ORIGINAL ARTICLE

Decreased Expression of Integrins by Hematopoietic Cells in Patients With Rheumatoid Arthritis and Anemia: Relationship With Bone Marrow Cytokine Levels

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Abstract

Background and Objectives: In order to gain a better insight into the pathogenesis of the anemia of chronic disease (ACD) accompanying rheumatoid arthritis, we analyzed the density of the integrins very late antigen (VLA) 4 and VLA-5 on the surface of erythroblasts from bone marrow in patients with rheumatoid arthritis. We also measured the concentration of interleukin (IL) 3 and tumor necrosis factor (TNF) in bone marrow. Finally, we analyzed the relationship between integrin expression on hematopoietic cells and the degree of anemia and concentration of cytokines in bone marrow in patients with rheumatoid arthritis.
Results: Patients with rheumatoid arthritis who also had ACD were found to have lower hemoglobin levels and higher C-reactive protein and erythrocyte sedimentation rate compared to patients who had rheumatoid arthritis without ACD or osteoarthritis of the hip. The mean bone marrow concentration of IL-3 was elevated in patients with rheumatoid arthritis and ACD compared to those without ACD or patients with osteoarthritis. IL-3 concentration in bone marrow showed a significant negative correlation with VLA-4 and VLA-5 expression on erythroblasts, but only in patients with rheumatoid arthritis and ACD.
Conclusion: Patients with rheumatoid arthritis and ACD have abnormal erythroblasts (decreased VLA density), possibly through an effect on early stages of erythroblast development. Increased levels of IL-3 and the negative correlation between IL-3 concentration in bone marrow and expression of the integrins VLA-4 and VLA-5 may suggest positive feedback between erythroblasts and IL-3, probably associated with decreased sensitivity of bone marrow erythroblasts to IL-3.

Key words: Rheumatoid arthritis. Anemia of chronic disease. Interleukin-3. Integrins. Bone marrow.

Resumen

Antecedentes y objetivos: Para adquirir un mayor conocimiento de la patogenia de la anemia de enfermedades crónicas (AEC) que acompaña a la artritis reumatoide, se analizó la densidad de las integrinas VLA (antígeno de expresión muy tardía) 4 y VLA 5 de la superficie de los eritroblastos de la médula ósea, en pacientes con artritis reumatoide. También se midió la concentración de interleucina (IL) 3 y el factor de necrosis tumoral (FNT) en la médula ósea. Por último, se analizó la relación entre la expresión de la integrinas en las células hematopoyéticas y el grado de anemia, y la concentración de citocinas en la médula ósea, en pacientes con artritis reumatoide.
Resultados: Se observó que los pacientes con artritis reumatoide que también presentaban anemia de las enfermedades crónicas tenían una concentración de hemoglobina inferior y unas concentraciones de proteína C reactiva y una velocidad de sedimentación globular superiores a la de los pacientes con artritis reumatoide sin anemia de las enfermedades crónicas o con coxartrosis. La concentración media de IL-3 en la médula ósea era elevada en pacientes con artritis reumatoide y anemia de las enfermedades crónicas, comparada con la de aquellos sin anemia de las enfermedades crónicas o pacientes con artrosis. Esta concentración media también mostró una correlación

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negativa significativa con la expresión de la VLA-4 y la VLA-5 en los eritroblastos, pero sólo en aquellos pacientes con artritis reumatoide y anemia de las enfermedades crónicas.
Conclusión: Los pacientes con artritis reumatoide y anemia de las enfermedades crónicas tienen unos eritroblastos anómalos (densidad reducida de VLA), posiblemente debido a una alteración en las primeras fases de desarrollo de los eritroblastos. Una mayor concentración de IL-3 y la correlación negativa entre la concentración de IL-3 en la médula ósea y la expresión de las integrinas VLA-4 y la VLA-5, pueden sugerir una retroalimentación positiva entre los eritroblastos y la IL-3 que probablemente esté asociada con la disminución de la sensibilidad de los eritroblastos de la médula ósea a la IL-3.

Palabras clave: Artritis reumatoide. Anemia de las enfermedades crónicas. Interleucina-3. Integrinas. Médula ósea.

Introduction

Rheumatoid arthritis is the most common chronic, autoimmune, inflammatory disease of the synovium. It is not only a disease of the joints, however. It is characterized by a number of extra-articular effects, including malaise, fever, weight loss, lymphadenopathy, hepatosplenomegaly, and abnormalities of the skin (rheumatoid nodules, vasculitis, chronic leg ulcers), nerves (carpal tunnel syndrome, peripheral neuropathy), eyes (episcleritis, keratoconjuctivitis sicca), and chest (pericarditis, pleural effusion, chest infection, Caplan syndrome) [1]. The disease is also associated with blood abnormalities such as anemia, neutropenia, and hypergammaglobulinemia [1,2].

