Concise report

Hyperuricaemia elevates circulating CCL2 levels and primes monocyte trafficking in subjects with inter-critical gout

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Abstract

Objective. To investigate the effect of hyperuricaemia on serum chemokine (C-C motif) ligand 2 (CCL2) levels and blood monocytes in people with gout.

Methods. Whole blood was collected from subjects with a history of acute or chronic gout but not currently experiencing an attack of gout, subjects with asymptomatic hyperuricaemia and healthy individuals with normouricaemia. Serum concentrations of CCL2 were measured by bead array and levels of CD14+/CD11b+ blood monocytes determined by flow cytometry.

Results. Subjects with gout and asymptomatic hyperuricaemia had higher serum levels of CCL2 and showed an increase in the percentage of circulating CD14+ monocytes compared with subjects with normouricaemia.

Conclusion. Hyperuricaemia causes elevated serum CCL2 levels and increased monocyte recruitment that may be driven by soluble uric acid-induced CCL2 production. Hyperuricaemia may initiate subclinical priming of circulating blood monocytes for adhesion and trafficking during a gout attack.

Key words: hyperuricaemia, CCL2, monocytes, inflammation, gout.

Introduction

Gout is generally regarded as an articular disease, with the manifestations related to the local inflammatory consequences of MSU crystal deposition.

Resident macrophages have been shown to be primarily responsible for initiation of inflammation and cytokine production in the early response to MSU crystals [1]. However, kinetic studies in mice have shown that monocytes are one of the initial infiltrating inflammatory cells [1, 2] and chemokine (C-C motif) ligand 2 (CCL2) is a key monocyte chemoattractant involved in controlling monocyte trafficking [3, 4]. Animal models of MSU crystal-induced inflammation have identified CCL2 as a key local chemoattractant in synovial recruitment of monocytes in gout [5] indicating that changes in CCL2 levels could impact significantly on monocyte recruitment during a gout attack.

Hyperuricaemia is the primary risk factor for gout. Soluble uric acid has been shown to induce CCL2 production in vascular smooth muscle cells [6] and hyperuricaemia has been linked with elevated levels of serum inflammatory markers, including CCL2 levels, in the absence of clinical inflammation [7, 8]. Together these data indicate that hyperuricaemia may directly contribute to elevated levels of CCL2 to facilitate monocyte release into the circulation of individuals with hyperuricaemia and established gout even in the absence of an inflammatory attack. In this study, we investigated the impact of hyperuricaemia on the levels of serum CCL2 and circulating CD14+ monocytes in subjects with established gout but no current inflammation, and asymptomatic hyperuricaemia.

Materials and methods

Study participants

Participants were recruited from advertisements to the public, primary care and a rheumatology clinic and was...
approved by the New Zealand Central Regional Ethics Committee (CEN/06/03/018). Informed, written consent was obtained from all participants according to the Declaration of Helsinki. The study has been previously described in Martin et al. [9]. Briefly, this study collected a peripheral blood sample from subjects who had acute gout \((n = 29)\), chronic tophaceous gout \((n = 16)\) (each with no current clinical signs of inflammation) hyperuricaemia with no history of gout \((n = 15)\) or healthy controls \((n = 31)\).

Serum collection

Peripheral blood was collected into additive free tubes. Serum was separated by centrifugation \((1900\, \text{g},\ 10\, \text{min})\) and stored at \(-70^\circ\, \text{C}\) for subsequent analysis.

Monocyte purification

Peripheral blood was collected in \(K_3\)EDTA tubes and peripheral blood mononuclear cells \((\text{PBMCs})\) were separated out by sedimentation using Polymorphprep \((\text{Invitrogen, Auckland, New Zealand})\) as per manufacturer’s instructions. Any contaminating red blood cells were removed with lysis buffer \((\text{Invitrogen})\) and the PBMCs were washed twice with PBS \((\text{Invitrogen})\). CD14\(^+\) monocytes were purified from PBMCs using positive immunomagnetic selection with MACS CD14 microbeads \((\text{Miltenyi Biotec, Pharmaco, New Zealand})\) as per manufacturer’s instructions. Monocyte \((\text{CD45}^+\text{/CD14}^+)\) purity \(>94\%\) was determined by flow cytometric analysis \((\text{FITC-Mouse anti-human-CD45, PE-CD11b, PE-Cy7-CD14; ebiosciences, San Diego, CA, USA})\).

Serum analysis

Serum was analysed for CCL2 using a Luminex multiplex bead array \((\text{Invitrogen})\), as per the manufacturer’s instructions. Serum uric acid levels were measured in a commercial laboratory \((\text{Aotea Pathology, Wellington, New Zealand})\).

Statistical analysis

Data were analysed using Prism \((\text{Version 5.0d; GraphPad, La Jolla, CA, USA})\). Exploratory data analyses confirmed that continuous variables were not normally distributed so serum concentrations of cytokines were assessed using non-parametric tests \((\text{Kruskal-Wallis test with Dunn’s post test, Mann-Whitney and Spearman’s } \rho)\). For all statistical tests a \(P < 0.05\) was considered statistically significant.

