Chemotaxis and chemiluminescence responses of synovial fluid polymorphonuclear leucocytes during acute reactive arthritis

Marjatta Leirisalo-Repo, Anneli Lauhio, Heikki Repo

Abstract
The chemotaxis and chemiluminescence responses of polymorphonuclear leucocytes (PMN) of synovial fluid and peripheral blood from patients with acute reactive arthritis were studied. Rates of chemotactic and chemokinetic migration of synovial fluid PMN were significantly decreased. In addition, chemiluminescence responses tended to be depressed, suggesting that the cells were deactivated for both chemotaxis and production of oxygen derived free radicals. Such deactivation has been described previously as a characteristic of synovial fluid PMN in rheumatoid arthritis. Compared with those with a mild disease, patients with severe acute reactive arthritis had higher chemiluminescence responses of synovial fluid PMN to phorbol myristate acetate during acute disease and developed increased migration of peripheral blood PMN towards zymosan treated serum after recovery from the disease. This supports the view that hyperreactive PMN contribute to the development of severe inflammatory symptoms in acute reactive arthritis.

Reactive arthritis, a sterile joint inflammation preceded by an infection elsewhere in the body, may be triggered by an enteric or urogenital infection. The microbes triggering reactive arthritis include yersinia, salmonella, shigella, campylobacter, chlamydia, and possibly gonococcus. The acute reactive arthritis is characterised by inflammatory signs that are both local (synovitis with polymorphonuclear cells predominating in the synovial fluid) and systemic (fever, conjunctivitis, iritis, urethritis, and mucocutaneous symptoms).

Peripheral blood polymorphonuclear leucocytes (PMN) of HLA-B27 positive patients with yersinia arthritis show increased chemotaxis, and PMN of patients who had recovered from a severe acute disease or those who have inflammatory sequelae tend to show enhanced chemiluminescence, suggesting that the cells are primed—that is, rendered hyperreactive—for instance, by mediators of inflammation. Chemotaxis of the peripheral blood PMN of patients with rheumatoid arthritis is depressed, and superoxide production is enhanced. Synovial fluid PMN, however, show depressed chemotaxis and depressed chemiluminescence responses. This indicates that the synovial fluid cells in rheumatoid arthritis tend to be deactivated for both chemotaxis and production of oxygen derived free radicals. In reactive arthritis the functional capacities of synovial fluid PMN are not known. Therefore, we set out to study chemotaxis and chemiluminescence responses of synovial fluid and peripheral blood PMN of patients with acute reactive arthritis, and to compare the responses with those of peripheral blood PMN of healthy controls.

Patients and methods
PATIENTS
We studied 12 patients with acute reactive arthritis, who presented with acute oligoarthritis or polyarthritis mainly affecting the lower extremities. The patients were treated at the Helsinki University Central Hospital or at the Aurora Hospital, Helsinki. Table 1 shows the characteristics of the patients. At the time when the synovial fluid and blood samples were taken the patients were treated with non-steroidal anti-inflammatory agents. Healthy HLA-B27 negative members of the hospital and laboratory staff served as controls. A patient and a control formed a fixed pair which was tested at the acute phase of the disease, one month later, and at six months after recovery. Two patients were tested at the acute phase only; one was not reached for subsequent testing and the other with acute colitis later developed Crohn’s disease. To study the relation between severity of inflammation and phagocyte function the patients were divided into two groups: those with severe acute disease (n=5) and those with mild acute disease (n=7, table 1).

