Association between increased CCL2 (MCP-1) expression in lesions and persistence of disease activity in giant-cell arteritis*

M. C. Cid¹, M. P. Hoffman³, J. Hernández-Rodríguez¹, M. Segarra¹, M. Elkin³, M. Sánchez², C. Vilardell¹, A. García-Martínez¹, M. Pla-Campo¹, J. M. Grau¹ and H. K. Kleinman³

Objective. Patients with giant-cell arteritis (GCA) usually respond dramatically to corticosteroid treatment. However, recurrences are frequent and corticosteroid requirements are highly variable among patients. The aim of our study was to identify genes potentially involved in disease persistence.

Methods. Gene expression was explored with cDNA arrays in temporal artery biopsies from six GCA patients with relapsing disease and six patients who easily achieved sustained remission. Differentially expressed genes of interest were subsequently analysed by quantitative real-time polymerase chain reaction (PCR) and immunohistochemistry in temporal artery biopsies from 35 patients with biopsy-proven GCA and nine controls.

Results. CCL2 (MCP-1) was up-regulated in temporal artery samples from relapsing individuals. In the extended series of patients, CCL2 mRNA concentration in lesions was significantly higher than in controls (31 ± 15.6 vs 0.44 ± 0.10, P = 0.0001). In addition, CCL2 was more abundant in patients who experienced two or more relapses during the first year compared with those who endured sustained remission (127 ± 82 vs 11 ± 5.5, P = 0.0233) and correlated with the cumulated prednisolone dose (R = 0.533, P = 0.0024). CCL2 mRNA concentration correlated with IL-1β (R = 0.45, P = 0.02), tumour necrosis factor-α (TNF-α) (R = 0.47, P = 0.013) and IL-6 (R = 0.52, P = 0.0053) mRNA. However, circulating CCL2 determined by ELISA was decreased in patients with strong systemic inflammatory response, suggesting that reduction in circulating CCL2 may reinforce the local gradient in lesions.

Conclusion. Increased CCL2 (MCP-1) expression in lesions is associated with persistence of disease activity in GCA.

Key words: Vasculitis, Inflammation, Chemokines.

Introduction

Patients with giant-cell arteritis (GCA) usually experience a dramatic relief of their symptoms with corticosteroid treatment [1]. However, corticosteroid requirements are highly variable among patients [2]. While some patients easily enter sustained remission with relatively short corticosteroid courses, others suffer from a relapsing disease requiring cumulative corticosteroid doses with their ensuing adverse effects [1, 2].

We have previously shown that the intensity of the acute-phase response is empirically associated with different disease outcomes [2]. Patients with weak systemic inflammatory response have higher risk of developing disease-related ischaemic events [3] but achieve a sustained remission more rapidly, suffer from fewer relapses, and require lower cumulated corticosteroid doses [2]. In contrast, patients with strong systemic inflammatory response have more refractory disease and require more prolonged corticosteroid treatment [2]. Associated with this persistent activity, these patients have higher tissue production and circulating levels of pro-inflammatory cytokines, and a more prominent angiogenic response with stronger expression of endothelial cell-adhesion molecules in their lesions compared with patients with weak systemic inflammatory reaction [2, 4–7]. These observations support the concept which some patients would develop an inflammatory process that would easily evolve to a healing stage with higher risk of ischaemic complications, perhaps facilitated by the scarring process, whereas other patients would develop persisting disease with sustained inflammatory cascades leading to a more refractory and relapsing outcome.

Pro-inflammatory cytokines IL-1β, tumour necrosis factor-α (TNF-α) and IL-6 may contribute to determining these different disease outcomes in GCA. As mentioned, their tissue expression correlates with the intensity of the systemic inflammatory response [4]. In addition, TNF-α and, to a lesser extent, IL-1β mRNA levels in lesions correlate with subsequent corticosteroid requirements [4]. Based on their known biological functions, pro-inflammatory cytokines may directly maintain inflammatory cascades leading to persistent disease activity [8–9]. However, pro-inflammatory cytokines may be downstream products regulated by other factors with stronger impact on the fate of the disease or may be coordinately regulated with other mediators that are also relevant in determining the disease expression and outcome.

