and activation-regulated chemokine (PARC, systematic name CCL18), is a member of the C-C chemokine family [12,13]. Chemokines are a large family of low molecular weight (7-12 kDa) proteins. They are characterized by the presence of four conserved cysteines involved in forming essential disulfide bonds. CXC and CC chemokines are distinguished according to the position of the first two cysteines [14,15]. Their main biological role is found in mediating chemotaxis of leucocytes, a process mediated by G protein-coupled receptors of which most of them recognize more than one chemokine [14,15].

We here report on CCL18 plasma levels in symptomatic patients with adult type I Gaucher disease. To test its value as surrogate disease marker, plasma CCL18 levels were analyzed in relation to parameters of disease severity, other surrogate markers, and the effect of therapy. The implications of chronically increased CCL18 for the pathophysiology of Gaucher disease are discussed.

Materials & methods

Patients

All patients with Gaucher disease (type I) studied (29 males and 26 females; 12-67 years old, at the initiation of therapy) were known to us either by contact with the Netherlands Gaucher Society or by referral to the Academic Medical Center. Of the 55 type I patients, 47 received enzyme replacement therapy (Ceredase[®], Cerezyme[®]: Genzyme (individualized dosing)), 2 patients received substrate reduction therapy (chronic oral administration of an imunosugar-inhibitor of glucosylceramide synthesis, Zavesca[®] (*N*-butyldeoxynojirimycin): Oxford Glycosciences) and 6 patients were not treated. The 36 controls consisted of healthy volunteers (males, n=16; females, n=20).

Assessment of disease severity

To assess the clinical severity of the Gaucher patients, the severity scoring index (SSI) was used. The score, accounting for a variety of clinical symptoms, is calculated according to Zimran et al. [16]. Several indicators of disease severity were separately assessed. They included: Hemoglobin, platelet count, spleen size, liver size and bone marrow fat fraction. Patients were also divided in two groups according to presence or absence of the spleen and presence or absence of skeletal symptoms.

SELDI-TOF MS

Plasma samples were surveyed for basic proteins using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Plasma samples were exposed to a weak cation exchange surface and bound proteins were subsequently analyzed on a PBSII reader (CIPHERGEN Biosystems, Fremont, CA, USA). Samples (10 μ L) were denatured in 9 M Urea, 2% CHAPS and 1% DTT at RT (60 min). An aliquot (10 μ L) of this solution was mixed with 90 μ L binding buffer (50 mM Tris HCl,

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pH 8.0, 0.1% Triton X100) and added to a WCX2 ProteinChip array. After 40 min at RT the ProteinChips were washed with binding buffer (2 times, 5 min) and 50 mM Tris HCl, pH 8 (2 times, 5 min). After a quick rinse with distilled water the ProteinChips were dried and matrix was added (sinapinic acid). ProteinChip arrays were read with laser intensity 200 and mass deflector set at 500 Da.

Immunocapture experiments were performed using a PS20 ProteinChip array precoated with anti-CCL18 or anti-TNF α monoclonal antibodies. Samples (10 μ L) were incubated with 90 μ L binding buffer and allowed to bind for 2 h. After washing (as before) ProteinChip arrays were dried, matrix was applied and arrays were read with laser intensity 180 and mass deflector set at 500 Da.

ELISA

Plasma CCL18 levels were measured by a sandwich ELISA assay using a commercially available CytoSet (Biosource International, California USA), consisting of a capture-antibody, a biotinylated detection-antibody, recombinant CCL18 standard and streptavidin-HRP conjugate. Assay conditions were exactly as described by the manufacturer.

Enzyme activity assays

Serum angiotensin converting enzyme (ACE) activity was measured using hippuryl-L-histidyl-L-leucine as substrate, as described [17]. The activity of serum β -hexosaminidase was measured using 4-methylumbelliferyl-*N*-acetylglucosamine (Sigma, St. Louis, MO) as substrate in citrate/phosphate buffer (0.1/0.2M) at pH 4.0. The standard enzyme activity assay for chitotriosidase with 4MU-chitotriose (4-methylumbelliferyl β -D-N,N',N''-triacetylchitotriose, Sigma) as substrate was performed at pH 5.2, as previously described [5].

