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Increased gene expression of integrin β7 in newly diagnosed rheumatoid arthritis patients

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ABSTRACT

Introduction: Integrin and chemokine receptors play an important role in leukocytes migration and recirculation during autoimmune disorders including rheumatoid arthritis (RA). Normally, gut-homing T cells express CCR9 and integrin α4β7 in order to home back from peripheral blood to the intestinal lamina propria. Our study was conducted to evaluate the chemokine receptor CCR9 and the integrin α4β7 gene expression in circulating leukocytes and lymphocytes among newly diagnosed RA patients and to compare these values with healthy individuals.

Methods: In this case-control study, 20 newly diagnosed patients with RA and 20 healthy controls were examined. Peripheral blood samples were acquired from patients and healthy individuals. The total RNA was extracted, then cDNA synthesis was performed. The expression of CCR9 and β7 genes were evaluated by quantitative real-time PCR. The t-test was used to compare gene expression between the groups.

Results: We found that RA patients had a significantly higher level of β7 gene expression compared to controls (P=0.007), while there was no significant difference in CCR9 gene expression between 2 groups (P=0.06).

Conclusion: Our data showed that the expression of β7 gene increases in RA patients and, similar to IBD, β7 gene can be a candidate target for therapy in RA patients.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of synovial joints that often results in joint damage and may lead to cartilage break down and bone destruction (1-3). RA has a variety of extra-articular manifestations including fatigue, lung involvement, vasculitis, pericarditis and hematological abnormalities (2, 4, 5). The synovium is the primary site of inflammation in RA and the accumulation of lymphocytes in the synovial tissue seems to be a critical step in the joint involvement in these patients (6). Synovial inflammation in RA results from infiltrating neutrophils, T cells, B cells, plasma cells, DCs, and macrophages. Inflammatory cells act synergistically with osteoclasts in bone destruction in the synovial membrane (7). Monocytes/macrophages play an important role in inflammation, cartilage destruction and bone erosion in RA. Activated macrophages produce several pro-inflammatory cytokines and chemokines including interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNFα), IL-6, chemokine ligands CXCL8, CCL2, and CCL3. The macrophage infiltration correlates with the radiological progression of joint destruction. Chemokine receptor CCR9 is expressed on T lymphocytes in the small intestine, thymus, lymph node, and spleen. Expression of CCR9 gene has been observed in the cells in the synovium (8). Interaction of CCL25 and CCR9 may contribute to the inflammatory cell migration into the RA synovial tissues (9). Integrins are a family of adhesion molecules that exist as transmembrane heterodimers, consisting of one α- and one β-subunit, that regulate cellular movement. In

mammals, eighteen α and eight β subunits have been identified. These subunits form 24 different integrin heterodimers (7). The a4β7 integrin, that is expressed on the cell surface of a small population of circulating T lymphocytes, involves in the recruitment of leukocytes to the gut. Mucosal addressin-cell adhesion molecule 1(MAdCAM-1) is the major ligand for a4β7 which is selectively expressed on the endothelium of the intestinal vasculature and is present in inflamed tissues (10). The accumulation of leukocytes within the rheumatoid synovium depends on the chemokine receptors and integrins (11, 12). The α4β7 integrin is highly expressed in the gut-homing lymphocytes and the synovium (13). Therefore, this integrin may connect rheumatoid arthritis with the intestinal immune system. It could raise the possibility that impaired gut lymphocyte recirculation and homing of these cells to synovium may precede the clinical onset of rheumatoid arthritis. Normally, in order to home back to the intestinal lamina propria, gut lymphocytes express the chemokine receptor CCR9 and the integrins α4β7. On the other hand, high endothelial venules (HEV) of the lamina propria express MAdCAM-1 and secret chemokine CCL25, which binds α4β7 and CCR9 respectively (14). Regarding the proposed link between Gut-associated lymphoid tissue (GALT) and synovium and presentation of some synovial involvement in IBD patients, it could be interesting to determine if the expression of gut-homing molecules alter in circulating blood leukocytes in RA patients (15, 16). The gut immune system is very extensive and actively communicates with the rest of the body. With this view, we can hypothesize that defect in imprinting gut-homing receptors on T cells may change their circulation pattern and lead to accumulation of these cells in other tissues including synovium. Regarding the important role of β7 and CCR9 in homing and migration of T cells to the gut, the current study was aimed to investigate whether the aberrant expression of β7 and CCR9 in circulation lymphocytes can be considered as factors involved in RA pathogenesis.

