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Enhanced expression of mRNA for FK506-binding protein 5 in bone marrow CD34 positive cells in patients with rheumatoid arthritis

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ABSTRACT

Objective. *Recent studies have disclosed that several genes are up-regulated in bone marrow (BM) mononuclear cells from rheumatoid arthritis (RA) patients. However, it remains unclear whether such abnormalities result from systemic inflammation or from abnormalities at stem cell level. The current study therefore examined the expression of several representative genes, including amphiregulin (AREG), chemokine receptor 4 (CXCR4), and FK506-binding protein 5 (FKBP5) in RA BM CD34+ cells.*

Methods. *BM samples were obtained from 52 patients with RA and 35 patients with osteroarthritis (OA) during joint operations. CD34+ cells were purified from the BM mononuclear cells by positive selection with magnetic beads. The mRNA expression for AREG, CXCR4, and FKBP5 was measured using quantitative real-time PCR.*

Results. *The expression of mRNA for FKBP5, but not that of AREG or CXCR4, was significantly higher in RA BM CD34+ cells than in OA BM CD34+ cells. The FKBP5 mRNA expression level was not correlated with serum CRP or treatment. In addition, tumour necrosis factor-α did not enhance the expression of FKBP5 mRNA in BM CD34+ cells from healthy donors.*

Conclusion. *The results suggest that the enhanced expression of FKBP5 in BM CD34+ cells might be an intrinsic abnormality of RA BM CD34+ cells, whereas the enhanced expression of AREG and CXCR4 in BM mononuclear cells might be secondary to systemic inflammation.*

Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory disease characterised by synovial hyperplasia (1). Type A synoviocytes are known to be derived from monocyte precursors in the bone marrow (BM), whereas type B synoviocytes have the morphological appearance of fibroblasts and produce a variety of factors, including cytokines and matrix metalloproteinases (MMPs) (1). We previously demonstrated that BM CD34+ cells from RA patients have abnormal capacities to respond to tumour necrosis factor (TNF) -α and to differentiate into fibroblast-like cells producing MMP-1, suggesting that abnormalities in BM CD34+ cells might play a role in the pathogenesis in RA (2).

Nakamura *et al*. recently disclosed that the expression of several genes including amphiregulin (AREG), chemokine receptor 4 (CXCR4), and FK506-binding protein 5 (FKBP5), was augmented in BM mononuclear cells from RA patients compared with those from osteoathritis (OA) patients (3). AREG has been implicated in the pathogenesis of RA, since it stimulates fibroblast-like synoviocytes to proliferate and produce proinflammatory cytokines (4). CXCR4, the receptor for stromal cell derived factor-1 (SDF-1), has been also shown to be involved in the pathogenesis of RA (5). FKBP5 binds heat shock protein 90 (Hsp90) and assembles glucocorticoid receptor heterocomplex (6). Interestingly, FKBP5 was found to be involved in nuclear translocation and activation of nuclear factor kappa B (NFκB) (7, 8). It is therefore possible that FKBP5 might be also involved in the pathogenesis of RA, in which the activation of NFκB plays a pivotal role (9).

Although the expression of mRNAs for AREG, CXCR4 and FKBP5 has been shown to be augmented in RA BM mononuclear cells (3), it remains unclear whether their expression in BM CD34+ cells might also be up-regulated. The current study, therefore, explored the expression of these genes in BM CD34+ cells from patients with RA and OA.

Materials and methods

Patients and samples

BM samples were obtained from 52 patients with RA (8 males and 44 females; aged 59.2±11.0 years) (mean ± SD), who satisfied the American College of Rheumatology 1987 revised criteria (10) and gave informed consent in accordance with the World Medical Assocciation Declaration of Helsinki Ethical Principles for Medical Reserch Involving Human Subjects, during joint operations via aspiration from the iliac crest. BM samples were similarly obtained from 35 patients with OA (3 males and 32 females; aged 71.2±6.9 years).

