Microheterogeneity of alpha 1-acid glycoprotein in patients with rheumatoid arthritis treated with methotrexate

The use of methotrexate (MTX) in the treatment of rheumatoid arthritis (RA) has been shown in many studies. The mode of action by which MTX suppresses disease activity and causes some of the beneficial effects associated with MTX is still unknown. Improvement during MTX therapy appears after a few weeks after the beginning of the treatment, and symptoms of the disease recur very quickly after cessation of the treatment. An anti-inflammatory mechanism of MTX action therefore is likely.

In our study we evaluated microheterogeneity of alpha 1-acid glycoprotein (AGP) in patients with RA treated with MTX. The initial study population comprised 35 patients (27 women, eight men) who met the RA criteria as defined by the ARA. The patients were aged 29-63 [mean(SD) 47-14(5)] years. The disease duration ranged from one to 24 years [mean(SD) 9-8(6-1)] years. All were treated with methotrexate (5-15 mg weekly) given in a single oral dose. Most of the patients, additionally took non-steroidal anti-inflammatory drugs (NSAIDs). The control population comprised 17 healthy subjects (seven women, 10 men), aged 22-45 years [mean(SD) of 31-6(4-6)] years. A second control population consisted of 31 RA patients with RA (24 women and seven men) treated with NSAIDs only [mean(SD) age 51-7(12-4) years, mean(SD) disease duration 11-8(9-9) years]. Disease activity was performed according to the method proposed by Malya and Macz. Patients with intercurrent infections or other severe illnesses were excluded from the study. AGP microheterogeneity was performed by crossed affinoimmunoelectrophoresis with Con-A as a ligand. The data were expressed as a reactivity coefficient (RC), which was calculated by dividing the sum of all Con-A reactive variants by a non-reactive one. The results were analysed using the Wilcoxon signed rank test.

In the majority of the patients, disease activity improved after six months of treatment with MTX. The number of painful joints* is still unchanged, however, this is likely due to the occurrence of methotrexate pneumonitis in rheumatoid arthritis. Arthritis Rheum 1990; 33 (suppl 9): S40.

Augmented expression of inflammatory cytokines and adhesion molecules in accelerated nodulosis during methotrexate therapy

The development of subcutaneous nodulosis during methotrexate (MTX) therapy has recently been reported by several investigators. The majority of the patients respond to MTX, and their disease activity is usually low when nodules, lupus-like plaques, or cutaneous vasculitis such as nailfold thrombi. The majority of patients respond to MTX, and their disease activity is usually low when nodules, lupus-like plaques, or cutaneous vasculitis such as nailfold thrombi. The majority of patients respond to MTX, and their disease activity is usually low when nodules, lupus-like plaques, or cutaneous vasculitis such as nailfold thrombi. It is well established that AGP-RC is decreased in patients with RA. Conversely, the low level of AGP-RC has been observed not only in RA, but also in chronic juvenile arthritis. The presence of AGP-RC in chronic inflammatory diseases is not clear whether AGP-RC may be affected by the disease activity. Preliminary studies showed that cyclophosphamide treatment does not modify AGP-RC in patients with RA. Therefore, the influence of MTX on AGP glycosylation should be considered.
The patient, a 62-year-old Japanese woman, had been suffering from RA since 1987. She was treated by gold thiomalate, bucillamine and salazopyrine, without dramatic response. There were no rheumatoid nodules or signs of cutaneous vasculitis at that time. Laboratory data revealed an erythrocyte sedimentation rate of 54 mm/h (Westergren), C-reactive protein of 5.4 mg/dl and positive rheumatoid factor (1:640 in RAHA test). Low dose corticosteroid therapy was started, and the response was moderate. MTX therapy was initiated in 1989, and the patient eventually responded to a weekly dose of 7.5 mg. Eighteen months after starting MTX, multiple painful nodules ranging from 5-15 mm in diameter gradually appeared and increased in number in the digits and soles. An infarcted area was observed in the centre of the nodules. Nailfold thrombi and periungal erythema were both present. However, no signs of visceral vasculitis or Raynaud’s phenomenon were observed. The biopsy specimen demonstrated small vessel obstruction as a result of fibrin thrombi and perivascular infiltration of neutrophils, lymphocytes and histiocytes, compatible with leukoclastic vasculitis. Granulomatous lesions with palisading layer were also observed. Immunohistochemical analysis revealed that ICAM-1 was strongly expressed in vascular endothelial cells of the vessels with pathological features of leukoclastic vasculitis (fig 1A). Some of the mononuclear cells around the vessels also reacted with anti-ICAM-1 antibody. Both VCAM-1 and ELAM-1 were faintly but exclusively expressed in the endothelial cells. IL-1, IL-6 and TNFα were identified in endothelial cells and cells in the palisading layer. Reverse-transcription polymerase chain reaction (RT-PCR) using primers for either inflammatory cytokines and adhesion molecules revealed that there was strong ICAM-1 mRNA expression together with upregulated expression of TNFα and IL-6 in the sample (fig 1B). IL-1 mRNA expression was extremely low. Both VCAM-1 and ELAM-1 mRNAs were more weakly expressed than that of ICAM-1.