CELLS
All the patients had effusion of the knee joint, which was aspirated. The leucocyte counts in the synovial fluid varied from 4×10⁶ to 12·4×10⁶/l (mean 7·7×10⁶/l). The proportion of PMN varied from 62 to 96% (mean 78%). The synovial fluid was immediately diluted (1:5) with RPMI 1640 (Orion Co, Helsinki) containing 1 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid) and centrifuged. The cells were then washed once with Hank’s balanced salt solution (HBSS). Buffy coat cells of peripheral blood of the patient and the control were separated from heparinised venous blood by dextran sedimentation. Polymorphonuclear leucocytes were separated from both buffy coat cells and synovial fluid cells by Ficoll-Isopaque density gradient centrifugation followed by hypotonic lysis of the red cells. The PMN were 95–99% pure. Synovial fluid PMN stained by the May-Grünwald-Giemsa technique were morphologically normal and the viability was >90% as judged by eosin exclusion test.
Table 1: Clinical characteristics of the patients

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>B27</th>
<th>White blood cell count</th>
<th>ESR (mm/h)</th>
<th>Extra-articular symptoms</th>
<th>Severe disease</th>
<th>Duration of symptoms (weeks)</th>
<th>Triggering factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>27</td>
<td>+</td>
<td>3</td>
<td>98</td>
<td>—</td>
<td>—</td>
<td>11</td>
<td>Campylobacter</td>
</tr>
<tr>
<td>F</td>
<td>23</td>
<td>—</td>
<td>6</td>
<td>92</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>Acute colitis</td>
</tr>
<tr>
<td>M</td>
<td>40</td>
<td>+</td>
<td>1</td>
<td>99</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>Gonococcus</td>
</tr>
<tr>
<td>M</td>
<td>44</td>
<td>—</td>
<td>5</td>
<td>86</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>Diarrhoea</td>
</tr>
<tr>
<td>M</td>
<td>28</td>
<td>+</td>
<td>7</td>
<td>125</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>Yersinia</td>
</tr>
<tr>
<td>M</td>
<td>26</td>
<td>+</td>
<td>7</td>
<td>103</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>Yersinia</td>
</tr>
<tr>
<td>M</td>
<td>18</td>
<td>+</td>
<td>9</td>
<td>110</td>
<td>—</td>
<td>—</td>
<td>6</td>
<td>Salmonella</td>
</tr>
<tr>
<td>M</td>
<td>27</td>
<td>+</td>
<td>6</td>
<td>125</td>
<td>—</td>
<td>7</td>
<td>2</td>
<td>Salmonella</td>
</tr>
<tr>
<td>F</td>
<td>24</td>
<td>+</td>
<td>6</td>
<td>102</td>
<td>Conjointicitis,urethritis</td>
<td>—</td>
<td>3</td>
<td>Yersinia</td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>+</td>
<td>1</td>
<td>15</td>
<td>—</td>
<td>—</td>
<td>16</td>
<td>Chlamydia</td>
</tr>
<tr>
<td>M</td>
<td>30</td>
<td>+</td>
<td>5</td>
<td>119</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>Chlamydia</td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>+</td>
<td>6</td>
<td>4</td>
<td>Balanitis</td>
<td>—</td>
<td>12</td>
<td>Chlamydia</td>
</tr>
</tbody>
</table>

*Severe acute disease = the presence of at least two of the three following characteristics: highest erythrocyte sedimentation rate (ESR) >110 mm/h, highest white blood cell count >10×10⁹/l, and presence of extra-articular manifestations (see ref 4).

POLYMORPHONUCLEAR LEUCOCYTE STIMULATING AGENTS
Zymosan treated serum (ZTS) was prepared from pooled normal human serum.¹⁶ N-formyl-methionyl-leucyl-phenylalanine (FMLP) and phorbol myristate acetate were purchased from Sigma Chemical Co, St Louis, Missouri. Opsonised zymosan particles were prepared as described previously.⁴

MEMBRANE FILTER ASSAY
The leading front modification⁷ of the Boyden chamber technique¹⁸ was applied according to Wilkinson¹⁰ using 2×10⁵ purified PMN for each 3 µm pore size Millipore filter. The attractants and reference solutions were used above and below the filters as follows: HBSS/HBSS in studies of spontaneous locomotion, 0.2% HSA/0.2% HSA (human serum albumin, AB Kabi, Stockholm, Sweden) and 12% ZTS/12% ZTS in studies of chemokinesis, and 3% ZTS/12% ZTS in studies of chemotaxis. The filters were incubated for 55 minutes at 37°C. The distance of the leading front of the cells migrating was determined in five microscopical fields of each of the triplicate filters.