Preparation of BM CD34+cells
CD34+ cells were purified from BM mononuclear cells by positive selection with magnetic beads (CD34 progenitor cell selection system; Dynal, Oslo, Norway), as previously described (2). BM CD34+ cells from healthy individuals (purity >95%) were purchased from BioWhittaker (Walkersville, MD, USA).

Real-time quantitative PCR
cDNA was prepared from 1 μg of total RNA isolated from purified BM CD34+ cells. Real-time quantitative PCR was performed with ABI Prism 7700 Sequence Detection System (Applied Biosystems Japan, Tokyo) and SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) with the following primers: 5′-GAT GTC TTC AGG GAG TGA GAT T-3′ (sense) and 5′-CCA GGT ATT TGT GGT TCG TT-3′ (antisense) for human AREG (GenBank accession no. BT019866); 5′-TGC CCT CCT GCT GAC TAT T-3′ (sense) and 5′-GTG CTG AAA CTG GAA CAC AAC-3′ (antisense) for human CXCR4 (BC020968); 5′-AGG CCC TTG GAC TGG ACA GT-3′ (sense) and 5′-CTG GGC TTC ACC CCT CCT AT-3′ (antisense) for human FKBP5 (BC111050; 5′-GCA AAG ACC TGT ACG CCA AC-3′ (sense) and 5′-CTA GAA GCA TTT GCG GTG GA-3′ (antisense) for human β-actin (X00351). Amplification was performed according to the standard protocol recommended by the manufacturer. The mRNA copy number of each gene was calculated using the plasmid DNAs of known copy numbers (Nihon Gene Research Laboratories, Miyagi, Japan).

Cell culture
BM CD34+ cells from healthy donors were cultured with or without TNF-α (10 ng/ml) (Peprotech EC, London) for 24 hours as previously described (11), after which the expression of various genes was analysed.

Statistics
Comparison between RA and OA patients, and that between patients with or without MTX or glucocorticoids was carried out using the Mann-Whitney U-test. Correlation of FKBP5 mRNA expression and serum C-reacting protein (CRP) was determined by Spearman's rank correlation test.

