Serum lysozyme: a potential marker of monocyte/macrophage activity in rheumatoid arthritis

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Abstract

Objective. Estimate the contribution of monocytes/macrophages to the disease process in rheumatoid arthritis (RA), by measuring the serum levels of the leucocyte-derived granular proteins: lysozyme, myeloperoxidase (MPO), lactoferrin and human neutrophil lipocalin (HNL).

Methods. Serum levels of these granular proteins were measured in patients with RA (n = 23) and in healthy controls (n = 27), and in 10 patients with RA after treatment with low-dose prednisolone. The serum levels of the granular proteins were also measured before and after treatment with metyrapone, a substance that inhibits the synthesis of cortisol in the adrenals.

Results. The serum levels of lysozyme and MPO were elevated in patients with RA, while the concentrations of lactoferrin and HNL were similar in both groups. Prednisolone treatment decreased the serum concentration of lysozyme and MPO. Metyrapone did not influence the level of the granular proteins measured.

Conclusions. The increased serum levels of lysozyme and MPO, but not of HNL and lactoferrin in RA could indicate a stimulated secretory activity of mononuclear phagocytes.

The measurement of serum lysozyme, as an indicator of monocyte/macrophage activity, might be used to study disease activity in RA.

Key words: Rheumatoid arthritis, Lysozyme, Myeloperoxidase, Prednisolone.
Glucocorticoids have been used in the treatment of RA for five decades [21] as effective anti-inflammatory agents with possible disease-modifying activity [22]. Glucocorticoids exert at least part of their action at the molecular level by binding to specific receptors in the cytoplasm and then migrating to the nucleus, where they bind to selective regulatory sites on DNA. This can result in increased or decreased expression of genes important for the inflammatory process [23].

The aim of the present study was to re-evaluate serum lysozyme as a measure of monocyte/macrophage activity in RA, using an immunological method, and to monitor the effect of exogenous and endogenous glucocorticoids on the secretory activity of macrophages.

Patients and methods

Patients

Twenty-three patients with definite RA (17 women, six men, mean age 61 yr, range 29–86 yr) according to the ARA criteria [24] were included in the study. The mean duration of disease was 63 months (range 1 month to 43 yr). Another six patients with seronegative polyarthritis not fulfilling the criteria for definite RA were also studied. None of the patients had received glucocorticoid treatment for the past 3 months prior to inclusion into the study and none of the patients had been treated with second-line drugs. Most of the patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs).

Ten of the patients with definite RA and three of the patients with seronegative polyarthritis were treated with prednisolone at an initial dose of 5–15 mg/day which was reduced to 5–7.5 mg/day during the first 2 weeks of treatment. In the majority of these patients, second-line drugs, chloroquine (n = 6), sulphasalazine (n = 2), methotrexate (n = 1) and oral gold (n = 1) were introduced as well. Blood samples were collected before the start of treatment and after 4–6 weeks. Blood samples were drawn at 10.00 a.m.

Eight of the patients with definite RA and three of the patients with seronegative polyarthritis were treated as in-patients with metyrapone for 2 days. Metyrapone inhibits the activity of the enzyme 11-β-hydroxylase in the adrenal cortex and thus decreases the synthesis of cortisol. On day 1, oral doses of metyrapone of 750 mg were given at 6.00 a.m., 12.00 a.m., 6.00 p.m. and 12.00 p.m. The first dose of 750 mg on day 2 was given at 6.00 a.m. Blood samples were collected the day before the start of the metyrapone treatment and on the second day of metyrapone treatment. All blood samples were drawn at 7.30 a.m. One of these patients was on treatment with sulphasalazine 500 mg × 1.

The patients were assessed according to the Thompson index [25] of joint inflammation and to the duration of early morning stiffness. The blood samples were analysed for the following variables: haemoglobin, erythrocyte sedimentation rate/h (ESR), C-reactive protein (CRP), white blood cell, neutrophil, monocyte and platelet counts.

Controls

Twenty-one healthy female and six healthy male individuals served as controls. Their mean age was 48 yr (range 26–65 yr). None of the controls had any symptoms of infection or any inflammatory disease. The patients and controls were informed according to the Declaration of Helsinki and the study was accepted by the local ethics committee.