Bone marrow fat fraction

Dixon quantitative chemical shift imaging (QCSI) was used to assess bone marrow fat fraction of the axial skeleton as described in detail by Maas et al. [18,19]. In short, bone marrow fat fraction is used as a reflection of severity of bone marrow involvement, since progressive infiltration of the bone marrow with Gaucher cells is associated with disappearance of normal adipocytes.

Immunohistochemistry

Immunohistochemistry was performed on frozen sections of Gaucher spleen to detect expression patterns of CCL18. The methodology of immunocytochemical procedures used here has been reviewed in detail previously [21]. In brief, frozen sections of 6 mm were cut and thawmounted on glass slides. Slides were kept overnight at room temperature in humidified atmosphere. After air-drying the slides for one hour, slides were fixed in fresh acetone containing 0.02% (v/v) H₂O₂. Slides were then air-dried for 10 min, washed with PBS and incubated with optimally diluted anti-CCL18 antibody (AB60, mouse

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monoclonal, R&D Systems) overnight at 4°C in humidified atmosphere. Incubations with secondary rabbit anti-mouse-Ig-biotin (Dako, Glostrup, Denmark) and tertiary HRP-labelled avidin-biotin-complex (ABC/HRP: Dako) were performed for 1 hour at RT. Between incubation steps slides were washed twice with PBS. Horseradish peroxidase (HRP) activity was revealed by incubation for 10 min at RT with 3-amino-9-ethyl-carbazole (AEC: Sigma, Zwijndrecht, the Netherlands), leading to a bright red precipitate. After washing, sections were counterstained with hematoxylin, and embedded with glycerol-gelatin. Primary antibody reagent omission control staining was performed.

RNA isolation and northern blot

Total spleen RNA was isolated using the RNazol B (Biosolve, Barneveld, The Netherlands) RNA isolation kit according to the manufacturers instructions. For Northern blot analysis, 15 µg samples of total RNA were run in 10 mM Hepes, 6% formaldehyde-agarose gels, transferred to Hybond N nylon membranes (Amersham, Buckinghamshire, UK) by the capillary method, and immobilized by UV cross-linking. The following probes were used: full length CCL18 cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a RNA control. The probes were radiolabelled with ³²P using the random priming method [20]. Hybridization conditions were exactly as described before [10].

Statistical analysis

Results are given as median and range. The data were analyzed using the Mann-Whitney *U*-test. Correlations were tested by the rank correlation test (Spearman coefficient, ρ). *P*-values less than 0.05 were considered statistically significant.

Results

In an attempt to identify novel factors present in plasma of Gaucher patients, we analyzed plasma samples of Gaucher patients before and after a few years of therapeutic intervention using surface-enhanced laser desorption/ionization mass spectrometry (SELDI-TOF MS). For this purpose Gaucher plasma was applied on a weak cation exchange ProteinChip array at different buffer conditions. A peptide of 7856 Da was virtually absent in control samples but prominent in a sample of an untreated symptomatic Gaucher patient (see Fig. 1A). The molecular mass and the basic isoelectric point of the peptide is remarkably similar to that of a member of the human C-C chemokine family named “pulmonary and activation-regulated chemokine” (PARC, systematic name CCL18), of which the mRNA was previously noted to be upregulated in the spleen of a Gaucher patient [11]. Immuno-capture experiments revealed that in serum of symptomatic Gaucher patients elevated levels of CCL18 occur (see Fig. 1B).

In order to accurately quantify the levels of CCL18 in plasma of Gaucher patients a sandwich ELISA assay was employed. Figure 2 shows the CCL18 levels in plasma samples from 36 controls and from 55 symptomatic Gaucher patients (prior to initiation of

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therapy). The median plasma CCL18 level in controls is 33 ng/mL (range 10-72 ng/mL), whereas the median level in the patients' samples is 948 ng/mL (range 237-2285 ng/mL). The levels of CCL18 in plasma of symptomatic Gaucher patients are thus on average 29 fold elevated. Importantly, there is no overlap between symptomatic patient values and control values. All patient plasma CCL18 values are considerably higher than the control mean + 3*SD (99.7% confidence interval).

