

Different expression of chemokines in rheumatoid arthritis and osteoarthritis bone marrow

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Abstract

Objectives: Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to joint destruction. In addition to involvement of the joints, there is growing evidence that inflammatory/autoimmune processes take place in bone marrow, beginning the disease onset. Activated T and B cells accumulate in bone marrow, where also effective antigen presentation takes place. An increased number of activated T cells was observed in RA in comparison to osteoarthritis (OA) bone marrow. In the present study we analyzed the levels of chemokines that may be responsible for accumulation/retention of T-cells in the bone marrow of RA and OA patients.

Material and methods: Bone marrow samples were obtained from RA and OA patients during total hip replacement surgery, and bone marrow plasma was obtained by gradient centrifugation. Levels of the chemokines CX3CL1, CCL5, CCL2, CXCL12 and CXCL1 were measured in bone marrow plasma by specific ELISAs. Comparison between the groups of patients and statistical significance were analyzed by the two-tailed Mann-Whitney U test.

Results: Increased levels of CX3CL1 (818 ±431 pg/ml vs. 502 ±131 pg/ml, $p < 0.0007$) and CCL5 (5967 ±1680 pg/ml vs. 4878 ±2360 pg/ml, $p < 0.05$) respectively in bone marrow plasma from RA in comparison with OA patients were observed. In contrast, similar levels of CCL2, CXCL12 and CXCL1 in RA and OA bone marrow suggest that these cytokines do not play a significant role in the observed T cell accumulation in RA bone marrow.

Conclusions: CX3CL1 and CCL5 overproduced in RA bone marrow may contribute to the accumulation of T cells observed in RA bone marrow.

Key words: chemokines, bone marrow, rheumatoid arthritis, T lymphocytes.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that leads to joint destruction and severe disability [1]. Histologically, it is characterised by synovial hyperplasia with bone and cartilage destruction. The pathogenesis of RA is complex and still unclear. Many cell types, cytokines, chemokines and growth factors have been implicated in RA pathogenesis. T cells are considered to be central for development

of RA [2], contributing greatly to joint pathology [3]. Chemokines are a family of chemoattractant cytokines that mediate leukocyte migration during homeostasis and the inflammatory process that is a feature of RA. Chemokines are also involved in T cell activation and proliferation [4].

A growing body of evidence indicates that bone marrow (BM) can participate in induction and/or perpetuation of the inflammatory process in RA. The

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pathological processes in BM of RA patients (named bone marrow oedema), together with production of autoantibodies, were shown to proceed joint destruction in RA [5, 6].

Our previous results indicate that biologically active TLR9 can participate in direct activation and proliferation of B cells from RA BM [7]. Moreover, locally overproduced IL-15 may be responsible for activation and proliferation of T cells in RA BM, reflected by an increased number of activated T cells in this tissue [8]. Several chemokines may also influence T cell infiltration and accumulation in the RA BM compartment. To test this hypothesis, we measured the concentrations of selected T cell-related chemokines in BM samples from RA and, for comparison, from OA patients.

Material and methods

Characteristics of patients: Bone marrow samples were obtained from RA and OA patients during total hip replacement surgery. Patients fulfilled the American College of Rheumatology revised criteria for RA [9] or for OA [10]. All individuals gave informed consent and the study was approved by the Institute of Rheumatology Ethics Committee. Twenty patients with RA (16 women, 4 men, mean age 57 ± 8.6) and 20 patients with OA (10 women, 10 men, mean age 56 ± 12) were enrolled in the study. Ten of the RA patients were treated with both methotrexate (MTX) and steroids, 2 received only MTX, and 3 received only steroids. The remaining patients were on non-steroidal anti-inflammatory drugs (NSAID).

Analysis of concentrations of chemokines: Bone marrow samples were diluted two-fold in PBS (Sigma, St Louis, Missouri, USA) with sodium citrate as an anti-coagulant. Bone marrow plasma samples were obtained by centrifugation. The concentrations of CX3CL1 (fractalkine), CCL5 (RANTES), CCL2 (MCP1), CXCL12 (SDF1)

and CXCL1 (GRO α) were measured in BM plasma by an enzyme-linked immunosorbent assay (ELISA) test (R&D Systems) according to the manufacturer's instructions.

Statistical analysis: Data were analysed using Statistica vol. 6.0 software. Comparison between RA and OA groups of patients was performed by the two-tailed Mann-Whitney U test. P values less than 0.05 were considered significant. Data are shown as the mean \pm SD.

Results

We found increased levels of CX3CL1 (818 ± 431 pg/ml vs. 502 ± 131 pg/ml, $p < 0.0007$) (Fig. 1) and CCL5 (5967 ± 1680 pg/ml vs. 4878 ± 2360 pg/ml, $p < 0.05$) (Fig. 2) in bone marrow plasma samples from RA, in comparison to OA patients.