AGAROSE ASSAY
An agarose assay was used as described previously.¹⁶ The agarose medium was 1% agarose (Biomedical Division of Marine Colloids, Rockland, Maine) and 1% HSA. The rates of random migration in HSA, those of chemokinetic migration in 4% ZTS, and in 1×10⁻⁸ M FMLP, and those of chemotactic migration in response to ZTS and 5×10⁻⁷ M FMLP were determined by measuring the distance of migration of 2.5×10⁵ PMN in quadruplicate tests.

CHEMILUMINESCENCE ASSAY
The chemiluminescence response was measured with an LKB luminometer (model 1250, Wallac) fitted with a 37°C water jacketed sample holder. Polymorphonuclear leucocytes (2×10⁵) in 100 µl of phosphate buffered saline (PBS) were kept at 37°C for one minute and mixed with 100 µl of PBS, 100 µl of FMLP (10⁻⁵ M), 100 µl of phorbol myristate acetate (1 µg/ml in PBS), or 100 µl of opsonised zymosan particles, and with 200 µl of the luminol solution (10 µg/ml in PBS; Fluka AG, Buchs SG, Switzerland). The height of the curve as millivolts (mV) at one minute intervals and the area under the curve (cm²) were determined.

STATISTICAL METHODS
Comparisons were made using the paired sample t-test. *p<0.01; **p<0.001. The significance of the difference between synovial fluid PMN and peripheral blood PMN is indicated by asterisks: **p<0.01; ***p<0.001. The difference between the patients’ peripheral and the control polymorphonuclear leucocyte migration in ZTS was statistically significant (p<0.05).

Figure 1: Migration of synovial fluid polymorphonuclear leucocytes (PMN) (open columns) and peripheral blood PMN of the patients (hatched columns) under agarose at the acute phase of the disease. HBBS=Hank’s balanced salt solution; ZTS=zymosan treated serum; FMLP=N-formyl-methionyl-leucyl-phenylalanine. Each bar indicates 1 SE. The significance of the difference between synovial fluid PMN and peripheral blood PMN is indicated by asterisks: **p<0.01; ***p<0.001. The difference between the patients’ peripheral and the control polymorphonuclear leucocyte migration in ZTS was statistically significant (p<0.05).

Figure 2: Migration of synovial fluid polymorphonuclear leucocytes (PMN) (open columns) and peripheral blood PMN of the patients (hatched columns) and controls (hatched columns) in the filter at the acute phase of the disease. HSA=human serum albumin; ZTS=zymosan treated serum. Each bar indicates 1 SE. The significance of the difference between synovial cells and peripheral blood cells is indicated by asterisks: ***p<0.001.
Neutrophil function in reactive arthritis

Table 2: Functions of peripheral blood polymorphonuclear leucocytes at one month and at six months (after recovery) of patients with acute reactive arthritis. The figures are expressed as means (SD)