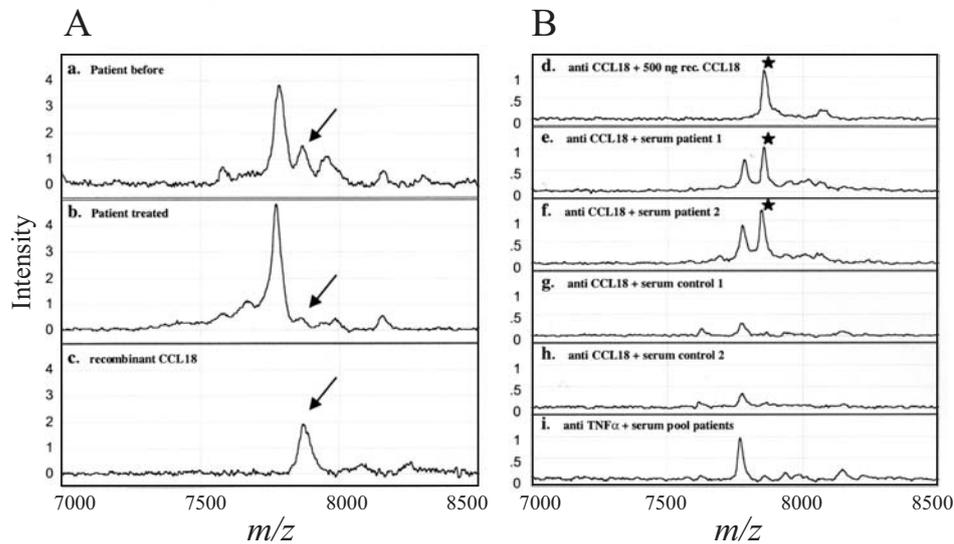


Figure 1. SELDI-TOF mass spectra of profiling (A) and antibody capture (B) experiments. Panel A. Weak cation exchange (WCX2) ProteinChip array: plasma samples of a Gaucher patient before (a) and after treatment (b) and a control experiment with recombinant CCL18 in 0.5% (w/v) BSA (c). Mass range 7000-8500 *m/z*. Panel B. PS20 ProteinChip array coated with monoclonal antibodies: anti-CCL18 (d-h) and (as a control) anti-TNF α (i). Recombinant CCL18 (d), sera of Gaucher patients (e and f), control sera (g and h) and a pool of patient samples (i) were exposed to the antibody-coated arrays. Mass range 7000-8500 *m/z*.

The relationship between plasma CCL18 levels and parameters of disease was examined. No strict correlation was noted with hematological abnormalities (hemoglobin levels and platelet count), degree of splenomegaly or hepatomegaly (data not shown). Moreover, we did not observe any difference between plasma CCL18 levels in patients with enlarged spleen or without spleen and no relation between chemokine levels and occurrence of skeletal disease (data not shown). The bone marrow fat fraction, as assessed with quantitative chemical shift imaging (QCSI) [18], did not correlate with plasma CCL18 levels either. In type I Gaucher patients with a mild degree of disease manifestation (individuals with a severity scoring index < 5) [16] plasma CCL18 levels tend to be lower than in more severely affected individuals (SSI \geq 5), median: 722 ng/mL (range: 405-1160 ng/mL) vs. median: 974 ng/mL (range: 303-2285 ng/mL) respectively, although not statistically significant. Similar relationships between plasma chitotriosidase activity and other Gaucher disease parameters were demonstrated previously [9]. Analysis of the present cohort of type I Gaucher patients showed that plasma chitotriosidase, β -

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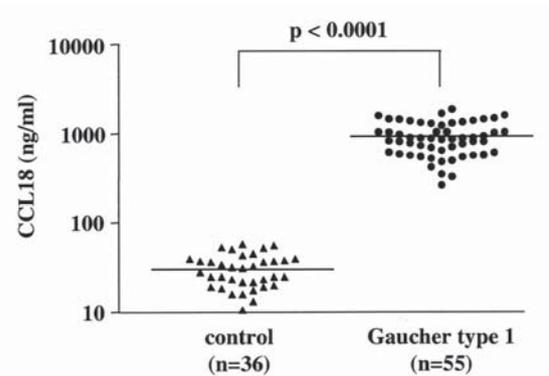