However, the levels of CCL2 (99 ± 63 pg/ml vs. 126 ± 100 pg/ml), CXCL12 (132 ± 145 pg/ml vs. 162 ± 154 pg/ml) and CXCL1 (1224 ± 914 pg/ml vs. 1254 ± 973 pg/ml) were comparable between RA and OA bone marrow plasma samples.

Discussion

Recent data indicate that bone marrow may participate in the pathogenesis of RA as a site where initiation and/or perpetuation of the inflammatory response can take place [5, 6, 11]. Bone marrow T cells may contribute to the pathogenesis of RA by production of pro-inflammatory and tissue destruction factors (tumor necrosis factor – TNF, interferon γ – IFN- γ , interleukin 17a – IL-17a, IL-17F, receptor activator for nuclear factor κ B ligand – RANKL) [12]. In our previous study we observed an increased number of activated CD3CD4 cells in RA bone marrow tissue, compared to OA patients [8]. This accumulation could be partially explained by increased concentration of IL-15 in RA BM, which supports T cell

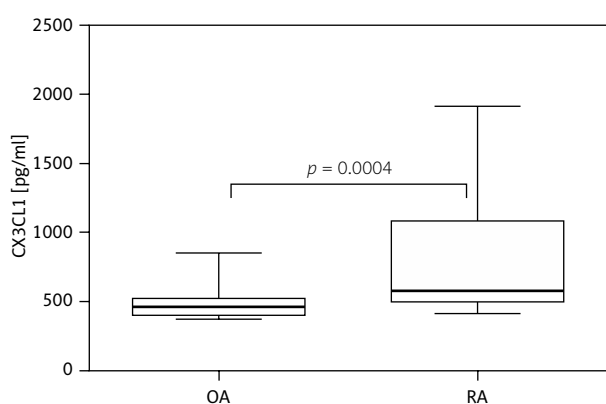


Fig. 1. Concentrations of CX3CL1 in BM plasma of RA ($n = 20$) and OA ($n = 20$) patients.

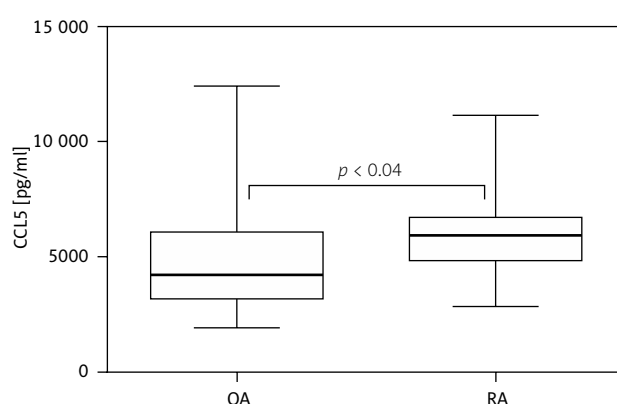


Fig. 2. Concentrations of CCL5 in BM plasma of RA ($n = 20$) and OA ($n = 20$) patients.

activation and proliferation. However, it is possible that other cytokines and chemokines may also be involved in this process.

It was shown that synovial fluid of RA patients contains elevated levels of CCL5 and CX3CL1, and this phenomena may be responsible for T cell homing and accumulation in RA synovial tissue [4, 13]. Interaction between chemokines and their receptors is needed to activate target cells. CCL5 has been shown to bind to CCR3, CCR5 and CCR1. Interestingly, T cells in synovial fluid and peripheral blood of RA patients were shown to express CCR1, CCR3 and CCR5 [14, 15]. In addition, the percentage of CD3-positive lymphocytes expressing CCR5 is significantly elevated in RA PB, in comparison to healthy donors [15]. Soluble CX3CL1 is a potent chemoattractant molecule for monocytes and T cells. Also, the percentage of CD3-positive lymphocytes expressing CX3CR1 is significantly elevated in RA PB [15]. To date, there is no information about the involvement of any chemokine in the accumulation of T cells in bone marrow of RA patients.

Our current study was undertaken to compare the levels of chemokines that are known to participate in T cell migration, in BM of RA and OA patients. Elevated levels of CCL5 and CX3CL1 were found in BM of RA patients (Figs. 1, 2). The levels of other tested chemokines – CCL2, CXCL12 and CXCL1 – were comparable between RA and OA bone marrow plasma samples. Higher levels of CCL5 and CX3CL1 in RA bone marrow may create the microenvironment that favours T cell attraction and retention in this tissue. On the other hand, the increased levels of CCL5 detected in RA bone marrow samples may reflect the activity of BM T cells itself, because T cells are known producers of this chemokine upon activation [13]. Therefore, by analogy to the synovial tissue, it is tempting to speculate that locally overproduced CCL5 and CX3CL1 may be involved in T cell homing and accumulation in bone marrow of RA patients.

Conclusion

The increased amounts of CCL5 and CX3CL1, but not other tested chemokines, in RA bone marrow tissue identify these chemokines as candidate factors that may play a role in T cell homing and/or retention in this tissue.

The authors declare no conflict of interest.

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