Anemia seems to be the most pronounced feature of rheumatoid arthritis since it is present in the majority of patients. The peculiar type of anemia accompanying the disease is known as anemia of chronic diseases (ACD) [3]. Its pathogenesis still remains uncertain, although a few contributing mechanisms have been identified [4-7].

The differentiation of hematopoietic cells depends upon the interaction of stem cells with stromal cells and extracellular matrix in the bone marrow [8]. Bone marrow from patients with rheumatoid arthritis and ACD is characterized by a lower percentage of erythroblasts at each step of development, a low number of sideroblasts (some of which are ring shaped), and a high number of siderocytes, along with infiltration of immunocompetent cells including plasmocytes, macrophages with cytoplasmic granules, and activated T lymphocytes [9].

It has been observed previously that integrin expression disturbances may lead to leukemia, myelodysplastic syndrome, and graft rejection [8,10-13]. Therefore, in order to gain a better insight into the pathogenesis of ACD accompanying rheumatoid arthritis we analyzed the density of integrins (very late antigen [VLA] 4 and VLA-5) on the surface of erythroblasts derived from the bone marrow of patients with rheumatoid arthritis. We also measured the bone marrow levels of interleukin (IL) 3 and plasma levels of tumor necrosis factor (TNF) . Finally, we assessed the relationship between integrin expression on hematopoietic cells and the degree of anemia and concentration of cytokines in bone marrow from patients with rheumatoid arthritis.

Methods

Patients

Twenty-eight patients fulfilling the 1987 criteria of the American Rheumatism Association [14] and undergoing hip surgery were included in the study. ACD was diagnosed in 16 patients with rheumatoid arthritis. Twelve patients with osteoarthritis of the hip also undergoing hip surgery were assigned as a control group. Bone marrow samples were taken during surgery after obtaining prior informed consent from the patients. Standard medical examination was performed in all patients. All patients received the standard treatment (15-22.5 mg/wk methotrexate and 6-10 mg prednisone daily) for at least 3 months before bone marrow extraction.

Laboratory Analysis

Integrin expression was analyzed by flow cytometry (FACStar Becton-Dickinson, San Jose, USA). The following monoclonal

Table 1. Clinical Parameters of Patients With Rheumatoid Arthritis, With or Without Anemia of Chronic Disease, and Patients With Osteoarthritis[a]

	Rheumatoid Arthritis With ACD	Rheumatoid Arthritis Without ACD	Osteoarthritis
Hemoglobin, g/L	10.7 (0.7)	13.0 (0.67)[b]	13.2 (0.97)[b]
CRP, mg/L	29.0 (21.0)	12.4 (7.9)[c]	5.8 (6.1)[b]
ESR, mm/h	47.4 (25.4)	19.1 (16.3)[d]	12.5 (9.4)[b]

Abbreviations: ACD, anemia of chronic disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
[a]Data are shown as means (SD).
[b]$P < .001$ compared with rheumatoid arthritis patients with ACD.
[c]$P = .007$ compared with rheumatoid arthritis patients with ACD.
[d]$P = .002$ compared with rheumatoid arthritis patients with ACD.

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Table 2. Cytokine Levels and Integrin Expression in Bone Marrow Taken From Patients With Rheumatoid Arthritis, With or Without Anemia of Chronic Disease, and Patients With Osteoarthritis[a]

	Rheumatoid Arthritis With ACD	Rheumatoid Arthritis Without ACD	Osteoarthritis
VLA-4, receptor density, FU	384 (133)	472 (110)	460 (101)
VLA-5, receptor density, FU	416 (122)	492 (186)[b]	511 (123)[c]
TNF- , pg/mL	382 (979)	101 (132)	43 (42)
IL-3, ng/mL	384 (581)	195 (106)[b]	173 (122)[c]

Abbreviations: ACD, anemia of chronic disease; VLA, very late antigen; FU, fluorescence units; TNF, tumor necrosis factor; IL, interleukin.
[a]Data are shown as means (SD).
[b]$P < .01$ compared with rheumatoid arthritis patients with ACD.
[c]$P < .01$ compared with rheumatoid arthritis patients with ACD.