Figure 2. Plasma CCL18 levels in controls and Gaucher patients. Plasma CCL18 levels in controls (n=36) and Gaucher patients (n=55). Chemokine concentrations were determined as described in Materials & Methods.

hexosaminidase and angiotensin converting enzyme (ACE) levels also tend to be lower in mildly affected individuals compared to severe patients, however also not statistically significant: (plasma chitotriosidase: (SSI < 5) median 8085 nmol/mL/h (range 4939-13358 nmol/mL/h) vs. (SSI \geq 5) median 18773 nmol/mL/h (range 2393-80074 nmol/mL/h); plasma β -hexosaminidase: (SSI < 5) median 1477 nmol/mL/h (range 756-2678 nmol/mL/h) vs. (SSI \geq 5) median 2580 nmol/mL/h (range 1072-7486 nmol/mL/h); plasma ACE: (SSI < 5) median 140 U/L (range 100-148 U/L) vs. (SSI \geq 5) median 202 U/L (range 116-324 U/L)). In the case of tartrate resistant acid phosphatase (TRAP) a more limited correlation with disease severity was observed (plasma TRAP: (SSI < 5) median 1053 nmol/mL/h (range 768-2307 nmol/mL/h) vs. (SSI \geq 5) median 2418 nmol/mL/h (range 616-14539 nmol/mL/h)). Plasma CCL18 values were weakly correlated to chitotriosidase, TRAP and ACE levels: CCL18 vs. chitotriosidase: n=51; Spearman $\rho=0.542$; $P < 0.0001$; CCL18 vs. chitotriosidase wild type: n=38; Spearman $\rho=0.571$; $P=0.0002$; CCL18 vs. TRAP: n=36; Spearman $\rho=0.558$; $P=0.0004$; CCL18 vs. ACE: n=29; Spearman $\rho=0.493$; $P=0.007$. The relation with β -hexosaminidase levels was even less strict: n=55; Spearman $\rho=0.391$; $P=0.003$. The four chitotriosidase-deficient Gaucher patients showed high plasma CCL18 values (863, 1122, 951, 329 ng/mL), consistent with their severe disease manifestations.

The effect of enzyme replacement therapy on plasma CCL18 in Gaucher patients was examined. Figure 3A shows the marked reduction in two representative patients that responded clinically well to enzyme replacement therapy. As observed for chitotriosidase, there was an initial prominent reduction in plasma CCL18, followed by a slowly progressing decrease over the successive years. Patients responding poorly to this treatment showed no sustained reduction in plasma CCL18 (see Fig. 3B). A decline in plasma CCL18 was also observed for patients treated with substrate reduction therapy (see Fig. 3C). In sharp contrast, patients that did not receive enzyme replacement therapy showed a gradual increase of CCL18 plasma levels in time (Fig. 3D).

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A weak correlation exists between the plasma levels of chitotriosidase and CCL18 in naïve Gaucher patients. There is striking similarity in corrections in both parameters in individual Gaucher patients during therapy. Fig. 4A & B shows that the reductions in plasma CCL18 and chitotriosidase upon enzyme replacement therapy are completely proportional. The relative corrections in both markers correlate significantly, see Fig. 4C: (n=47 individual Gaucher patients; Spearman $\rho=0.706$; $P<0.0001$). The relative reduction in excess plasma ACE and excess plasma β -hexosaminidase during therapy also correlated to that in CCL18 (ACE; n=40 individual Gaucher patients, Spearman $\rho=0.579$, $P<0.0001$; plasma β -hexosaminidase: n=39 individual Gaucher patients, Spearman $\rho=0.6218$, $P<0.0001$). Interestingly, the relative reduction in plasma CCL18 during therapy also closely correlates with the corrections in the fat fraction of the lumbar spine bone marrow (Fig. 5). All the findings suggest that reductions in CCL18 reflect corrections in the presence of Gaucher cells.