¹Department of Internal Medicine and ²Department of Pathology, Hospital Clinic, Vasculitis Research Unit, University of Barcelona, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain and ³Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda MD, USA.

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In this study, we used cDNA arrays to screen for differential gene expression in tissue samples from untreated patients with different subsequent disease outcomes in order to identify additional genes with potential prognostic significance. We characterized CCL2 (MCP-1) as a significant factor associated with disease persistence and identified other potentially relevant genes that deserve further study. Some of the differentially expressed genes have previously unknown functions in inflammation or in vascular biology.

**Patients and methods**

**Patients**

We consecutively selected two groups of patients from our prospectively recorded database of patients with biopsy-proven GCA. All the patients were essentially required to have had a temporal artery biopsy excised prior to the administration of corticosteroids, and stored frozen at −80°C in guanidine-thiocyanate (GTC) to preserve RNA. For group 1, we selected six patients with weak systemic inflammatory response (<2 inflammatory parameters as described) [2, 3] who had tolerated corticosteroid reduction with no relapses, successfully achieving a stable maintenance prednisolone dose <10 mg/day in less than 35 weeks. Four of the patients had ischaemic events at the time of diagnosis. For group 2, we selected six patients with strong systemic inflammatory reaction (3–4 inflammatory parameters). All these patients required more than 50 weeks to achieve a stable maintenance prednisolone dose <10 mg/day and none of them developed ischaemic complications. The main clinical characteristics of these patients are summarized in Table 1. The study was approved by our local ethics committee and all patients signed informed consent.

**RNA extraction from temporal artery biopsies**

Temporal artery biopsies were obtained for diagnostic purposes. Part of the specimen was snap frozen in isopentane pre-chilled in liquid nitrogen and stored at −80°C. This fragment was used for histopathological examination and immunohistochemical studies.

About 0.3–0.5 cm of the biopsies were frozen in GTC and stored at −80°C. These samples were thawed in TRIzol reagent (Life Technologies) and RNA extraction and purification were performed according to the manufacturer’s instructions. The yield was 2.75 µg RNA ± 0.77 (mean ± SEM) per patient in group 1 and 2.15 µg RNA ± 0.58 in group 2. RNAs from patients in each group were pooled in order to have enough RNA to hybridize the cDNA arrays after DNase treatment. Pooling of samples was performed to minimize non-specific changes in the expression of genes modulated by ischaemia or involved in tissue repair, due to unavoidable variations either in tissue manipulation during the excision, duration of the surgical procedure or delay in tissue processing.

**cDNA array hybridization**

We used Human Atlas 1.2 I nylon cDNA arrays (Clontech, BD Biosciences) containing 1176 known genes (list available at http://bioinfo.clontech.com/atlasinfo).

Pooled RNA samples were treated with DNase to remove contaminating genomic DNA and 2 µg of total RNA per condition were 32P-labelled using Moloney murine leukaemia virus (MMLV) reverse transcriptase and 3’ primers corresponding to the genes represented on the Atlas 1.2 I array (Atlas Pure Labeling System protocol, Clontech). After the recommended blocking steps and purification of the labelled probes, cDNA array membranes were hybridized overnight at 68°C with 25 × 10⁹ cpm of each labelled probe. Membranes were then stringently washed and exposed for 6 days to a Phosphor Imager screen. With this exposure time, low abundance transcripts may not be detected. Hybridization of both the control and the housekeeping cDNAs was equivalent in both filters and contamination by genomic DNA was excluded by internal controls. Negative controls (plasmid and phage DNA) did not provide any radioactive signal, excluding non-specific binding. After the subtraction of the background and global normalization, the intensity of the radioactive signals was compared using the Atlas-Image software (Clontech).

