Monocyte activation and relationship to anti-proteinase 3 in acute vasculitis

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Abstract

Background. Monocytes have been suggested to play a role in antineutrophil cytoplasmic antibody (ANCA)-positive vasculitis, but their state of activation in vivo in patients is still unknown.

Methods. Twelve consecutive patients with acute anti-proteinase 3 (PR3)-positive vasculitis were included prospectively, and blood samples were drawn at diagnosis. As controls, peripheral blood was obtained from a group of patients with acute infection (n = 12) and from healthy controls (n = 12). Monocyte activation was estimated from the expression of adhesion molecules (CD62L and CD11b), production of oxygen radicals and serum concentrations of soluble inflammation markers and adhesion molecules [intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1)].

Results. Compared with monocytes from healthy subjects, monocytes from patients with acute vasculitis expressed upregulated CD11b (P < 0.05) but had reduced production of oxygen radicals (P < 0.01). A high concentration of anti-PR3 correlated with decreased expression of CD62L (r = 0.71, P = 0.009) and increased expression of CD11b (r = 0.63, P = 0.02). The serum concentrations of soluble inflammation markers [soluble CD14, interleukin (IL)-6, tumour necrosis factor receptor 1 (TNFR1), IL-10 and IL-8] as well as soluble adhesion molecules (sVCAM-1 and sICAM-1) were increased. Monocytes in patients with acute vasculitis displayed a reduced production of oxygen free radicals (P < 0.01) but similar serum concentrations of soluble inflammation markers and adhesion molecules, as compared with the control group of patients with acute infection and negative PR3-ANCA.

Conclusions. High anti-PR3 concentration in patients with acute vasculitis correlated with an activated adhesion molecule phenotype (CD62L<sub>1</sub>/CD11b<sub>high</sub>) in circulating monocytes, indicating a potential pathophysiological role for anti-PR3. An impaired production of oxygen radicals in monocytes in patients with vasculitis compared with those with acute infection may mirror the longer time interval from onset of first symptoms to admission, in patients with vasculitis.

Keywords: adhesion molecules; ANCA; cytokines; monocytes; proteinase 3; vasculitis

Introduction

Primary or idiopathic vasculitic syndromes constitute a group of inflammatory disorders characterized by inflammation and necrosis of blood vessels, frequently in combination with granuloma formation. Small vessel diseases, such as Wegener’s granulomatosis (WG), necrotizing crescentic glomerulonephritis (GN), microscopic polyangiitis and Churg–Strauss syndrome, are associated with the presence of antineutrophil cytoplasmic antibodies (ANCAs). The target antigen for ANCs in WG is most often proteinase-3 (PR3), whereas necrotizing crescentic GN is associated more often with ANCs directed against myeloperoxidase (MPO) [1,2]. Although the pathophysiological role of ANCA is not fully elucidated, several lines of evidence suggest that ANCA is involved in the development of small vessel vasculitis [3]. Relapses frequently are preceded by a rise in ANCA titres and can be prevented by treatment interventions based on changes in those titres [4]; however, different opinions exist on this matter. Furthermore, ANCA can activate primed neutrophils to increased production of reactive oxygen species and release of lytic enzymes, and

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also to activate and damage cultured endothelial cells [5,6]. The subsequent local inflammatory reaction results in an early influx of neutrophils, followed by extravasation of mononuclear cells which contributes to granuloma formation seen preferentially in patients with WG.

The pathogenic role of the neutrophils has been explored in patients with WG, but the specific role of the monocyte has yet to be determined. Monocytes, which contain both PR3 and MPO, are frequently located in vascular infiltrates, and are proposed to be involved in the pathogenesis of vasculitis [7]. This notion is supported by in vitro studies showing that ANCA can trigger monocytes, even without tumour necrosis factor-α (TNF-α) priming, to produce oxygen radicals [8], as well as monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8) [9,10]. However, the clinical relevance of these in vitro finding is not clear, and clinical and experimental studies in newly diagnosed, previously untreated patients with WG are scarce.

Influx of leukocytes into surrounding tissues requires a sequential upregulation and activation of adhesion molecules on both leukocytes and endothelial cells. Adhesion molecules expressed on monocytes (CD62L and CD11b) and on endothelial cells [intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)] together with acute-phase and regulatory cytokines and chemokines is key in the recruitment of mononuclear cells to sites of inflammation.