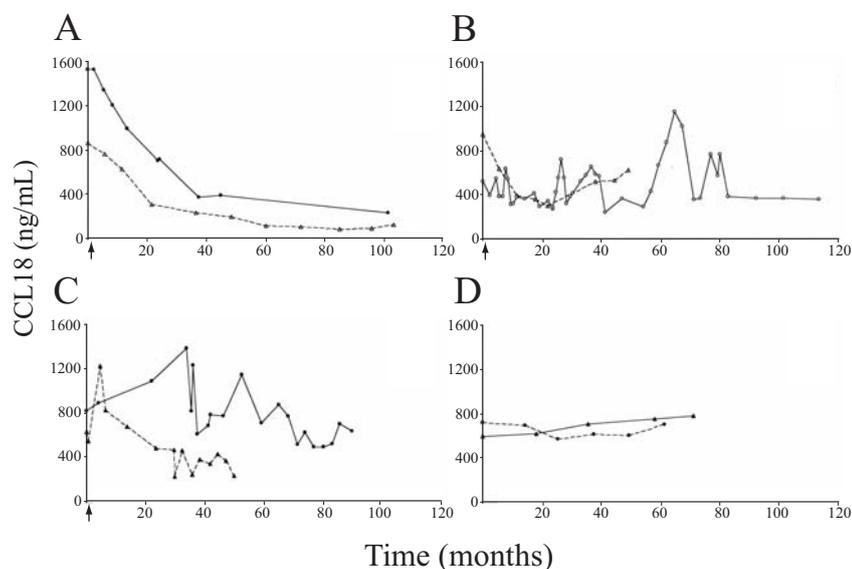


Figure 3. Effect of therapeutic interventions on plasma CCL18 levels. Panel A: plasma CCL18 levels in two individuals responding well to enzyme replacement therapy. Panel B: plasma CCL18 levels in two individuals responding clinically poorly to enzyme replacement therapy. Panel C: plasma CCL18 levels in two individuals on substrate deprivation therapy. Panel D: plasma CCL18 levels in two untreated Gaucher patients. Closed symbols represent chitotriosidase wild type individuals; open symbols chitotriosidase deficient individuals. The arrow indicates the initiation of therapy.

To investigate whether CCL18 is produced directly by Gaucher cells, the presence of CCL18 mRNA in Gaucher spleen was studied by northern blot analysis. As can be seen in Fig. 6A, CCL18 mRNA is highly expressed in the patient's spleen and almost absent in control tissue. Next, immunohistochemistry on sections from spleens of two Gaucher patients unequivocally revealed the specific expression of CCL18 protein in all Gaucher cells (see Fig. 6 B and C). Expression of CCL18 was virtually absent in other cells in these spleen sections. Similar labeling was observed for chitotriosidase (not shown).

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CCL18 is supposed to be involved in initiation of an adaptive immune response, recruitment of naïve T and B cells towards antigen presenting cells, and as such may contribute to development of antibody producing plasma cells. We therefore investigated whether the levels of CCL18 in plasma correlate with the presence of gammopathy. No obvious difference was noted between plasma CCL18 values in patients with or without detectable monoclonal gammopathy: mean: 1305 ng/mL (range: 525-2005 ng/mL, n=9) vs. mean: 932 ng/mL (range: 101-2285 ng/mL, n=46) respectively. However, the plasma concentration of CCL18 (1878 ng/mL) was relatively high in the case of one Gaucher patient who had uncontrolled proliferation of monoclonal plasma cells in the bone marrow.