Assays

The serum concentrations of lysozyme and HNL were measured by a double-antibody radioimmunoassay described in detail elsewhere [26]. Briefly, 50 µl of either sample or standard was mixed with 50 µl of specific anti-lysozyme/anti-HNL antibodies, diluted in assay buffer, and incubated for 3 h at room temperature. Thereafter, 2 ml of decanting suspension containing Sepharose anti-rabbit IgG was added and the incubation continued for 30 min at room temperature. Lysozyme– or HNL–antibody complexes bound on Sepharose anti-rabbit IgG were separated and pelleted by means of centrifugation for 10 min at 4000 r.p.m. After decantation the radioactivity was measured in a gamma counter.

The serum concentration of MPO was assayed by means of a double-antibody radioimmunoassay (Pharmacia and Upjohn, Diagnostics AB, Uppsala, Sweden).

The serum concentration of lactoferrin was measured by means of radioimmunoassay as previously described [27].

Statistics

Values are given as medians and inter-quartile ranges or total range. Non-parametric tests, Mann–Whitney U-test, Wilcoxon matched-pair test and the Spearman rank correlation coefficient were used to analyse the data. A P-value of <0.05 was considered significant. The statistical calculations were carried out using the software Statistica (StatSoft. Inc., Tulsa, OK, USA).

Results

**Serum concentration of lysozyme, MPO, HNL and lactoferrin**

The disease activity of the patients with RA was defined by the joint index (median Thompson index 82, range 0–225) and various laboratory data; median ESR 32 mm/h (range 8–120 mm/h), median CRP 24 mg/l (≤10–184 mg/l), median white blood cell count 7.5 × 10^9/l (range 4.3 × 10^9/l–18.1 × 10^9/l) and median platelet count 341 × 10^9/l (range 179 × 10^9/l–896 × 10^9/l). Figure 1 shows that the serum concentrations of lysozyme and MPO were significantly elevated in the patients with RA compared with the control individuals (P < 0.00001; P = 0.001, respectively). In Table 1, peripheral blood neutrophil count, monocyte count, serum concentration of HNL and lactoferrin are presented.

No difference was observed between the concentration
Serum lysozyme in RA

The serum cortisol level at 7.30 a.m. on day 2 of metyrapone treatment was significantly lower compared with the day before the start of treatment at 7.30 a.m. (162 nmol/l compared with 438 nmol/l, \( P < 0.01 \)). The serum levels of lysozyme and MPO (Table 3) were not influenced by metyrapone treatment, nor were those of HNL and lactoferrin (data not shown).

Discussion

In the present study, elevated serum levels of lysozyme together with elevated serum levels of MPO were observed in patients with RA compared with controls. In contrast the concentrations of lactoferrin and HNL were similar in both groups. Based on the results of previous investigations indicating that lysozyme in serum predominantly originates from monocytes/macrophages and to a lesser degree from neutrophils [13, 15, 16], that MPO originates from monocytes and neutrophils [17], and that lactoferrin and HNL originate from neutrophils [18–20], the elevated serum level of lysozyme can be assumed to derive from monocytes/macrophages. Thus, the increased lysozyme level probably reflects an increased secretory activity by monocytes/macrophages in RA. This interpretation is in concordance with previous studies indicating peripheral blood monocyte activation in RA measured as increased monocyte cell surface expression of \( \beta2 \)-integrins and as increased adhesion [28–30].

Lysozyme is produced continuously by monocytes and macrophages, although macrophages have a higher basal production of lysozyme [7]. Tumour necrosis factor-\( \alpha \) (TNF-\( \alpha \)) stimulates lysozyme production by monocytes and macrophages [7] and the release of lysozyme and MPO by neutrophils [31]. Furthermore, elevated levels of TNF-\( \alpha \) in serum have been measured in RA [32]. The increased secretion of lysozyme into the peripheral blood could thus be mediated by TNF-\( \alpha \).

Ten of the patients with RA included in the study were treated with low-dose prednisolone for approximately 1 month. The decrease in the serum levels of lysozyme and MPO after treatment suggests a diminished secretory activity of the monocytes/macrophages. These results are in concordance with our previous results, where low-dose prednisolone treatment decreased the expression of integrin, complement and \( \mathrm{Fc}_{\gamma} \)-receptors on peripheral blood monocytes in RA [30]. Macrophages contribute to the cartilage and bone destruction in RA. The reported disease-modifying effect of low-dose prednisolone treatment in RA [22] could

<table>
<thead>
<tr>
<th></th>
<th>HNL (( \mu )g/l)</th>
<th>Lactoferrin (( \mu )g/l)</th>
<th>Blood neutrophils (( \times 10^9 )l)</th>
<th>Blood monocytes (( \times 10^9 )l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>115 (62–300)</td>
<td>379 (158–1057)</td>
<td>4.7 (1.2–9.9)</td>
<td>585 (150–1460)</td>
</tr>
<tr>
<td>Healthy individuals</td>
<td>103 (56–220)</td>
<td>266 (121–1239)</td>
<td>4.0 (2.0–8.8)</td>
<td>456 (337–878)</td>
</tr>
</tbody>
</table>