The pathogenic role of the neutrophils has been explored in patients with WG, but the specific role of the monocyte has yet to be determined. Monocytes, which contain both PR3 and MPO, are frequently located in vascular infiltrates, and are proposed to be involved in the pathogenesis of vasculitis [7]. This notion is supported by in vitro studies showing that ANCA can trigger monocytes, even without tumour necrosis factor-α (TNF-α) priming, to produce oxygen radicals [8], as well as monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8) [9,10]. However, the clinical relevance of these in vitro finding is not clear, and clinical and experimental studies in newly diagnosed, previously untreated patients with WG are scarce.

To test the hypothesis that the nature and magnitude of monocyte activation differ between ANCA-positive and ANCA-negative acute inflammation, we included consecutive patients with positive PR3-ANCA and clinical and laboratory signs of acute WG together with two control groups, one comprised of patients with ANCA-negative acute infection and one comprised of healthy subjects. At diagnosis, the expression of the adhesion molecules CD62L and CD11b on monocytes and the monocyte’s capacity for production of oxygen radicals were determined. In serum, the concentrations of soluble adhesion molecules, acute-phase cytokines [IL-6, soluble TNF receptor 1 (TNFR1)], regulatory cytokines [IL-10, transforming growth factor-β (TGF-β)], chemokines (IL-8 and MCP-1) and soluble CD14 (sCD14) were analysed.

Subjects and methods

Patients

During the period November 1999–December 2001, all patients with a new positive PR3-ANCA and signs of renal involvement were asked if they agreed to participate in the study. Twelve consecutive patients gave informed consent and were included prospectively in the study. In five patients, immunosuppressive treatment with steroids and/or cytotoxic agents was started on clinical indication, before blood sampling. All patients had a diagnosis of systemic vasculitis based on clinical, serological and histological criteria. All patients had signs of kidney involvement including haematuria and proteinuria, and 10 had renal biopsy-proven crescentic GN. The clinical characteristics and laboratory test results of the patients at the time of inclusion are presented in Table 1. The time interval from the first clinical symptoms and signs of vasculitis to the time of admission to hospital and between the start of immunosuppressive treatment and blood sampling are summarized in Table 1. All samples were ANCA-positive as shown by indirect immunofluorescence on ethanol-fixed granulocytes with cytoplasmic-ANCA [1]. The specificity was confirmed by an enzyme-linked immunosorbent assay (ELISA) (Wieslab AB, Ideon, Lund, Sweden). Quantification in units was performed by ELISA. The study was approved by the Ethical Committee of the Karolinska Institutet.

Control groups

Peripheral blood was obtained from a group of patients prospectively included among patients acutely admitted to the Infectious Disease Clinic at Karolinska Hospital because of acute clinical and laboratory signs of infection. The inclusion criteria for these patients were C-reactive protein (CRP) >50 mg/l, fever >38.5 °C and normal urine sediment. A second control group consisted of voluntary healthy blood donors. All samples in the control groups were ANCA negative. Characteristics of the patients and the two control groups are summarized in Table 2.

Preparation of blood leukocytes

Peripheral blood from the subjects was collected into glass tubes containing citrate (Vacutainer, 5 ml, with 1 ml of 0.129 M 9NC, Becton Dickinson). The blood was haemolysed in 100 µl portions by addition of 2 ml of 4°C isotonic NH4Cl-EDTA lysis solution (154 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA pH 7.2) and incubated for 5 min at 15°C. After centrifugation at 300 g for 5 min at 4°C, the supernatant was aspirated. The leukocyte suspensions were then washed with 2 ml of 4°C phosphate-buffered saline (PBS).

Serum samples

Serum was centrifuged and frozen in 0.5 ml portions within 4h of sampling, and stored at ~70°C until analysis.