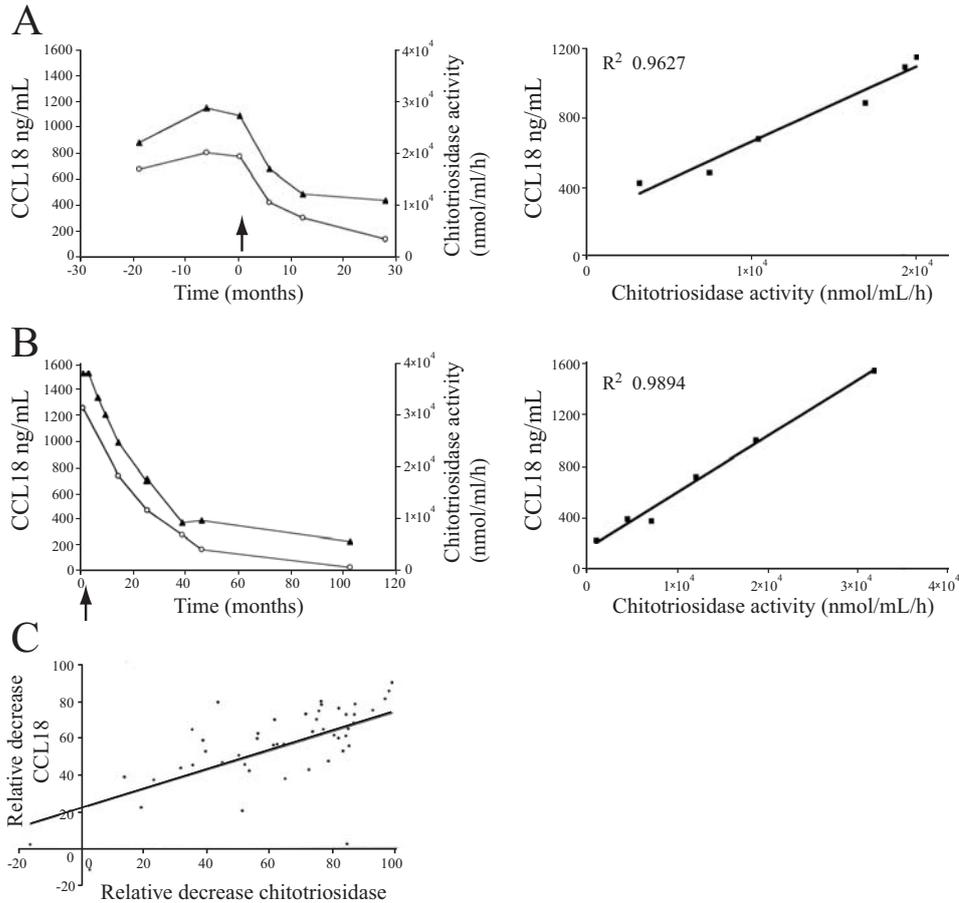


Figure 4. Relationship between decrease in CCL18 plasma levels and chitotriosidase plasma activity. Left panel A & B: Decrease in plasma CCL18 (filled triangle) levels and plasma chitotriosidase activity (open circle) in individual patients upon enzyme replacement therapy. Right Panel A & B: relation between the fractional change in markers. Panel C: Relation between fractional changes in markers following enzyme replacement therapy for at least 1 year. Excess activity at t=0 was set at 100%. Depicted are results for 47 individual Gaucher patients.

Discussion

Our study describes a marked increase in plasma levels of the CC chemokine CCL18 in symptomatic Gaucher patients. This chemokine, originally identified as a T cell chemoattractant, also attracts CD38-negative mantle zone B-lymphocytes. It has been speculated that CCL18 plays a role in the recruitment of T and B-lymphocytes toward antigen presenting cells (APC), a crucial step in the initiation of adaptive immune responses [12,13,22,23]. The chemotactic response of T cells to CCL18 is abolished by treatment of the cells with pertussis toxin, indicating a role for heterotrimeric G protein-coupled receptors. The exact receptor, however, remains unidentified [12]. There have been several reports on elevated levels of CCL18 in human disease, for example atherosclerosis, active hepatitis C infection, hypersensitive pneumonitis, allergic contact hypersensitivity, sepsis as well as rheumatoid arthritis and ovarian carcinoma [24-30]. Different detection methods have been employed in the previous studies, in some investigations CCL18 mRNA was detected either by RT-PCR analysis or in situ hybridization [24,25,27,29,30] and in other studies ELISA's were used to measure CCL18 protein in synovial or ascitic fluid [26,28]. To our knowledge this is the first report on chronically elevated plasma levels of CCL18 in a disease condition. Interestingly, the recurrent theme of all disease states in which CCL18 is over-expressed seems to be inflammation.