These data clearly indicated that MTX-induced nodulosis originated from inflammatory vascular lesions. We have already reported that significant amounts of IL-1 and TNFα were produced from rheumatoid nodules. In this sense, the pathophysiology of rheumatoid nodules and those induced by MTX may be quite similar. Augmented production of inflammatory cytokines in situ can cause upregulation of ICAM-1 on vascular endothelial cells, resulting in further recruitment of mononuclear cells, although an initial event that triggers endothelial cell injury remains to be clarified in MTX-induced nodulosis. At present we do not know how MTX induces nodulosis, as MTX itself did not directly stimulate the expression of either inflammatory cytokines or adhesion molecules of vascular endothelial cells and synovial cells in vitro in our preliminary experiments (data not shown). It is obvious, however, that MTX-induced nodulosis is a dose-dependent phenomenon.  

Sensitivity to MTX-induced nodulosis might be related to genetic factors, as most of the reported cases have had HLA-DR. Further study is necessary to elucidate the pathogenesis.

NOBUYUKI MIYASAKA  
ICHIRO SAITO  
Division of Immunological Diseases, Department of Virology and Immunology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan  
TOMOKO UEUMURA  
SADAO KASHIWAZAKI  
Institute for Rheumatology, Tokyo Women’s Medical College, Tokyo, Japan

Correspondence to: Dr Nobuyuki Miyasaka, Division of Immunological Diseases, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo, Japan 113.


Effect of milk product deprivation on spondyloarthropathy

Certain faecal gram negative bacteria have been incriminated as provocative agents in the pathogenesis of ankylosing spondylitis and related spondyloarthropathies (SA). Despite ultra high temperature, milk and milk products might still contain bacterial fragments which could play an allergenic or activating role on the immune system. An attempt was therefore made to show whether in SA a diet where patients were told to exclude milk, cheese, yoghurt, ice cream and butter, could exert a beneficial effect on the course of the disease.

Twenty-five patients with SA and 10 with RA according to the ESSG and ACR criteria were enrolled in this study (they were paired for sex, age, duration of disease).

All patients complained of morning stiffness over 30 minutes, inflammatory low back pain and/or multiple joint swelling (at least one joint in SA).

Eighteen of 25 patients with SA and seven of 10 with RA also needed nonsteroidal anti-inflammatory drugs (NSAIDs). Some patients (7/25 SA and 4/10 RA) were receiving other disease modifying drugs for more than six months. At each visit (after six weeks, three, six and nine months) the patients were asked the following questions about changes (worse, unchanged, better by comparison with the enrollment visit):

1) How do you feel?
2) What is the severity of your pain?
3) What is the duration of your morning stiffness?

(A) Immunohistochemical analysis revealed that vascular endothelial cells were highly reactive with anti-ICAM-1 antibody. (B) RT-PCR analysis showed a strong expression of IL-6 and ICAM-1 mRNAs, and a weak expression of TNFα, VCAM-1 and ELAM-1 (E-selectin) mRNAs in a biopsy specimen of MTX-induced nodule.