Immunofluorescence staining of cell adhesion receptors

The cell adhesion receptor expression (CD62L and CD11b) was determined by adding 10 µl of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) anti-CD62L (Becton Dickinson, San Jose, CA) and 5 µl of phycoerythrin (PE)-conjugated anti-CD11b (Dako A/S, Glostrup, Denmark). Isotype-matched control antibodies, PE-conjugated IgG2 and FITC-conjugated IgG1 (Becton Dickinson), were used as backgrounds to define the value for positive fluorescence. All cell suspensions containing mAb were incubated for 30 min at 4°C and then washed once in PBS, before flow cytometric analysis.
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>Symptom duration before diagnosis</th>
<th>Main organ involvement</th>
<th>Other organ involvement</th>
<th>Kidney biopsy % crescents</th>
<th>Treatment at sampling</th>
<th>Anti-PR3 conc. (units)</th>
<th>CRP mg/l</th>
<th>Leukocyte count $\times 10^9$/l</th>
<th>Monocyte count $\times 10^9$/l</th>
<th>$\text{s-Creatinine} \text{ mol/l}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>F</td>
<td>6 weeks</td>
<td>Joints, skin</td>
<td>Kidneys</td>
<td>Crescentic GN 13%</td>
<td>Prednisolone + cyclo$^a$ 7 days</td>
<td>326</td>
<td>12</td>
<td>13.7</td>
<td>0.2</td>
<td>83</td>
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<tr>
<td>2</td>
<td>41</td>
<td>F</td>
<td>8 weeks</td>
<td>Joints, skin</td>
<td>Kidneys</td>
<td>Crescentic GN 3%</td>
<td>0</td>
<td>30</td>
<td>16</td>
<td>13.7</td>
<td>0.7</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>M</td>
<td>6 weeks</td>
<td>Kidneys</td>
<td>ENT$^a$, lungs</td>
<td>Crescentic GN 100%</td>
<td>0</td>
<td>335</td>
<td>226</td>
<td>12.0</td>
<td>0.3</td>
<td>518</td>
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<tr>
<td>4</td>
<td>74</td>
<td>M</td>
<td>6 weeks</td>
<td>Kidneys</td>
<td>ENT, joints, polymyalgia</td>
<td>Crescentic GN 29%</td>
<td>Prednisolone 1 day</td>
<td>404</td>
<td>280</td>
<td>14.9</td>
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<td>5</td>
<td>17</td>
<td>F</td>
<td>1 week</td>
<td>Lungs</td>
<td>0</td>
<td>Crescentic GN 45%</td>
<td>Prednisolone 7 days</td>
<td>220</td>
<td>362</td>
<td>10.5</td>
<td>0.3</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>89</td>
<td>F</td>
<td>8 weeks</td>
<td>Kidneys</td>
<td>0</td>
<td>ND</td>
<td>Prednisolone 7 days</td>
<td>363</td>
<td>26</td>
<td>7.0</td>
<td>0.4</td>
<td>713</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>F</td>
<td>8 weeks</td>
<td>Kidneys</td>
<td>ENT, lungs</td>
<td>Crescentic GN 90%</td>
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<td>320</td>
<td>226</td>
<td>13.8</td>
<td>0.3</td>
<td>402</td>
</tr>
<tr>
<td>8</td>
<td>61</td>
<td>F</td>
<td>4 weeks</td>
<td>Kidneys</td>
<td>Skin, joints, ENT, lungs</td>
<td>Crescentic GN 75%</td>
<td>0</td>
<td>410</td>
<td>310</td>
<td>25.4</td>
<td>0.1</td>
<td>862</td>
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<tr>
<td>9</td>
<td>11</td>
<td>F</td>
<td>2 weeks</td>
<td>Joints</td>
<td>Kidneys, skin, ENT</td>
<td>Crescentic GN 17%</td>
<td>0</td>
<td>376</td>
<td>313</td>
<td>16.3</td>
<td>0.4</td>
<td>63</td>
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<tr>
<td>10</td>
<td>41</td>
<td>M</td>
<td>&gt; 8 weeks</td>
<td>Skin</td>
<td>Kidneys</td>
<td>ND</td>
<td>0</td>
<td>636</td>
<td>10</td>
<td>3.9</td>
<td>ND</td>
<td>107</td>
</tr>
<tr>
<td>11</td>
<td>39</td>
<td>M</td>
<td>4 weeks</td>
<td>Joints</td>
<td>Kidneys, skin, ENT</td>
<td>Crescentic GN 33%</td>
<td>0</td>
<td>107</td>
<td>56</td>
<td>8.4</td>
<td>0.4</td>
<td>81</td>
</tr>
<tr>
<td>12</td>
<td>79</td>
<td>M</td>
<td>6 weeks</td>
<td>Kidneys</td>
<td>ENT, joints</td>
<td>Crescentic GN 100%</td>
<td>Prednisolone + cyclo$^a$ 7 days</td>
<td>590</td>
<td>37</td>
<td>19.7</td>
<td>0.5</td>
<td>513</td>
</tr>
</tbody>
</table>

$^a$ENT = ear, nose and throat.