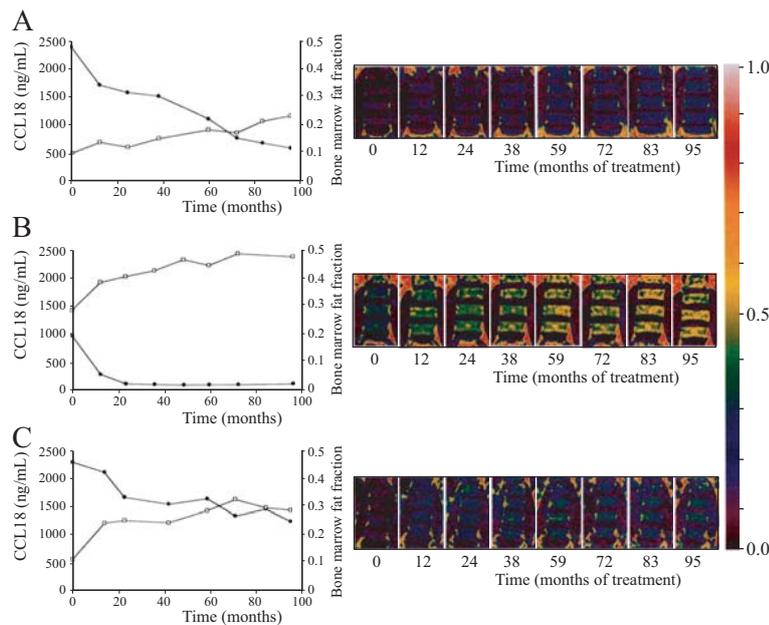


Figure 5. Relationship between decrease in CCL18 plasma levels and lumbar spine bone marrow fat fraction. Panel A, B and C: Inverse relationship between the decrease in plasma CCL18 levels and increase in lumbar spine marrow fat fraction upon enzyme replacement therapy. Patient A: Spearman $\rho=-0.952$; $P=0.0011$, patient B: Spearman $\rho=-0.802$; $P=0.0218$, patient C: Spearman $\rho=-0.815$; $P=0.0108$. (filled circle = CCL18, open square = bone marrow fat fraction). Right hand side, changes in the bone marrow fat fraction of the lumbar spine during enzyme replacement therapy as visualized by quantitative chemical shift imaging.

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Our findings suggest that plasma CCL18 can act as reliable surrogate disease marker that is useful in the case of Gaucher disease for further confirmation of diagnosis, demonstration of disease onset and monitoring of efficacy of therapeutic intervention. The increase in plasma CCL18 is far more pronounced than that in ACE, β -hexosaminidase and TRAP. Very mild affected patients still show abnormal plasma CCL18 in contrast to the other markers mentioned above. As compared to CCL18, chitotriosidase is more spectacularly increased in symptomatic Gaucher patients, provided that they do not carry the chitotriosidase gene defect. In the case of Gaucher patients that are deficient in chitotriosidase activity, monitoring of plasma CCL18 seems a good and reliable alternative that can aid in the clinical management of Gaucher patients. Immunohistochemical analysis of spleen sections from two Gaucher patients have indeed revealed that Gaucher cells are the prominent source of CCL18 as well as chitotriosidase. Fractional corrections in plasma CCL18 levels and of other disease markers like chitotriosidase, ACE and β -hexosaminidase are similar in Gaucher patients following therapy. Corrections in bone marrow fat fraction, an indirect assessment of Gaucher cell infiltration of the marrow, are also paralleled by corrections in plasma CCL18 (or chitotriosidase).