Methods

Sample collection

A total of 20 patients who were newly diagnosed with RA based on the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) were recruited in this case-control study. In order to prevent the effects of the therapeutic regimen on study variables, we selected newly diagnosed RA patients. These patients were selected by an expert rheumatologist in order to ensure that all patients were in the initial stage of the disease. Patients were excluded if they were pregnant, used any medications (especially medications that interfere with RA and alter study variables), and had other autoimmune disorders. Based on laboratory results and physician investigation, 20 healthy controls were selected from healthy subjects who matched the RA patients in terms of age and sex. Furthermore, the control group had no history of chronic and autoimmune diseases.

Ethical consideration

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. Participants will be provided with enough information to make an informed decision regarding participation and will be free to withdraw from the experiment at any time without having to provide a reason.

RNA extraction and cDNA synthesis

5 mL of the peripheral blood sample in an EDTA vacutainer was collected from each subject. Total RNA was extracted from fresh whole blood using Roche, high pure RNA isolation kit according to the manufacturer's protocol. The quantity and purity of the RNA samples were assessed by spectrophotometry using the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific NanoDrop) and the RNA integrity was assessed by gel electrophoresis. Total RNA was reversed transcribed using anchored oligo -dT/random hexamer primers (Roche Transcriptor First Strand cDNA Synthesis Kit).

Quantitative Real-Time RT-PCR

The relative gene expression of CCR9 and β7 was measured using real-time PCR. After designing the primers for the gene of interest and reference gene, samples were tested through a BLAST program to ensure specificity of the real-time PCR results and to exclude the possibility of cross-hybridization with other genes or formation of primer dimers. The primers for the reference gene and gene of interest are listed in table 1. The PCR reactions were normalized against GAPDH. We performed real-time PCR with SYBR Green qPCR Master Mix (EURX, Poland) on a Roche Life Science LightCycler® 96 Instrument. PCR reaction was performed in a final volume of 25 µL according to the manufacturer protocol. The thermal cycling conditions of all genes included a temperature profile of preincubation at 50 °C for 2 minutes, followed by incubation at 94 °C for 5 minutes. This protocol was continued by 3 step amplification, including 94 °C for 45s, 58 °C for the 30s, 72 °C for 40s for 40 cycles. A standard curve was constructed for each gene to check

PCR efficiencies.

Statistical analysis

The normality of data was assessed using the Kolmogorov-Smirnov (KS) test. Mann–Whitney U-test/independent sample t-test was used to compare gene expression between groups. Statistical analysis was performed using the statistical package for social sciences (SPSS) software, version 18 (IBM Inc., Chicago, IL, USA). The significant level was considered as $P< 0.05$.

Results

The comparison of variables in patient and control groups was shown in table 2. The patients and control groups were matched in terms of age and sex and there was no statistically significant difference in age and sex between groups ($P>0.05$). In this study, we had 20 patients with the mean age of 44 years old (19 females, 1 male) and 20 healthy subjects with the mean age of 45 years old (19 females, 1 male) with the same situation. We normalized the expression of β7 and CCR9 mRNA relative to the GAPDH as internal control and compared the expression of these genes between RA and healthy groups. Statistical analysis revealed a significant upregulation in the expression of β7 gene in RA patients compared to healthy subjects ($P=0.007$). The relative gene expression of β7 is shown in figure 1. In case of CCR9, we could not find any significant difference in the mRNA expression of CCR9 between RA and healthy groups ($P=0.06$). The relative gene expression of CCR9 is shown in figure 2. The PCR products on a 1.5% agarose gel is shown in figure 3.