<table>
<thead>
<tr>
<th>Function</th>
<th>At one month (n=11)</th>
<th>After recovery (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
</tr>
<tr>
<td>Migration under agarose (mm):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In HBSS†</td>
<td>0.68 (0.51)*</td>
<td>0.84 (0.53)</td>
</tr>
<tr>
<td>In 4% ZTS</td>
<td>1.61 (0.52)</td>
<td>1.67 (0.62)</td>
</tr>
<tr>
<td>In FMLP (10⁻⁴ M)</td>
<td>2.62 (0.58)</td>
<td>2.41 (0.65)</td>
</tr>
<tr>
<td>Toward ZTS</td>
<td>1.47 (0.76)</td>
<td>1.63 (0.59)</td>
</tr>
<tr>
<td>Toward FMLP (5×10⁻⁹ M)</td>
<td>3.13 (0.73)</td>
<td>3.02 (0.65)</td>
</tr>
<tr>
<td>Migration into membrane filter (μm):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In HBSS</td>
<td>40 (11)</td>
<td>39 (13)</td>
</tr>
<tr>
<td>In 4% ZTS</td>
<td>74 (17)</td>
<td>69 (19)</td>
</tr>
<tr>
<td>Toward 4% ZTS</td>
<td>71 (23)</td>
<td>69 (27)</td>
</tr>
<tr>
<td>Chemiluminescence response, peak (mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>0.5 (0.4)</td>
<td>0.6 (0.7)</td>
</tr>
<tr>
<td>FMLP</td>
<td>4.8 (2.9)</td>
<td>5.9 (3.8)</td>
</tr>
<tr>
<td>PMA</td>
<td>6.3 (7.0)</td>
<td>6.9 (7.3)</td>
</tr>
<tr>
<td>Zymosan</td>
<td>11.7 (10.4)</td>
<td>11.8 (7.8)</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.02; paired sample t test between patients and controls.
†HBSS=Hanks's balanced salt solution; ZTS=zymosan treated pooled human serum; FMLP=N-formyl-methionyl-leucyl-phenylalanine; HSA=human serum albumin; PBS=phosphate buffered saline; PMA=phorbol myristate acetate.
‡Performed in only five patients.

At one month both the chemotactic and chemokinetic migration rates of the patients' PMN and the control PMN were much the same, whereas the spontaneous migration of the patients' PMN under agarose, but not in the filter, was decreased (table 2).

At six months after recovery, the chemokinetic and chemotactic responses of the patients' PMN to ZTS were in the agarose assay significantly lower and in the membrane filter technique significantly higher than those of the control PMN (table 2).

CHEMILUMINESCENCE
At the acute phase the peak chemiluminescence response of the synovial PMN to opsonised zymosan particles was significantly lower than that of control PMN (fig 3). The response to phorbol myristate acetate, but not to FMLP, also tended to be decreased, though not significantly (fig 3). The responses of the patients' peripheral blood PMN and control PMN were much the same at the acute phase, at one month, and at six months (fig 3 and table 2). The shapes of the chemiluminescence curves of the three groups were similar and analysis of the areas under the curves showed findings identical to the peak responses (data not shown).

Figure 3: Peak chemiluminescence responses of synovial fluid polymorphonuclear leucocytes (PMN) (open columns) and peripheral blood PMN of the patients (black columns) and the controls (hatched columns) at the acute phase of the disease. PBS=phosphate buffered saline; FMLP=N-formyl-methionyl-leucyl-phenylalanine; PMA=phorbol myristate acetate; OZP=opsonised zymosan particles. Each bar indicates 1 SE. The significance of the difference between synovial cells and peripheral blood cells is indicated by asterisk: *p<0.05.

EFFECT OF THE SEVERITY OF THE DISEASE
At the acute phase the chemiluminescence response induced by phorbol myristate acetate of the synovial fluid PMN of the patients with severe disease, mean (SD), was significantly higher than that of patients with mild disease: 76 (3-3) mV v 3-8 (2-6) mV, p<0.05. At six months the peripheral blood PMN of the group with severe disease showed significantly higher rates of migration, mean (SD), than those of the group with mild disease: under agarose in ZTS 1.98 (0.49) mm v 1.06 (0.5) mm, p<0.02; in the filter in the presence of HSA 48 (9) mm v 35 (5) mm, p<0.02; and in the filter toward ZTS 88 (26) v 56 (18) mm, p<0.05.
Discussion

These findings indicate that chemotactic and chemokinetic migration of synovial fluid PMN of patients with acute reactive arthritis is depressed, and oxygen radical production determined by the luminol enhanced chemiluminescence assay is also depressed or similar to that of peripheral blood PMN of the patients and healthy control subjects. These functional aberrations suggest that the patients’ synovial fluid PMN are deactivated.6