Results

Study participants

Full demographic data have been previously reported [9]. The mean \((95\% \text{ CI})\) serum uric acid levels of the four subject groups were control \(0.35\, \text{mM (0.33, 0.36 mM)}\), asymptomatic hyperuricaemic \(0.45\, \text{mM (0.44, 0.47 mM)}\), acute gout \(0.46\, \text{mM (0.44, 0.48 mM)}\) and chronic gout \(0.48\, \text{mM (0.45, 0.51 mM)}\). Uric acid levels represent \(5.9\, \text{mg/dl, 7.6 mg/dl, 7.7 mg/dl and 8.1 mg/dl, respectively}\).

Elevated serum CCL2 correlates with elevated serum uric acid

Analysis of serum CCL2 levels from the four study groups showed higher concentrations of CCL2 in subjects with acute and chronic gout and asymptomatic hyperuricaemia compared with healthy controls \((\text{Fig. 1A})\). Due to the presence of elevated serum CCL2 levels in subjects with asymptomatic hyperuricaemia, serum CCL2 was stratified based on the presence of hyperuricaemia \(\geq 0.42\, \text{mM/7.1 mg/dl}\). As shown in Fig. 1B there was an increase in serum CCL2 in subjects with hyperuricaemia.

Elevated serum CCL2 correlates with increased circulating CD14\(^+\) monocytes and up-regulation of adhesion molecule CD11b

CCL2 is considered to be the primary chemoattractant for monocyte/macrophages. Analysis of monocyte composition in total white blood cells revealed an increase in circulating CD14\(^+\) blood monocytes associated with serum CCL2 levels \((\text{Fig. 1C})\) that was independent of gouty disease \((\text{Fig. 1D})\).

Elevated levels of CCL2 have also been linked to up-regulation of the adhesion molecule CD11b on monocyte/macrophages [10]. As shown in Fig. 1E, there was a positive correlation between serum CCL2 levels and monocyte CD11b expression levels. When CD11b was analysed by subject group we saw a significant increase in CD11b expression on monocytes from acute gout subjects and a trend towards elevated CD11b on CD14\(^+\) monocytes from individuals with asymptomatic hyperuricaemia and chronic gout \((\text{Fig. 1F})\).

Discussion

Here we show that serum CCL2 levels positively correlate with serum uric acid in both individuals with gout and with asymptomatic hyperuricaemia. Soluble uric acid has been shown to induce CCL2 production in vascular smooth muscle cells [6, 11] and may represent one source of CCL2 in subjects with hyperuricaemia.

This study also showed that elevated serum CCL2 was associated with an increase in both circulating CD14\(^+\) monocytes and monocyte CD11b expression. CD14\(^+\) monocytes are one of the first leucocytes to migrate to inflammatory sites during a gout attack. The observed increase in circulating blood monocytes associated with elevated serum uric acid and CCL2 levels in the absence of active inflammation may serve to provide a pool of monocytes primed for rapid trafficking in response to inflammatory insults. Monocyte migration is mediated by the up-regulation of adhesion molecules such as CD11b, which binds intercellular adhesion molecule 1 \((\text{ICAM-1})\) on endothelial cells to facilitate cell adhesion. CCL2 has been shown previously to increase CD11b expression [10]. Consistent with this, CD11b was also up-regulated on monocytes in this study. Interestingly, the most significant increase in CD11b expression was observed on monocytes from acute gout subjects and was not directly related to uric acid levels. Increased CD11b expression
on monocytes in acute gout subjects could indicate heightened responsiveness of these cells to migration signals triggered by local inflammatory stimuli such as MSU crystals. Up-regulation of CD11b would facilitate monocyte adhesion to the vascular wall and therefore the process of diapedesis. In this way, up-regulation of CD11b could accelerate monocyte migration to sites of acute MSU crystal deposition leading to rapid inflammation such as that observed during an acute attack of gout.

Our findings may also have relevance to the elevated cardiovascular risk observed in gout subjects [12–14]. Hyperuricaemia, elevated serum CCL2 levels and increased circulating leucocyte numbers are commonly associated with higher risk of cardiovascular disease [15, 16]. The migration of monocytes to inflamed sites on the arterial wall and their subsequent transformation into foam cells plays a pivotal role in plaque formation in atherosclerosis [17, 18]. The presence of higher numbers of circulating monocytes in subjects with hyperuricaemia and gout could be a contributing factor in the higher risk of cardiovascular disease in these individuals [19] where increased expression of CD11b on monocytes could act to facilitate monocyte adhesion and migration into the vascular endothelium.

In conclusion, we show elevated levels of serum CCL2 in subjects with hyperuricaemia, with and without gout, resulting in increased CD14+ monocytes in the circulation. These findings indicate an important role for serum uric acid in the modulation of monocyte trafficking and function and may be of particular importance in acute gout attacks. Future prospective studies examining these inflammatory processes in the context of gout, hyperuricaemia and the potential relationship with atherosclerosis will be necessary to confirm and expand the current findings.
Rheumatology key message

- Hyperuricaemia elevates serum CCL2 production and monocyte trafficking in patients with gout.

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References