Discussion

Various factors have been identified in RA pathogenesis (17). In this study, we focused on factors associated with leukocyte migration. We found that the expression of the β7 gene, which codes a β7 subunit of the α4β7 integrin, significantly increases in the peripheral blood of the newly diagnosed RA patients. Increased expression of β7 gene may lead to overexpression of the α4β7 integrin on the surface of leukocytes in RA patients. Considering the study that showed the presence of α4β7 on lymphocytes within the synovial tissue of RA patients (18), it could be concluded that

Table 1. Forward and reverse primers of genes for real-time PCR amplification

Primer		Sequence	Amplicon size
CCR9	forward	5'- AGCCCAGGACTAACACAAGC -3'	150
	reverse	5'- GGAGGAAATGGCTCGCAAAC - 3'	
β7	forward	5'- TGCAGCTCATCATGGATGCTTA -3'	120
	reverse	5'- CCGTCTTCTCAGGACCCTTACA -3'	
GAPDH	forward	5'-CTTCCAGGAGCGAGATCCCT-3'	107
	reverse	5'-AATGAGCCCCAGCCTTCTC-3'	

Table 2. Demographic characteristics of RA patients and HS

Characteristics	RA n=20	HC n=20	P value
Mean age (year)	44±3	45±3	0.230
Sex (F/M)	19/1	19/1	0.541
β7	5	4.1	0.007
CCR9	0.81	0.83	0.06