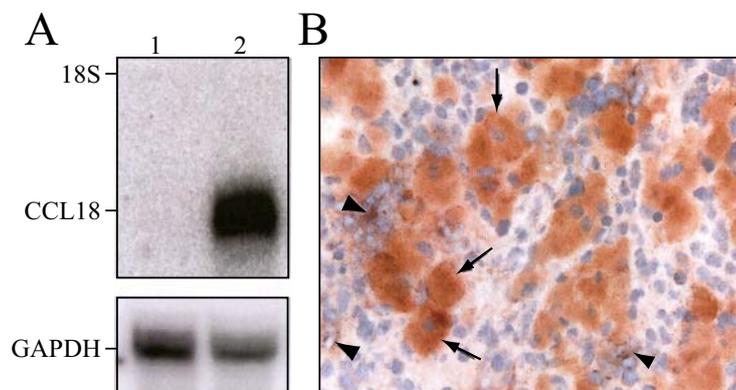


Figure 6. Expression of CCL18 in Gaucher spleen. Panel A: Detection of CCL18 mRNA in Gaucher spleen by northern blot analysis. Control and Gaucher spleen total RNA was analyzed by northern blotting as described in Material and Methods. Lane 1: Control spleen; Lane 2: Gaucher spleen. The probes used: full length CCL18 cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a RNA control. The 18S ribosomal band is indicated. Panel B: Detection of CCL18 protein by immunohistochemistry in Gaucher spleen. Clustered large swollen cells are Gaucher cells and label massively for CCL18 protein (arrows). Some surrounding spleen cells also show some labeling (arrowheads). (magnification, x400).

The CCL18 in plasma is derived from Gaucher cells in various body locations. This is suggested by the lack of correlation between the plasma CCL18 levels and splenic volume. As is the case for plasma chitotriosidase levels, the concentration of CCL18 in the circulation does not reflect one particular clinical sign of the disease but reflects the total body burden of Gaucher cells. Plasma CCL18 (or chitotriosidase) concentrations do not strictly correlate with the severity scoring index. Several factors may contribute to this. Firstly, the scoring index does not reflect the actual body burden on storage cells but rather scores the incidence of pathological events. Secondly, Gaucher cells in different body

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locations may contribute differently to the chronic CCL18 concentration in the circulation. Thirdly, the kinetics of elimination of CCL18 from the circulation by mechanisms such as receptor mediated uptake and excretion via the kidney may vary among individual patients.

The potential role of chronically elevated CCL18 in the pathophysiology of Gaucher disease is of interest. Abnormalities concerning serum immunoglobulins and other manifestations of disturbed B-cell function, like monoclonal or oligoclonal gammopathies, occur frequently in Gaucher patients. However, we did not observe any correlation between the plasma levels of CCL18 and the presence of a monoclonal gammopathy. Since all symptomatic Gaucher patients show at least 10-fold increased plasma levels of CCL18, it can be envisioned that this constitutes a risk factor for the development of disturbed B-cell function with other factors influencing the eventual outcome.

It has been reported that CCL18 can function as a natural antagonist of the CCR3 chemokine receptor [31,32]. CCR3 is expressed by eosinophils, Th2 cell subsets, basophils, mast cells, neural tissue, some epithelia and CD34⁺ progenitor cells [33,34]. Eosinophil chemotaxis induced by the most potent CCR3 agonists, like eotaxin and macrophage chemoattractant protein-4 (MCP-4) can be inhibited by CCL18 at concentrations as low as 10 nM [31,32]. The CCL18 plasma levels in symptomatic Gaucher patients exceed these inhibitory concentrations considerably (3-27 times). It seems likely that tissues rich in Gaucher cells contain even higher CCL18 concentrations. At this moment it is not clear whether Gaucher patients show abnormalities in CCR3 mediated chemotaxis of eosinophils. Moreover, it can not be excluded that the high concentrations of CCL18 in plasma and tissues block also other chemokine receptors, explaining neutrophil chemotaxis abnormalities in Gaucher disease [35,36].

Increased plasma chitotriosidase levels have been found to be extremely useful as surrogate marker for Gaucher cell burden in Gaucher patients. The extent of elevation in plasma CCL18 in symptomatic patients is far less compared to that in chitotriosidase. The application of plasma CCL18 is therefore particularly of interest for those Gaucher patients in which chitotriosidase is lacking due to a homozygosity for the common gene defect. At present it can not be excluded that plasma CCL18 can also be markedly increased due to other pathologies. It should be clear that measurement of plasma CCL18 for primary diagnosis of Gaucher disease should therefore not be advocated. Only in those cases in which the disease has been confirmed by demonstration of glucocerebrosidase deficiency or gene defects the monitoring of plasma CCL18 is useful to obtain an impression of Gaucher cell burden.

In conclusion, our finding of markedly elevated plasma CCL18 levels in symptomatic Gaucher patients warrants further investigations regarding its applicability in the clinical management of Gaucher disease as well as its role in the peculiar pathophysiology of the disorder.

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