**Real-time quantitative RT–PCR measurement of cytokines in temporal artery samples**

CCL2 (MCP-1) was one of the genes differentially expressed in both groups of patients. CCL2 mRNA was subsequently measured in a wider series of 35 consecutively diagnosed patients with biopsy-proven GCA, who had been diagnosed and treated by the authors according to a previously reported schedule [2] and prospectively followed for at least 1 yr. Patients were unselected; all of them fulfilled the American College of Rheumatology (ACR) classification criteria for GCA, and encompassed the entire classical spectrum of disease manifestations. TNF-α, IL-1β and IL-6 cytokine mRNAs were also measured for comparison purposes. One microgram of total RNA obtained from temporal artery samples was reverse transcribed to cDNA with the Superscript® II First Strand Synthesis kit (Life Technologies), using random hexamers as a priming method. Quantitative real-time PCR amplification of the above cytokine cDNAs was performed using Pre-Developed TaqMan Target kits from Applied Biosystems (Foster City, CA, USA), as described [4, 5]. The reaction was monitored by measuring the fluorescence signal.
after each cycle with ABI Prism TM 7700 Sequence Detection System (Applied Biosystems). Values were expressed as relative units. CCL2 mRNA was also quantified in nine normal temporal artery biopsies obtained from patients in whom the disease was initially suspected but not confirmed, being subsequently diagnosed with other conditions. The ultimate diagnoses in these patients were: isolated polymyalgia rheumatica (two patients), anaemia-related to diabetic nephropathy (two patients), monoclonal gammopathy (one patient), non-vasculitic anterior ischaemic neuropathy (one patient), temporomandibular osteoarthitis (one patient), ophthalmoplegia due to orbital myositis (one patient), and acute lymphoblastic leukaemia (one patient). Specimens from these patients also served as controls for immunohistochemistry.

**Immunohistochemical detection of CCL2 and its cell receptor CCR2 in temporal artery biopsies**

Expression of CCL2 and its receptor CCR2 was subsequently analysed by immunohistochemistry in temporal artery sections from 50 patients and in normal temporal artery biopsies from nine patients eventually diagnosed with the conditions listed in the previous section. Seven out of the 50 GCA samples exhibited only adventitial inflammation, whereas in the remaining 43 specimens inflammatory infiltrates extended through the media and the intima.

Serial 4–6 μm cryostat sections were air dried, fixed with cold acetone, and incubated with the following primary monoclonal antibodies for 30 min at room temperature: mouse anti-human CCL2 (clone 23002 from R&D Systems at 5 μg/ml) and mouse anti-human CCR2 (clone 48607.121 from R&D Systems at 10 μg/ml). Immunoreactivity was detected with a horseradish peroxidase (HRP)-bound polymer coupled to a secondary anti-mouse antibody (Envision system from Dako). Peroxidase activity was visualized by 0.02% 3-3 diaminobenzidine (Sigma) and 0.05% hydrogen peroxide. Mouse immunoglobulin (Dako) at the same concentration as the primary antibodies and incubated with the detection system served as the negative control. All sections were slightly counterstained with Harris' haematoxylin.

Immunostaining in the granulomatous areas at the media and intima/media junction was measured with a previously published score [4, 5], based on the percentage of infiltrating cells positively stained. The score was as follows: 0 = negative immunostaining; 1 = ≤ 25%; 2 = 25–50%; 3 = 51–75%; 4 = 76–100%). Sections were evaluated by two investigators (M.C.C. and J.H.R.) blinded to the clinical data.

**CCL2, TNF-α and IL-6 detection in sera from patients with GCA**

Circulating levels of CCL2 were determined in 56 patients (40 females and 16 males) with biopsy-proven GCA using enzyme-linked immunosorbent assays (ELISAs; R&D Systems), following the instructions of the manufacturer. These patients had been prospectively evaluated at diagnosis, treated and followed by the authors according to a predefined schedule [2]. Serum samples were obtained before starting corticosteroid treatment in 47 patients, and the remaining nine had received a single prednisolone dose (60 mg) the day before serum collection. Eighteen age- and sex-matched healthy donors were used as controls. Each sample was tested in duplicate wells. TNF-α and IL-6 were also measured by ELISA (R&D Systems) in sera from 48 of these patients and in 15 controls for comparison purposes.

**Statistical analysis**

The Mann–Whitney U-test was applied to qualitative data. Fisher's exact test was used for contingency tables and Pearson or Spearman's rank coefficients for correlations. Time required to achieve a stable maintenance prednisolone dose <10 mg/day was analysed by the Kaplan–Meier survival analysis method and compared by the log-rank test.