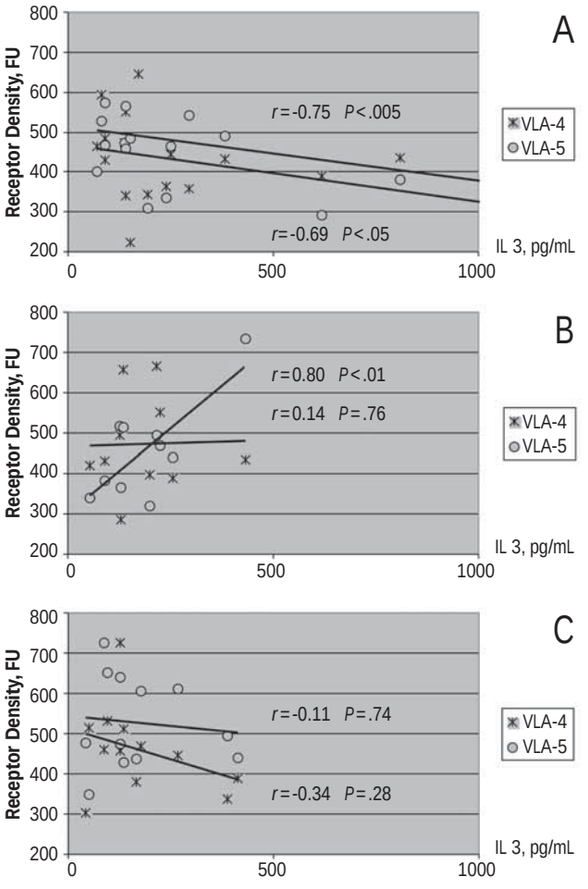

Figure. Correlation between interleukin (IL) 3 levels in bone marrow and density of the integrins very late antigen (VLA) 4 and VLA-5 density on bone marrow erythroblasts derived from rheumatoid arthritis patients with anemia (A), without anemia (B), and patients with osteoarthritis (C). Receptor density is shown as mean fluorescence units (FU).

antibodies were used for staining: anti-HPCA-2(CD34)/PerCP and anti-Leu-45RO(CD45RO)/FITC for hematopoietic cells, and anti-VLA-5(CD49e)/PE and anti-VLA-4(CD49d)/PE for integrins. Cytokines (TNF- and IL-3) were analyzed by enzyme-linked immunosorbent assay using standard methods [15,16].

Statistical Analysis

Data from patients in the different study groups were compared by Mann-Whitney test. The relationship between the variables was examined using Pearson's correlation coefficient for continuous variables. Values of *P* less than .05 were considered statistically significant.

Results

Analysis of clinical parameters showed that patients with ACD accompanying rheumatoid arthritis had lower hemoglobin levels, and higher C-reactive protein levels and erythrocyte sedimentation rate than patients who had rheumatoid arthritis without ACD or patients with osteoarthritis (Table 1). The differences between rheumatoid arthritis patients without ACD and osteoarthritis patients were not found to be statistically significant.

The density of VLA-4 and VLA-5 was found to be lower in patients with rheumatoid arthritis and ACD compared to rheumatoid arthritis patients without ACD and osteoarthritis patients (Table 2). However, only the changes in VLA-5 were found to be statistically significant. The mean bone marrow level of IL-3 was elevated in rheumatoid arthritis patients with ACD compared to those without ACD and patients with osteoarthritis. There was no correlation between hemoglobin level and the density of VLA-4 and VLA-5 or the cytokines analyzed in our group of patients.

There was a positive correlation between the density of VLA-4 and VLA-5 ($P < .05$, $r = 0.66$) in patients with ACD accompanying rheumatoid arthritis. A positive correlation was also found between TNF- and IL-3 ($P < .05$, $r = 0.76$) and between TNF- and VLA-5 ($P < .05$, $r = 0.73$). Moreover, IL-3 levels displayed a significant negative correlation with VLA-4 and VLA-5 (Figure), but only in the group of rheumatoid arthritis patients with ACD.

Discussion

Rheumatoid arthritis, like all autoimmune diseases, is characterized by a number of immunological and genetic

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changes. The association with *HLA DRB1* gene polymorphisms and the so-called shared epitope is well described [17,18]. Moreover, rheumatoid arthritis is considered a polygenic disease and cytokines are thought to play a crucial role in the pathogenesis of the disease; consistent with this, a number of single-nucleotide polymorphisms have been described in genes encoding cytokines [19-23]. However, in addition to pathogenic effects, both proinflammatory and anti-inflammatory cytokines are involved in bone marrow differentiation and formation. Although the origin of ACD would appear to be strictly determined by factors promoting rheumatoid arthritis, we still do not know the rules governing the process.

We observed decreased expression of VLA-4 and VLA-5 by CD34-positive hematopoietic cells in patients with anemia accompanying rheumatoid arthritis. This diminished expression of integrins may affect the attachment of hematopoietic cells to stromal cells and the extracellular matrix, and disturb maturation and differentiation of those cells.

Based on the data obtained in this study, we conclude that patients with ACD accompanying rheumatoid arthritis have abnormal erythroblasts (decreased VLA density) and that early stages of erythroblast development may be primarily affected. Increased levels of IL-3 in the bone marrow and a negative correlation between IL-3 and integrin expression may suggest positive feedback between erythroblasts and IL-3, probably associated with decreased sensitivity of bone marrow erythroblasts to IL-3.

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Manuscript received March 1, 2007; accepted for publication April 30, 2007.

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