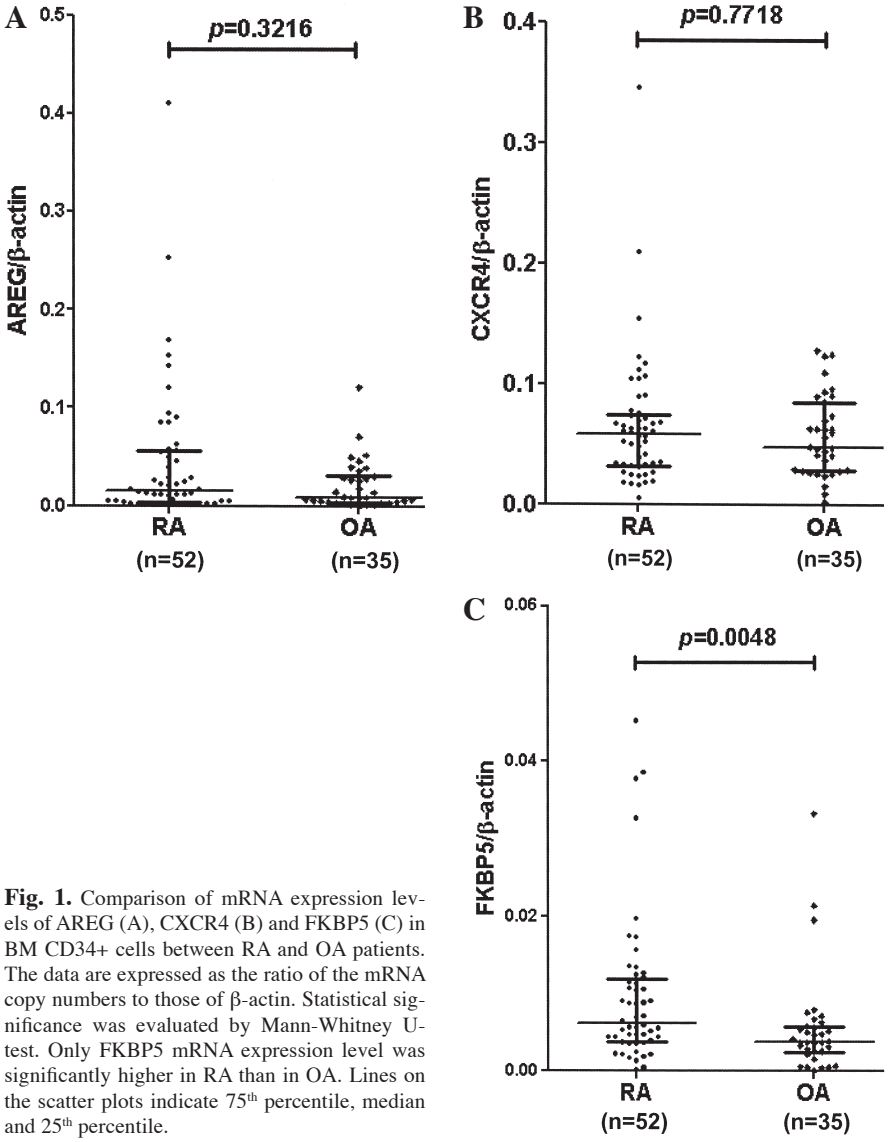

Fig. 1. Comparison of mRNA expression levels of AREG (A), CXCR4 (B) and FKBP5 (C) in BM CD34+ cells between RA and OA patients. The data are expressed as the ratio of the mRNA copy numbers to those of β-actin. Statistical significance was evaluated by Mann-Whitney U-test. Only FKBP5 mRNA expression level was significantly higher in RA than in OA. Lines on the scatter plots indicate 75th percentile, median and 25th percentile.

Results

mRNA expressions of AREG, CXCR4 and FKBP5 in BM CD34+ cells from RA and OA patients
The expression of mRNAs for AREG, CXCR4 and FKBP5 is shown as the ratio of the copy number of each gene to that of β-actin. There was no significant difference in AREG mRNA expression (Fig. 1A) and CXCR4 mRNA expression (Fig. 1B) in BM CD34+ cells between RA and OA (AREG/β-actin: (1.537±1.014) × 10^{-2} and (0.8718±0.4237) ×10^{-2} (median ± standard error), respectively, p=0.3216; CXCR4/β-actin: (5.770±0.7639) × 10^{-2} and (4.777±0.5723) ×10^{-2}, respectively, p=0.7718). By contrast, FKBP5 mRNA expression was significantly higher in RA BM CD34+ cells than that in OA BM CD34+ cells (FKBP5/β-actin: (6.248±1.341) × 10^{-3} and (3.794±1.111) ×10^{-3}, respectively, p=0.0048) (Fig. 1C). FKBP5 mRNA expression was not significantly correlated with serum CRP (Fig. 2). Of note, 29 or 37 of the 52 RA patients were taking methotrexate (MTX) or glucocorticoid, whereas none of the OA patients were taking either. However, as shown in Figure 3, there were no significant differences in FKBP5 mRNA expression in BM CD34+ cells between RA patients with or without MTX or glucocorticoid (FKBP5/