**Results**

**Patients with different disease outcomes exhibit differentially expressed genes in their temporal artery lesions**

Among the 1176 genes screened, 14 were differentially expressed in both groups of patients. Since the hybridization conditions of the arrays favoured specificity rather than sensitivity, it is possible that additional relevant genes were not detected. In fact, the expression of the pro-inflammatory cytokines IL-1β, TNF-α and IL-6, as well as the endothelial adhesion molecules, which we have previously found to be up-regulated in GCA [4, 7], were barely or not detected.

The known biological functions of some of the up-regulated genes suggest that their increased expression may be related through different pathways to the persistence of inflammatory activity. Among them, the chemokine CCL2 may contribute to the perpetuation of the inflammatory process through the recruitment and activation of monocytes, which, when becoming tissue macrophages, are significant effector cells in GCA [10]. As shown in Table 2, other genes differentially expressed in this screen were transcriptional factors or transcriptional regulators involved in inflammatory responses [11, 12], genes involved in cell migration [10, 13–15] and cell survival [16], and genes expressed during cell response to stress [17, 18]. Intriguingly, some genes involved in bone remodelling [19] and in neural transmission [20] or neural development [21] were also differentially expressed. The function of these genes in either inflammation or vascular biology is unknown.

To validate the array findings, mRNA from one of the genes differentially expressed, CCL2 was measured by quantitative real-time PCR in three individual samples from group 1 and 4 individual samples from group 2. In agreement with the cDNA array results, CCL2 transcripts were more abundant in patients in group 2 (178 relative units ± 117) (mean ± SEM) than in group 1 (1.59 relative units ± 0.94).

**CCL2 (MCP-1) gene expression in vascular inflammatory lesions from patients with GCA**

Since CCL2 was over-expressed in the pooled samples from patients with a more refractory outcome, and given its potential contribution to the perpetuation of lesions, we investigated CCL2 expression in temporal artery samples from a larger series of patients. Since pro-inflammatory cytokines have been demonstrated to induce CCL2 in different cell types, IL-1β, TNF-α and IL-6 expression was also assessed in the same samples.

CCL2 mRNA could be detected in normal biopsies even at low levels, indicating baseline CCL2 expression in normal temporal arteries. However, CCL2 transcripts were much more abundant in GCA samples (31.4 ± 15.6 relative units) (mean ± SEM) than in normal specimens (0.44 ± 0.10 relative units) (P = 0.0001) (Fig. 1A). Twenty-six out of the 35 patients studied had received no treatment at the time of the temporal artery excision, whereas nine had received prednisolone at 1 mg/Kg for a median of 2 days (range 1–6). No significant differences were observed in CCL2 values between untreated patients and patients who had received corticosteroids. These findings indicate that, contrarily to what has been observed for pro-inflammatory cytokines and endothelial adhesion molecules [5, 7], corticosteroid treatment for <1 week is not sufficient to significantly down-regulate CCL2 expression.

A significant correlation was found between CCL2 and IL-1β (R = 0.45, P = 0.02), TNF-α (R = 0.47, P = 0.013) and IL-6
CCL2 and persistence of disease activity in GCA