$^b$Cyclo = cyclophosphamide.
Production of hydrogen peroxide (H₂O₂)

Leukocyte pellets were incubated for 15 min at 37°C with 5 μM dichlorofluorescein diacetate (DCFH) (Eastman Kodak Company, Rochester, NY) suspended in PBS-glucose. Thereafter, 1 ml of 4°C PBS glucose was added, before analysis. When DCFH penetrates the leukocyte membrane, it is oxidized by hydrogen peroxide to highly fluorescent 2′,7′-dichlorofluorescein. The production of hydrogen peroxide was determined by flow cytometry analysis. Hydrogen peroxide production after in vitro stimulation was determined by adding a receptor-dependent stimulus, 5 × 10⁻⁷ M N-formyl-methionyl-phenylalanine (fMLP) (Sigma Chemicals, St Louis, MO) or a receptor-independent stimulus, 2.5 × 10⁻⁹ M phorbol 12-myristate 13-acetate (PMA) (Sigma Chemicals) suspended in PBS-glucose, for 15 min at 37°C. The cells were suspended in 1 ml of 4°C PBS-glucose before analysis by flow cytometry.

Cell surface expression of PR3

To determine the surface expression of PR3 on monocytes, 10 μl of mouse anti-human PR3 mAb, designated clone 4A5 (Wieslab, Lund, Sweden), diluted 1:5 in PBS, was added to 150 μl of leukocyte suspension and incubated for 30 min at 4°C. A 30 μl aliquot of isotype-matched control IgG1 (Dako A/S) was used as background control to determine non-specific binding. After two washes with 3 ml of PBS at 300 g for 6 min, FITC-conjugated rabbit anti-mouse IgG (Dako A/S) diluted 1:60 was added and incubated for 30 min at 4°C. The optimal dilutions were determined by titration. After one wash, the leukocytes were resuspended and analysed.

Analysis by flow cytometry

The mixed leukocyte populations were analysed and counted in an EPICS XL flow cytometer (Beckman Coulter Inc., Hialeah, FL). In the flow cytometer, cells are distinguished by their light-scattering properties, and gates were set around the granulocyte and monocyte clusters in a two-parameter histogram with linear amplification. To optimize the gating strategy, gates were adjusted individually for each sample, since the light-scattering properties may vary between individuals and following in vitro stimulation. The purity of monocytes within the monocyte gate, judged by CD14-positivity, was >90% for both unstimulated and in vitro stimulated cells. FITC-conjugated monoclonal anti-CD14, clone RMO52 (Immunotech, Marseille, France) was used to determine CD14-positive cells.

The instrument was calibrated daily with standardized 10 mm fluorospheres, Flow-Check (Beckman Coulter). Flow-set (Beckman Coulter), another fluorosphere with controlled fluorescence intensity, was used to standardize the mean fluorescence intensity (MFI) before each experiment. The percentage and absolute number of positively immunostained leukocytes were determined, and a quantification of antigen was obtained by measuring the MFI units of the positive cell population.

Soluble inflammation markers

The serum concentration of soluble adhesion molecules sICAM-1 and sVCAM-1, and of cytokines and chemokines IL-6, IL-10, TGF-β, IL-8, MCP-1, soluble TNFR1 and soluble CD-14 were determined by ELISA (R&D Systems, Europe, Ltd, Oxon, UK). The detection limit was <2.0 ng/ml for sVCAM-1, 0.35 ng/ml for sICAM-1, 0.70 pg/ml for IL-6, 3.9 pg/ml for IL-10, 7 pg/ml for TGF-β, 10 pg/ml for IL-8, 5 pg/ml for MCP-1, 3.0 pg/ml for sTNFR1 and 125 pg/ml for sCD14. Analyses were performed according to the manufacturer’s instructions.

Statistics

Data are presented as medians, interquartile range and range. Statistical comparisons were made by using the non-parametrical Kruskal–Wallis ANOVA and Mann–Whitney U-test. Differences were considered statistically significant at P < 0.05. Multiple linear regression was used for correlation analysis.