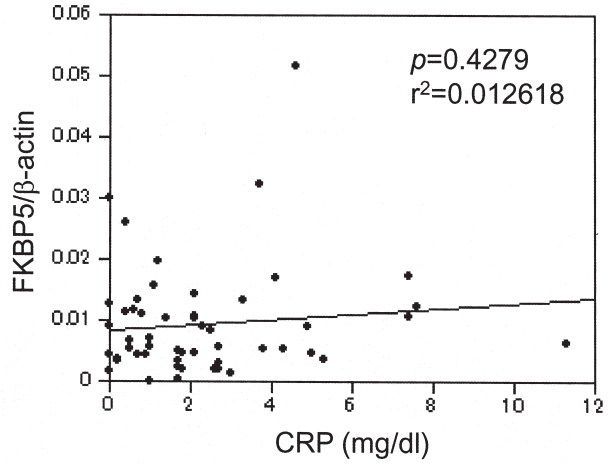

Fig. 2. Lack of correlation between serum CRP levels and FKBP5 mRNA expression levels in BM CD34+ cells in RA patients. FKBP5 mRNA expression levels are expressed as the ratio of the mRNA copy numbers to those of β-actin. Statistical significance was evaluated by Spearman's rank correlation test.

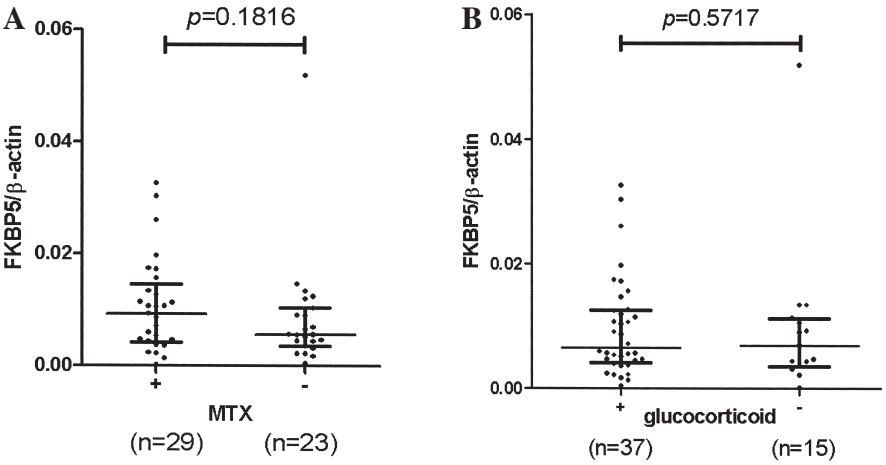

Fig. 3. Relevance of treatment to the expression of mRNA for FKBP5 in BM CD34+ cells. FKBP5 mRNA expression levels are expressed as the ratio of the mRNA copy numbers to those of β-actin. No significant difference was detected in FKBP5 mRNA expression levels between RA patients with or without MTX (A) or glucocorticoid (B). Statistical significance was evaluated by Mann-Whitney U-test.

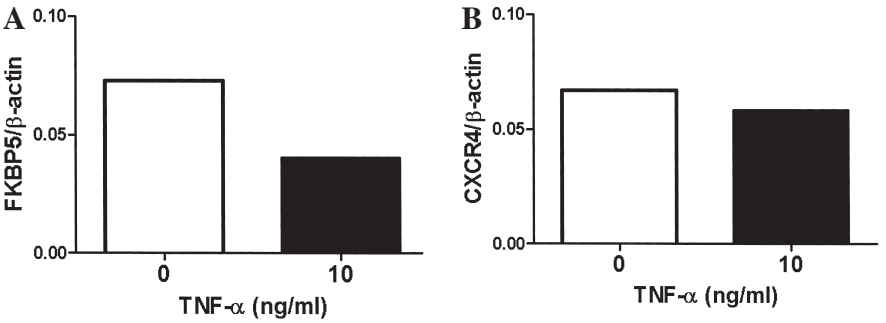

Fig. 4. Effect of TNF-α on the expression of mRNAs for CXCR4 (A) and FKBP5 (B) in BM CD34+ cells. BM CD34+ cells from healthy individuals were incubated in culture medium with or without TNF-α for 24 h. After incubation, total RNA was isolated for evaluation of the expression of mRNAs for AREG, CXCR4, FKBP5 and β-actin by real-time quantitative PCR. The data are expressed as the ratio of the mRNA copy numbers to those of β-actin. AREG mRNA was not detected.