Table 2. Genes differentially expressed in temporal artery samples from relapsing patients (group 2) compared with remitting patients in (group 1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>GeneBank accession#</th>
<th>Status</th>
<th>Fold change</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRL-1</td>
<td>U48296</td>
<td>Up</td>
<td>94x</td>
<td>Increases migration and invasiveness</td>
<td>[13]</td>
</tr>
<tr>
<td>TREB 36</td>
<td>X55544</td>
<td>Up</td>
<td>10.6x</td>
<td>Transcription factor                                                     –</td>
<td></td>
</tr>
<tr>
<td>RAD 52</td>
<td>U12134</td>
<td>Up</td>
<td>31x</td>
<td>DNA repair                                                               [18]</td>
<td></td>
</tr>
<tr>
<td>Neuronatin</td>
<td>U250033</td>
<td>Up</td>
<td>29x</td>
<td>Brain developmental gene (protects from injury)                          [21]</td>
<td></td>
</tr>
<tr>
<td>PCAF 65β</td>
<td>AF069736</td>
<td>Up</td>
<td>16.8x</td>
<td>Transcriptional activity regulator                                       [11, 12]</td>
<td></td>
</tr>
<tr>
<td>MRP8</td>
<td>X06234</td>
<td>Up</td>
<td>174x</td>
<td>Expressed by early phagocytes                                             [15]</td>
<td></td>
</tr>
<tr>
<td>CCL2</td>
<td>M24545</td>
<td>Up</td>
<td>53.5x</td>
<td>Chemotaxis                                                               [24, 25]</td>
<td></td>
</tr>
<tr>
<td>RhoA</td>
<td>L25080</td>
<td>Up</td>
<td>2x</td>
<td>Cell migration                                                           [14]</td>
<td></td>
</tr>
<tr>
<td>RhoB</td>
<td>X06820</td>
<td>Up</td>
<td>2.9x</td>
<td>Cell migration                                                           [14]</td>
<td></td>
</tr>
<tr>
<td>HSP 27</td>
<td>M11717</td>
<td>Up</td>
<td>2.87x</td>
<td>Stress protein                                                           [17]</td>
<td></td>
</tr>
<tr>
<td>PTP-2C</td>
<td>L08807</td>
<td>Down</td>
<td>Undetected</td>
<td>Decreases haematopoetic cell survival                                    [16]</td>
<td></td>
</tr>
<tr>
<td>NK-3R</td>
<td>M89473</td>
<td>Down</td>
<td>53.5x</td>
<td>Neurotransmission                                                        [20]</td>
<td></td>
</tr>
<tr>
<td>OSF</td>
<td>U63717</td>
<td>Down</td>
<td>Undetected</td>
<td>Bone remodelling                                                         [19]</td>
<td></td>
</tr>
<tr>
<td>PNAT/MNAT</td>
<td>X14672/ X17059</td>
<td>Down</td>
<td>Undetected</td>
<td>Drug resistance –                                                        –</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aminoacid transporter                                                    –</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. (A) CCL2 (MCP-1) mRNA levels in temporal artery samples from 35 patients compared with nine controls. (B) CCL2 (MCP-1) mRNA concentrations in temporal artery biopsies from patients with or without disease-related ischaemic complications at diagnosis. (C) CCL2 (MCP-1) mRNA levels in patients who presented two or more disease flares during the first year of follow-up compared with those who did not relapse. Bars represent mean (x) ± SEM. (D) Percentage of patients requiring a maintenance dose ≥10 mg prednisolone/day over time (total follow-up 53 weeks) according to the CCL2 mRNA concentration in their biopsies.

\( R = 0.52, \ P = 0.0053 \) transcripts, supporting an interrelated regulation of these cytokines in GCA. Interestingly, and as observed for IL-6 [7], CCL2 transcripts were significantly less abundant in samples from patients with disease-related ischaemic events (3.1 ± 1.5 vs 39.7 ± 20, \( P = 0.0243 \)) (Fig. 1B).

CCL2 mRNA levels were significantly higher in patients who suffered ≥2 relapses than in patients who did not relapse during the first year of treatment (Fig. 1C) and correlated with the cumulated dose of prednisolone during this period of time (\( R = 0.533, \ P = 0.0024 \)). In addition, the time required to achieve a stable maintenance dose of prednisolone <10 mg/day, was significantly longer in patients with CCL2 mRNA levels higher than three relative units (Fig. 1D). These data suggest that CCL2 expression may be involved in the persistence of inflammatory activity in GCA.
Immunohistochemical detection of CCL2 (MCP-1) and its receptor CCR2 in temporal artery samples