HC: healthy contro

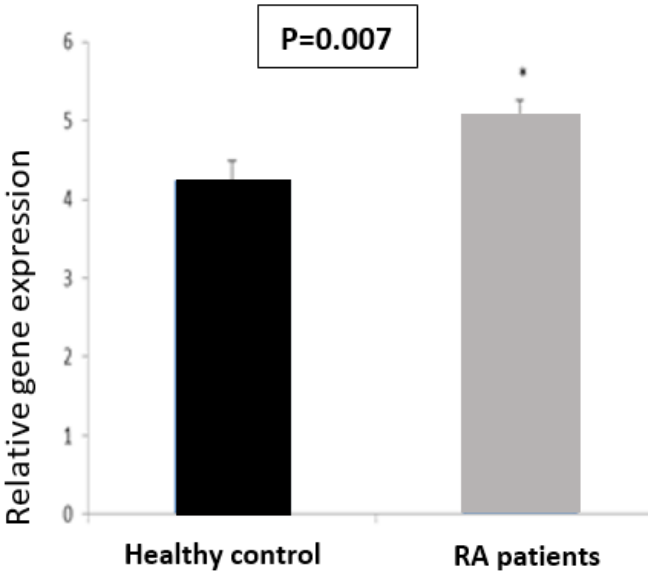

Figure 1. Expression of β7 mRNA in RA and healthy control

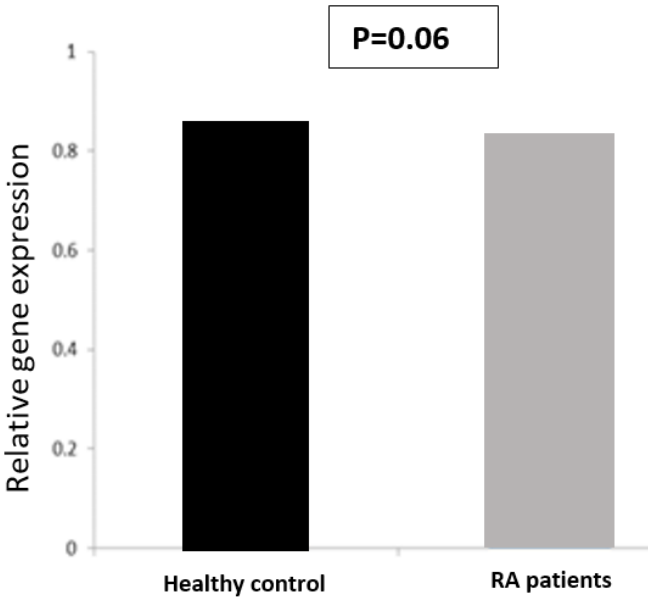

Figure 2. Expression of CCR9 mRNA in RA and healthy control

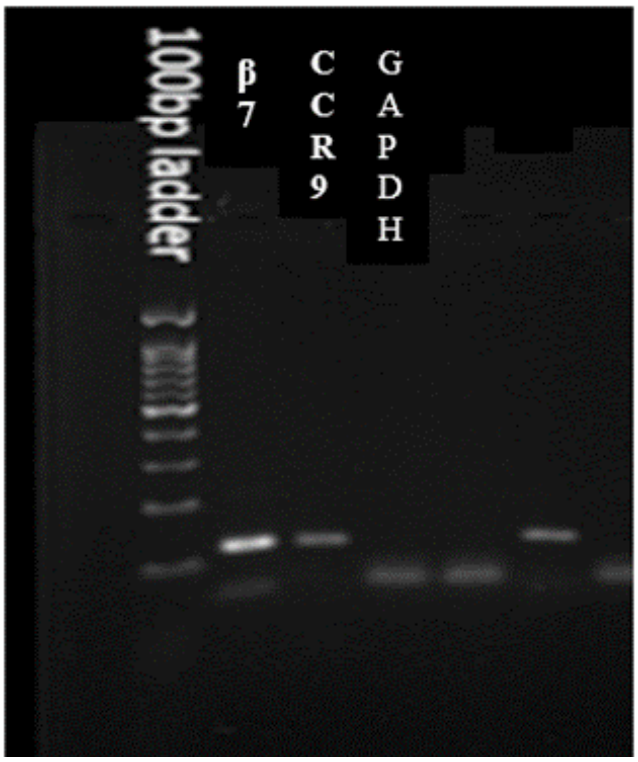

Figure 3. Gel electrophoresis of PCR products on a 1.5% a

the integrin α4β7 may contribute to the adhesion of lymphocytes to synovial HEV and induce the accumulation of these cells at this site. The study that was performed on the inflammatory bowel disease (IBD) intensified the possible significance of α4β7 in the pathogenesis of RA. It was appreciated that patients with inflammatory bowel disease (IBD) have a higher incidence of arthritis (16, 19). Considerably, a recent large-scale population study showed the significant association between IBD and RA which may suggest that IBD and RA share similar etiopathogenesis (20). Vedolizumab, a novel anti-integrin agent which is used for the treatment of IBD, targets the α4β7 molecule (21). In our study, the expression of the β7 gene, which codes a β7 subunit of α4β7, increased in the circulating peripheral blood leukocyte of RA patients. Regarding the joint involvement associated with IBD and similar etiopathogenesis with RA (20, 22), the increased gene expression of the β7 integrin may also play a role in the pathogenesis of RA and, as for IBD, it could be a potential target for biologic agents including vedolizumab. On the other hand, the expression of CCR9 was not significantly different between the two groups; however, an increase in the expression of CCR9 was shown in RA patients in a previous study. In one study, a high CCR9 expression was reported on monocytes/macrophages (23). Park et al. indicated that lower expression of CCR9 attenuated migration abilities in the intestinal immune system (24). The results of another study showed that CCL25/CCR9 interactions regulate inflammatory immune responses in the large intestinal mucosa by balancing different subsets of dendritic cells. These findings implicate the use of CCR9-inhibitors in the therapy of human IBD as they indicate a potential risk for patients with large intestinal inflammation (25). We can also use these same features to treat other autoimmune diseases. We should have more robust methods to examine the expression of the CCR9 and β7 integrin genes and their resultant proteins. Finally, one of the limitations of this

study was that our sample size was small due to the small number of new cases of RA patient without no medication history and also due to the ethical considerations we could not access synovial fluid samples.

Conclusion

In general, although the β7 gene expression was higher among RA patients compared to the control group, there was no significant difference in CCR9 gene expression of in RA patients compared to the control group. According to the reasons that were discussed before on the IBD disease, these two molecules can be used for therapeutic purposes in RA. We can declare that the increased level of β7 expression has a role in the pathogenesis of RA and can be a candidate target for biologic therapy in RA patients.

Ethical disclosure

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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Author contributions

All authors are individually responsible for the content and writing of the paper.

Conflict of interest

The authors declare no conflicts of interest.

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