Results

Expression of adhesion molecules

The expression of CD62L on monocytes was significantly lower in the group with acute anti-PR3-positive vasculitis compared with the group with acute infection (Figure 1). The expression of CD11b was upregulated in both groups with acute inflammation compared with

<table>
<thead>
<tr>
<th>Group</th>
<th>Acute anti-PR3-positive vasculitis (n = 12)</th>
<th>Acute infection (n = 12)</th>
<th>Healthy controls (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45 (13–89)</td>
<td>46 (16–88)</td>
<td>58 (22–67)</td>
</tr>
<tr>
<td>Sex M/F</td>
<td>5/7</td>
<td>3/9</td>
<td>6/6</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>141 (10–362)</td>
<td>205 (92–542)</td>
<td>&lt;7 (&lt;7–40)</td>
</tr>
<tr>
<td>Leukocyte count ×10⁹/l</td>
<td>13.7 (3.9–25.4)</td>
<td>13.5 (3.3–28)</td>
<td>5.1 (3.9–7.2)</td>
</tr>
<tr>
<td>Monocyte count ×10⁹/l</td>
<td>0.4 (0.1–0.7)</td>
<td>0.4 (0.2–2.0)</td>
<td>ND</td>
</tr>
<tr>
<td>s-Creatinine (μmol/l)</td>
<td>134 (54–862)</td>
<td>70 (50–111)</td>
<td>74 (64–112)</td>
</tr>
<tr>
<td>Anti-PR3 (units)</td>
<td>335 (30–636)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
</tbody>
</table>
healthy controls. Furthermore, the anti-PR3 concentration in the group with vasculitis correlated with a decrease in CD62L expression ($r = 0.71, P = 0.009$) and an increase in CD11b expression ($r = 0.65, P = 0.02$) (Figure 2A and B) and to the monocyte CD62L/CD11b ratio ($r = 0.74, P = 0.006$).

Analysis with the exclusion of the patients on therapy did not alter the statistically significant results. The correlation between anti-PR3 levels and CD62L and CD11b expression was $r = 0.81, P < 0.01$ and $r = 0.7, P = 0.07$, respectively, with the exclusion of five patients.

Production of oxygen radicals before and after in vitro stimulation with fMLP and PMA

Production of $H_2O_2$ in resting monocytes obtained from the peripheral circulation was decreased in the vasculitis patients compared with both control groups. This was also true for receptor-dependent fMLP-induced stimulation (Figure 3). When the receptor-independent stimulus PMA was used, $H_2O_2$ production in monocytes was similar in the three groups, with an MFI of 46.1 (range 23.8–101.0), 44.0 (9.0–111.0) and 47.3 (24.9–72.7) for acute vasculitis, acute infection and healthy controls, respectively. There was a tendency that patients with acute vasculitis who had had symptoms for a prolonged period of time had a more pronounced reduction in monocyte oxygen radical production compared with those with a shorter duration of symptoms, but the difference did not reach statistical significance ($r = 0.5, P = 0.089$).
Monocyte activation in acute vasculitis

Table 3. Serum concentrations of pro-inflammatory cytokines (IL-6, sTNFR1), anti-inflammatory cytokines (TGF-β, IL-10), chemokines (IL-8, MCP-1), soluble adhesion molecules (sICAM-1, sVCAM-1) and sCD14 in patients with acute anti-PR3 positive vasculitis, acute infection and healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Acute anti-PR3-positive vasculitis (n = 12)</th>
<th>Acute infection (n = 12)</th>
<th>Healthy subjects (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 pg/ml</td>
<td>15.1 (0.9–54.9)**</td>
<td>40.0 (11.4–337.0)**</td>
<td>0.2 (0–0.9)</td>
</tr>
<tr>
<td>sTNFR1 pg/ml</td>
<td>3737 (1612–16 144)**</td>
<td>2023 (1886–7667)**</td>
<td>1062 (891–1526)</td>
</tr>
<tr>
<td>TGF-β pg/ml</td>
<td>53 982 (32 344–134 042)</td>
<td>40 757 (26 796–92 051)</td>
<td>49 789 (42 570–67 260)</td>
</tr>
<tr>
<td>IL-10 pg/ml</td>
<td>5.5 (0–19.9)**</td>
<td>4.6 (0.4–171.6)**</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>IL-8 pg/ml</td>
<td>25.9 (0–73.5)**</td>
<td>11.6 (0–95.9)</td>
<td>0 (0–12.7)</td>
</tr>
<tr>
<td>MCP-1 pg/ml</td>
<td>304 (25.3–617)</td>
<td>321 (150–662)</td>
<td>267 (184–619)</td>
</tr>
<tr>
<td>sICAM-1 ng/ml</td>
<td>328 (199–1007)**</td>
<td>352 (216–821)**</td>
<td>219 (119–276)</td>
</tr>
<tr>
<td>sVCAM-1 ng/ml</td>
<td>632 (340–2041)**</td>
<td>526 (261–1107)**</td>
<td>335 (244–480)</td>
</tr>
<tr>
<td>sCD14 ng/ml</td>
<td>1673 (1128–2372)*</td>
<td>2747 (1795–4433)**</td>
<td>1279 (285–1624)</td>
</tr>
</tbody>
</table>