We next investigated the expression of CCL2 protein and its receptor, CCR2, in temporal artery lesions from 50 patients and nine normal controls. In normal specimens, slight CCL2 and, to a lesser extent, CCR2 expression by vascular smooth muscle cells (VSMCs) could be detected. In samples from patients with GCA, CCL2 was strongly expressed by infiltrating leucocytes and also by VSMCs (Fig. 2). Expression predominated in the granulomatous areas in fully developed lesions (Fig. 2). This distribution is in accordance with a previously published study performed by other investigators in a small series of patients [22]. CCR2 expression was also observed in infiltrating leucocytes and in VSMCs (Fig. 2). Interestingly, in samples with only initial adventitial inflammation, adventitial infiltrating leucocytes strongly expressed CCR2, whereas in full-blown lesions, CCR2 expression predominated in the granulomatous areas (Fig. 2). Given that inflammatory cells are thought to reach and invade the temporal artery wall through the adventitial vasa vasorum, this distribution suggests a relevant role for CCL2/CCR2 interactions in the recruitment of inflammatory cells into GCA lesions. A significant correlation was observed between CCL2 and CCR2 immunohistochemical scores, suggesting coordinated regulation of CCL2 and its receptor (R = 0.29, P = 0.012), as demonstrated in vitro and in inflammatory models [23].

Interestingly, CCL2 expression by VSMCs was stronger in patients than in controls and in patients with fully developed inflammatory lesions compared with those with only adventitial inflammation (Fig. 2). This finding suggests, in agreement with in vitro studies with cultured cells [23, 24] that, in GCA, pro-inflammatory cytokines or other mediators released by inflammatory cells contribute, in a paracrine manner, to up-regulate CCL2 expression by VSMCs.

Circulating CCL2 (MCP-1) in patients with GCA

Serum CCL2 concentrations were similar in patients than in age and sex-matched healthy donors (137 ± 11 pg/ml vs 206 ± 53 pg/ml, P = 0.26). However, when patients were stratified according to the severity of the acute-phase response, prospectively evaluated at the time of diagnosis [2, 3], a significantly negative association with CCL2 levels was observed (Fig. 3A). We have previously shown that serum IL-6 and, to a lesser degree, TNF-α correlate with the intensity of the systemic inflammatory response [2]. Although a negative correlation between individual CCL2 and IL-6 or TNF-α values did not reach statistical significance, serum TNF-α and IL-6 concentrations showed a positive association with the intensity of the acute-phase response in the same cohort of patients, following a pattern opposite to CCL2 (Fig. 3B and C).

Discussion

In this study, we show that patients with weak systemic inflammatory response who have higher prevalence of ischaemic complications and a better response to corticosteroid treatment have different gene expression in their lesions than patients with strong systemic inflammatory reaction who are more refractory to therapy. Among the 1176 genes analysed, differential expression of 14 genes was significantly detected. Although their potential role in the pathogenesis of GCA needs to be investigated more extensively, the characterized functions of many of these genes suggest their relationship to the persistence of vascular inflammation [10–21].

Interestingly, one of the most significantly up-regulated genes was CCL2, a potent chemotactic factor for monocytes and activated Th1 lymphocytes, the most prominent cell types in GCA inflammatory infiltrates [10]. CCL2 is known to be expressed by vessel wall components, such as VSMCs and endothelial cells, and may contribute to the continuous recruitment and activation of Th1 lymphocytes and monocytes [23–26]. Although CCL2 mRNA and protein expression were detected at low levels in VSMCs from normal temporal arteries, a much stronger expression was detected in inflamed arteries from patients with GCA. In addition, CCL2 expression by VSMCs was more intense in patients with inflammatory lesions extending through the media than in those
with solely adventitial inflammatory infiltrates, suggesting that inflammatory mediators released by infiltrating leucocytes have a major role in amplifying vascular CCL2 expression. In fact, a significant correlation between CCL2 and proinflammatory cytokine mRNAs was observed in an extended series of patients. CCL2 can also be expressed by activated macrophages [26] and, in fact, the strongest immunostaining for CCL2 was observed in the granulomatous areas. This may constitute an amplificatory feed-back mechanism through which activated macrophages promote the recruitment of additional monocytes. Supporting this concept, MRP-8, an S-100 related molecule expressed by early recruited phagocytes, was also up-regulated in relapsing patients.

We have previously shown that MRP-8 is mainly expressed by infiltrating cells located at the adventitia and in the surrounding vasa vasorum, the sites through which inflammatory cells are thought to penetrate the vessel wall [15]. Up-regulated MRP-8 may reflect an increased presence of freshly recruited monocytes. The elevated expression of RhoA, RhoB and PRL-1, pivotal elements in cell migration would also represent the active influx of inflammatory cells into the vessel wall [13, 14], and the down-regulation of PTP-2C, a molecule promoting apoptosis of haematopoietic cells, might, in turn, prolong survival of infiltrating leucocytes [16].