Deactivation of synovial fluid PMN occurs in patients with rheumatoid arthritis,13, 14 possibly owing to the ingestion of immune complexes by PMN. Under experimental conditions PMN exposed to small size aggregated IgG show decreased stimulated migration20 and decreased chemiluminescence responses,21, 22 whereas large aggregates are stimulatory.20 Some patients with yersinia arthritis have circulating yersinia specific immune complexes of small size23 and also yersinia specific immune complexes in synovial fluid.24 The presence of microbial structures in the joint fluid is further supported by the finding of chlamydia and yersinia antigens in the synovial fluid cells of patients with reactive arthritis triggered by chlamydia urethritis25 and yersinia enteritis.26 Thus deactivation is a characteristic of synovial fluid PMN in both rheumatoid arthritis13, 14 and sero-negative reactive arthritis as shown in this study and, possibly, immune complexes contribute to both disorders.

The mechanism of deactivation is not known but it may entail auto-oxidation of PMN; this is supported by the findings that oxygen radical scavengers potentiate chemotaxis27 and that PMN of patients with chronic granulomatous disease, which cannot generate oxygen radicals, do not become deactivated.28 It is also possible that the non-steroidal anti-inflammatory drugs taken by our patients may inhibit chemotaxis29 and production of oxygen derived free radicals.30 This explanation is not supported by the finding that the patients’ peripheral blood PMN were functionally normal and not deactivated.

In this study chemotactic/chemokinetic migration of the patients’ purified peripheral blood PMN was similar to that of control PMN during the acute disease, whereas after recovery the chemokinetic responses of the patients’ PMN to ZTS were depressed in the agarose assay and increased in the filter assay. These findings differ from those in our previous study,31 which showed that both at the acute phase and after recovery buffy coat PMN of HLA-B27 positive patients with yersinia arthritis showed increased migration in response to ZTS. Buffy coat does also contain monocytes which increase migration of PMN in vitro.32 Monocytes of the patients with previous yersinia arthritis generate increased amounts of tumour necrosis factor.33 Tumour necrosis factor may contribute to increased chemotaxis/chemokinesis of the patients’ buffy coat PMN by inducing generation of monocyte derived chemotactic factors for PMN.34 Furthermore, recombinant tumour necrosis factor has been reported by some35 but not all36 authors to be chemotactic for PMN.

After recovery the peripheral blood PMN of patients with severe acute disease showed higher chemotactic migration than did those with mild disease, which agrees with our previous study.4 We have also previously shown that PMN of the patients who have sequelae tend to show increased migration.5 These findings support the view that among patients with reactive arthritis there are subgroups with hyperreactive neutrophils, which may contribute to the development of severe inflammatory symptoms. Clinical studies also provide evidence of subgroups: according to the 10 year follow up study about 30% of patients with yersinia arthritids develop sacroiliitis, even ankylosing spondylitis.28 It is interesting that purified PMN of patients with ankylosing spondylitis show increased chemotactic responses to ZTS20, 40 but not to FMLP,41 both under agarose and in the filter. Thus the data suggest that increased migration of PMN in response to ZTS is most pronounced, and can be shown also using purified PMN, in patients with severe inflammatory tissue injury, which occurs in ankylosing spondylitis and possibly in reactive arthritis characterised by severe acute disease or late complications. On the other hand, in patients with mild, self limited reactive arthritis, enhanced migration of PMN would be more evident in the presence of both PMN and monocytes. Because microbial antigens may occur42, 43 and persist in43 the host tissues, an interesting possibility is that such bacterial structures modify monocyte/macrophage function and thereby also functions of PMN in patients with reactive arthritis.

The skilful technical assistance of Mrs. Eins Vololainen and Mrs Paula Ruhikainen is gratefully acknowledged. The study was supported by grants from the Paulo Foundation, the Academy of Finland, the Rheumatism Research Foundation, the Finnish Medical Foundation, and the Finnish Cultural Foundation.

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Ann Rheum Dis 1990 49: 615-619
doi: 10.1136/ard.49.8.615

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