The values are given as medians with ranges in parentheses.
Table 2. (A) Clinical and laboratory data of the 10 patients with definite rheumatoid arthritis before and after treatment with prednisolone for 4–6 weeks. (B) Clinical and laboratory data from eight patients with rheumatoid arthritis before and after treatment with metyrapone

<table>
<thead>
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<th></th>
<th>ESR (mm/h)</th>
<th>CRP (g/l)</th>
<th>WBC ( \times 10^9/l )</th>
<th>Blood monocytes ( \times 10^9/l )</th>
<th>Blood neutrophils ( \times 10^9/l )</th>
<th>Thompson index</th>
<th>Early morning stiffness (min)</th>
</tr>
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<tr>
<td>(A) Patients treated with prednisolone</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Before treatment</td>
<td>24 (8–59)</td>
<td>16 (≤10–30)</td>
<td>7.6 (4.3–10.1)</td>
<td>575 (150–840)</td>
<td>5.1 (2.7–6.3)</td>
<td>68 (0–100)</td>
<td>90 (0–300)</td>
</tr>
<tr>
<td>After treatment</td>
<td>6 (2–20)*</td>
<td>10 (&lt;10–13)*</td>
<td>7.6 (1.9–14.2)</td>
<td>495 (360–920)</td>
<td>4.7 (0.6–12.0)</td>
<td>0 (0–32)**</td>
<td>0 (0–100)**</td>
</tr>
<tr>
<td>(B) Patients treated with metyrapone</td>
<td></td>
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<tr>
<td>Before treatment</td>
<td>34 (9–110)</td>
<td>10 (&lt;10–184)</td>
<td>6.3 (4.3–18.1)</td>
<td>530 (150–810)</td>
<td>3.4 (1.2–9.9)</td>
<td>86 (5–162)</td>
<td>60 (0–180)</td>
</tr>
<tr>
<td>After treatment</td>
<td>38 (10–125)</td>
<td>10 (&lt;10–128)</td>
<td>6.0 (3.5–11.2)</td>
<td>530 (100–740)</td>
<td>3.4 (2.0–6.8)</td>
<td>104 (5–227)</td>
<td>60 (0–180)</td>
</tr>
</tbody>
</table>

The values are given as medians with ranges in parentheses. WBC, white blood cell count. Reference intervals: ESR 2–15 mm/h, CRP ≤10 mg/l, WBC 4.0–9.0 \( \times 10^9/l \).

Thus be explained by down-regulation of monocyte/macrophage activity. Measurement of serum lysozyme in RA might be a simple way to estimate monocyte/macrophage activity and to follow the efficacy of treatment. Glucocorticoids have been shown to inhibit lysozyme production \emph{in vitro} [8, 33]. The effect of glucocorticoids in the present study could thus be a direct effect on the production of lysozyme by leucocytes or indirect through down-regulation of cytokine production.

Inhibition of endogenous cortisol by metyrapone did not influence the circulating levels of the various granular proteins measured before and after treatment. One explanation could be the short duration of treatment. Another possible explanation is that the release of lysozyme and MPO is not affected by endogenous cortisol. Our previous report showed that metyrapone treatment increased the expression of complement receptor 1 and CD49f on peripheral blood monocytes [30]. The monocyte expression of these receptors seems to be more sensitive to lowered serum cortisol concentrations compared with the secretory activity by monocytes/macrophages.

Most of the patients receiving low-dose prednisolone treatment were also treated with second-line drugs. The second-line drugs, i.e. chloroquine, methotrexate, sulphasalazine and gold salts affect leucocyte function in different ways. Auronofin was reported to inhibit the cytokine-induced release of lysozyme by leucocytes [34].
No reports are available on the effect of other second-line drugs on lysozyme secretion by leucocytes. However, an effect of the second-line drugs on protein secretion by leucocytes cannot be excluded.

In summary, our findings have suggested an increased secretory activity of the mononuclear phagocytes, but not of the neutrophils in patients with RA. The secretory products of macrophages are thought to contribute to the destructive process in RA, and the normalizing effect of glucocorticoids on serum lysozyme might thus reflect one of the mechanisms behind the reported disease-modifying effect of low-dose prednisolone treatment in RA [22].

Acknowledgements

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References