**P < 0.01 and *P < 0.05 vs healthy controls.

The decreased H₂O₂ production in monocytes from patients with PR3-ANCA-positive vasculitis did not correlate with the anti-PR3 concentration in serum (r = 0.22, P = 0.5).

Expression of PR3 on the cell surface

There were no differences in the proportion of PR3-positive monocytes from patients with ANCA-positive and ANCA-negative acute inflammation, or compared with the healthy control group. The proportion was 2.6% (range 0–17.7%) for patients with acute vasculitis, 2.4% (0–9.0%) for patients with acute infection and 2.5% (0–12.7%) for healthy controls.

Concentration of soluble inflammation markers

The concentrations of soluble inflammation markers in serum are given in Table 3. The concentration of IL-6 was increased in patients both with acute vasculitis and with acute infection, with the highest value in the latter group, which was also true for sCD14. sTNFR1 was increased in both acute vasculitis and acute infection. The concentration of IL-10 was increased in acute vasculitis, whereas the TGF-β concentration did not differ from that in healthy controls. The concentrations of sICAM-1 and sVCAM-1 were increased in both groups of patients with acute inflammation compared with healthy controls. The concentration of IL-8 was increased in the vasculitis group, whereas the concentration of MCP-1 was similar in all groups.

The concentration of the soluble inflammation markers analysed in this study did not correlate with the anti-PR3 concentration. The proportions of crescents in glomeruli correlated with s-creatinine (r = 0.79, P = 0.006) but did not correlate with any of the levels of soluble adhesion molecules and inflammatory markers.

Discussion

In this study, monocyte activation and soluble inflammation markers were analysed in consecutive patients with acute anti-PR3-positive vasculitis. Compared with monocytes from healthy subjects, monocytes from patients with acute anti-PR3-positive vasculitis expressed upregulated CD11b and reduced production of oxygen radicals, and compared with monocytes from patients with ANCA-negative acute infection they showed a reduced production of oxygen radicals. Moreover, a high concentration of anti-PR3 correlated with the expression of an activated adhesion molecule phenotype in monocytes, with low CD62L and high CD11b. In serum, the concentrations of soluble inflammation markers (sCD14, sTNFR1, IL-6, IL-10, IL-8, sVCAM-1 and sICAM-1) were increased compared with serum from healthy subjects. Compared with the control group with acute infection, the concentrations of sCD14 and IL-6 were lower in the patients with vasculitis. These patients had had clinical symptoms and signs of inflammatory disease for 1 to >8 weeks. Blood samples were collected prior to immune suppressive treatment in seven patients. However, in five patients, treatment had to be instituted on the clinical indications before blood samples could be collected for flow cytometric analysis. Analysis with the exclusion of the patients on therapy did not alter the statistically significant results, neither for expression of adhesion molecules nor for production of H₂O₂.

The contribution of neutrophils to the lesions that characterize vasculitis has been appreciated for many years. One hypothesis suggests that proinflammatory cytokines induce surface expression of intracellularly localized PR3 antigens, rendering them available for interaction with ANCA [11]. Only recently has attention been paid to monocytes as potential effector cells. Monocytes and tissue macrophages participate in granuloma formation, which is characteristic of WG, and monocytes/macrophages accumulate in the proliferative lesions and in crescents in patients with necrotizing GN. The accumulation of monocytes/macrophages is a complex process that involves the coordinated action of a variety of adhesion molecules and cytokines. The subsequent activation of monocytes/macrophages implies generation of oxygen radicals and release of both pro- and anti-inflammatory mediators, thereby giving these cells an essential role in orchestrating the inflammatory process.
In our study, circulating monocytes from patients with acute vasculitis expressed normal CD62L and upregulated CD11b, which is in concordance with previous reports on neutrophils from patients with newly diagnosed vasculitis [12]. An interesting finding was the correlation between the anti-PR3 levels and the CD62L/CD11b ratio. This may have relevance for the attachment of monocytes to the endothelium, and it corresponds to in vitro findings on the activating capacity of anti-PR3 and anti-MPO [13]. The expression of CD11b on monocytes was increased to a comparable degree in the patients with ANCA-negative acute infection, while the expression of CD62L was increased. Increased CD62L expression in the group with acute infection may reflect a rapid monocyte turnover from the bone marrow.