β-actin: (9.302±1.558) $\times 10^{-3}$ (with MTX) and (5.654±2.135) $\times 10^{-3}$ (without MTX), p=0.1816; (6.529±1.288) $\times$ 10^{-3} (with glucocorticoid) and (6.957±3.181) $\times 10^{-3}$ (without glucocorticoid), p=0.5717). These results indicate that FKBP5 mRNA expression was up-regulated in RA BM CD34+ cells independently of medication or systemic inflammation.

Effect of TNF-α on the expression of FKBP5 mRNA in BM CD34+ cells from healthy donors

Previous studies have demonstrated that TNF-α plays a critical role in the pathogenesis of RA (12). To explore whether the up-regulation of FKBP5 mRNA in RA BM CD34+ cells might be due to the action of TNF-α, the effect of TNF-α on FKBP5 mRNA expression in BM CD34+ cells from healthy individuals was examined. As can be seen in Figure 4, TNF-α did not up-regulate the expression for FKBP5 and CXCR4 mRNAs. Instead, TNF-α appeared to suppress the expression of FKBP5 mRNA. The results suggest that the increased expression of FKBP5 mRNA in RA BM CD34+ cells might not be accounted for by the action of TNF-α.

Discussion

Accumulating evidences suggest that abnormalities in BM play a role in the pathogenesis of RA (11, 13). Nakamura *et al*. revealed that several genes were up-regulated in BM mononuclear cells in RA patients compared with OA patients (3). In the present study, we have demonstrated that only FKBP5 mRNA expression was significantly up-regulated in BM CD34+ cells from RA. It should be pointed out that the expression of mRNA for AREG and CXCR4 were up-regulated in both BM mononuclear cells and peripheral blood mononuclear cell from RA patients compared with those from OA patients (3). In addition, neither AREG mRNA nor CXCR4 mRNA was up-regulated in RA BM CD34+ cells. Therefore, it is suggested that the up-regulation of the expression of mRNAs for AREG and CXCR4 in RA BM mononuclear cells might be sequelae of systemic inflammation of RA, but not primary abnormalities.

In contrast with AREG or CXCR4, FKBP5 was up-regulated only in BM mononuclear cells, but not in peripheral blood mononuclear cells in RA (3). It is therefore likely that the up-regulation of FKBP5 mRNA expression in RA BM CD34+ cells might not be secondary to systemic inflammation, but a primary abnormality in BM CD34+ cells.

Previous studies demonstrated that BM CD34+ cells from RA patients have

abnormal capacities to respond to TNF-α and to differentiate into type B synoviocyte-like cells (2). TNF-α is one of the first triggers to be found effective for the activation of NFκB (14). Of note, we have recently demonstrated that RA BM CD34+ cells showed enhanced expression of NFκB1 (p50), silencing of which resulted in prevention of their differentiation into fibroblast-like cells (11). Interestingly, FKBP5 was found to be involved in nuclear translocation and activation of NFκB by degradation of inhibitor of NFκB alpha (IκBα) in a human megakaryoblastic leukemia cell line (7, 8). It is therefore suggested that the up-regulated expression of both NFκB1 and FKBP5 mRNAs in BM CD34+ cells from RA patients might be involved cooperatively in their abnormal responses to TNF-α to differentiate into Type B synoviocyte-like cells.

It has been previously shown that TNF-α enhanced NFκB1 mRNA expression in BM CD34+ cells from healthy individuals (11). However, TNF-α did not enhance FKBP5 mRNA expression in BM CD34+ cells from healthy individuals in the present study. The results indicate that the regulation of FKBP5 mRNA expression is different from that of NFκB1 mRNA expression in BM CD34+ cells. Further studies to explore the mechanism of up-regulation of FKBP5 mRNA would be helpful for delineation of the etiology of RA.

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