CCL2 may act by multiple mechanisms to maintain inflammation in GCA. In addition to its chemotactic effects on the main leucocyte populations present in GCA infiltrates, CCL2 is also chemotactic for endothelial cells and may stimulate angiogenesis, a potent amplificatory mechanism in chronic inflammatory diseases [27]. Interestingly, CCR2 null mice exhibit decreased IFNγ production and deficient granuloma formation, indicating that besides participating in inflammatory cell recruitment, CCL2/CCR2-mediated interactions regulate functional activities which are thought to be relevant in the pathogenesis of GCA [28, 29]. The pro-inflammatory role of CCL2/CCR2-mediated interactions has been demonstrated in several animal models of inflammatory diseases [30–33] and is thought to contribute to the inflammatory component of atherosclerosis and graft vasculopathy [34, 35].

Similar to IL-6 [5], CCL2 expression was associated with lower risk of ischaemic events. The mechanisms underlying this observation are not clear at present; CCL2 may be coordinately regulated with other protective factors or may have a protective function by itself. As mentioned, and similarly to IL-6, CCL2 has angiogenic properties [5, 27] and may help to prevent ischaemia by contributing to compensatory neovascularization.

Our patients did not exhibit elevated levels of circulating CCL2 compared with age and sex-matched controls. This was unexpected since other investigators have reported elevated concentrations of CCL2 in patients with GCA [22] and in patients with other chronic inflammatory conditions such as rheumatoid arthritis, psoriatic arthritis and Kawasaki disease [36–38], although there is no complete agreement in these studies. In the previously existing study performed in patients with GCA, a young population, aged 43 (range 33–59) was used as control and it has been demonstrated that, as with other inflammatory molecules, circulating CCL2 increases with age [39]. Other chronic inflammatory disorders usually occur in younger individuals and differences from healthy controls may be more apparent.

Interestingly, when patients were stratified according to the number of inflammatory parameters, CCL2 concentration in sera was negatively related to the intensity of the systemic acute-phase response. It has been demonstrated that there is an increased population of circulating activated monocytes in GCA, which actively produce IL-6 [40]. Elevated serum concentrations of TNFα and IL-6 may then reflect an increased number of activated monocytes. CCR2 is up-regulated in activated monocytes and may sequester circulating CCL2, as it has been demonstrated in experimental models [35]. Sequestering by activated mononuclear cells at the sites of inflammation has been postulated to explain the low levels of CCL2 found in cerebrospinal fluid from patients with active multiple sclerosis compared with patients with inactive disease or patients with non-inflammatory neurological disorders [41]. Similar to other chemokines, local CCL2 concentrations are more functionally relevant than circulating levels since CCL2 acts in an autocrine/paracrine manner determined by a local gradient, facilitated by interactions with proteoglycans. Reduced circulating CCL2 may help to maintain an efficient local gradient at the sites of inflammation.

**Fig. 3.** Serum concentration of CCL2 (MCP-1) (A), TNFα (B) and IL-6 (C) in patients with GCA stratified according to the intensity of the systemic inflammatory response. Inflammatory parameters are fever (T° >37°C), weight loss (≥4 kg), anaemia (Hb <110 gm/l) and ESR ≥85 mm, as previously defined [2, 3].
Several strategies addressed to block CCL2 expression or biological activity are being developed as potential new therapeutic interventions. These are being tested in animal models of atherosclerosis, graft vasculopathy and chronic inflammatory diseases [30–33]. Our findings indicate that CCL2 expression is associated with persistent disease activity and suggest that CCL2 might contribute to the perpetuation of inflammatory lesions in GCA. Therefore, GCA may be considered among the diseases that could potentially benefit from CCL2/CCR2 blockade. However, definitive confirmation of the role of CCL2 in disease persistence can be obtained only from clinical trials. The negative association between CCL2 expression and ischaemic events suggests caution about blocking CCL2/CCR2 as first-line therapy, and also that it might be better to test it in relapsing/refractory patients.

The authors have declared no conflicts of interest.

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References