A combination of increased CD11b expression and a normal to low CD62L expression on monocytes may favour both rolling and adhesion to activated vascular endothelium. An existing activated endothelium is reflected by our data on increased levels of both sICAM-1 and sVCAM-1, an observation reported by others [14]. In the integrin-mediated adhesion step, CD11b binds to its ligand ICAM-1, which promotes further activation by contact with local inflammatory mediators.

In the present study, monocytes from patients with acute vasculitis had a decreased capacity to produce oxygen radicals, which contrasts with previous in vitro data [9; own unpublished data]. Vasculitis is a chronic inflammation, and in many cases the patient has symptoms for a long time before a correct diagnosis is established. This may explain why these patients’ circulating monocytes, which have been under continuous inflammatory activation for weeks, were unable to respond adequately with production of oxygen free radicals. In our study, there was a tendency that patients with acute vasculitis who had had symptoms for a prolonged period of time had a more pronounced reduction in monocyte oxygen radical production compared with those with a shorter duration of symptoms, but the difference did not reach statistical significance. Impaired production of oxygen radicals was demonstrated in fresh monocytes as well as after fMLP stimulation. However, they responded just as well as monocytes from healthy controls when given a receptor-independent stimulus; this indicates that another signal may evoke a proper response. A down-regulated fMLP response has been identified in other clinical settings and has been attributed to desensitization. Circulating neutrophils from patients with vasculitis have been reported to have impaired [15] as well as enhanced superoxide production [16]. It must be emphasized that a dynamic situation exists and that the findings noted in circulating cells may not reflect the nature and magnitude of the state of activation at the local inflammatory site.

Monocytes from patients with anti-PR3-positive acute vasculitis did not express higher amounts of PR3 on the cell surface compared with controls. This is in concordance with previously reported results where neutrophils—but not monocytes—from patients with active WG displayed increased PR3 expression [17]. This may suggest that monocyte activation is mediated via Fc receptor binding of antibodies or antibody-coated neutrophils, rather than by direct ANCA–antigen binding.

The CD14 molecule expressed on monocytes is a receptor for lipopolysaccharide. High levels of sCD14 reflect monocyte activation, and immunosuppressive drugs down-modulate the antigen [18]. sCD14 previously has been reported to be elevated in serious infectious diseases and in Kawasaki’s disease to even higher levels than in acute Gram-negative infection [19]. In our study, the highest concentration of sCD14 was seen in patients with acute infection. Also the acute-phase cytokine IL-6 was lower in the patients with anti-PR3-positive inflammation compared with those with acute infection. These observations support our previous assumption that the patients in the anti-PR3-positive group have had ongoing inflammatory reaction for a longer period. Although there are many reports supporting pathological activation of the cytokine cascades in patients with vasculitis [20], we were not able to identify any clinically relevant pattern in how the cytokines and soluble adhesion molecules responded to acute vasculitis compared with acute infection.

The chemokines IL-8 and MCP-1 are important in recruiting and activating neutrophils and monocytes. The vasculitis patients had significantly higher serum levels of IL-8, but not of MCP-1, than the healthy controls. IL-8 may be an important factor in maintaining the inflammation.

In our study with focus on monocyte activation and related soluble inflammation markers, we compared patients with acute anti-PR3 vasculitis with patients with acute ANCA-negative infection and healthy controls. The main difference between the inflammatory response of monocytes in acute vasculitis and acute infection was that in patients with acute vasculitis, circulating monocytes showed a reduced capacity to produce oxygen radicals, which may be a consequence of a prolonged period of immune activation. High concentrations of anti-PR3 in the vasculitis group correlated with decreased CD62L and increased CD11b expression on monocytes, which may have pathophysiological implications for the endothelial damage seen in vasculitis. Further studies in a larger number of patients are needed to reach conclusions with clinical applicability.

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