Does the anti-herpesviral effect of leflunomide play a role in the treatment of rheumatoid arthritis?

Sirs,

Human herpes viruses, including Epstein-Barr virus (EBV) and Cytomegalo virus (CMV) are candidate triggers for RA (1-4). Leflunomide exerts antiviral effect *in vitro* and *in vivo* against CMV and possibly against other herpes viruses (5-7). The aim of this study was to assess the effect of leflunomide on peripheral blood EBV and CMV viral load in RA patients and to analyse whether prevalence of CMV or EBV DNA in whole blood (WB) predicts treatment responsiveness to leflunomide.

Twenty-two RA patients, to whom leflunomide was started *de novo* as part of routine treatment, were recruited to this observational study. Patients were eligible if they had active disease defined as four or more tender joints, four or more swollen joints and CRP ≥10mg/l or ESR ≥15mm/h. The dose of leflunomide was 20mg daily, started without loading. If leflunomide was administered in combination with other disease modifying antirheumatic drugs (DMARDs) changes to the concomitant therapy with DMARDs and/or oral steroids could be made whenever considered appropriate. WB samples collected at 0 and 6 months were analysed by quantitative PCR for DNA prevalence and viral load of CMV and EBV (8). Disease activity was assessed using validated DAS28 (ESR).

The patients were predominantly female (82%) with a median (range) age of 61 years (26 to 78 years) and a median (range) disease duration of 11 years (0.4 to 32 years). Twenty out of twenty-two (91%) of the patients were rheumatoid factor or anti-CCPAb positive. The mean (SD) DAS28 score was 5.4 (0.8) at baseline. All but one patient were given leflunomide in combination with one or more concomitant DMARDs at baseline. Twelve patients (55%) used leflunomide in combination with methotrexate and 14 (64%) received concomitant prednisone. A total of 14 patients (64%) withdrew from leflunomide before the 6-month visit due to adverse drug reaction. At the time of withdrawal the patients had been taking leflunomide for a median (range) of 2.3 months (0.2 to 5 months).

EBV DNA was detected in 15/22 of the baseline WB samples. In these 15 patients (including 10 patients who withdrew from leflunomide before study termination) EBV viral load (median, IQR) declined from 2.69 (2.10–3.41) log copies/ml to 2.51 (0–3.05) at six months, p=0.2. Clearance of EBV DNAemia was observed in four cases. These 4 patients did not differ from the other 11 in terms of adherence to leflunomide or use of methotrexate or prednisone. Significant response (DAS28 change ≥1.2) was observed in 10/15 of the EBV DNA-positive *vs*. 3/7 EBV DNA-negative patients, p=0.38 (Fig. 1). CMV DNA was not found in any of the patient samples.

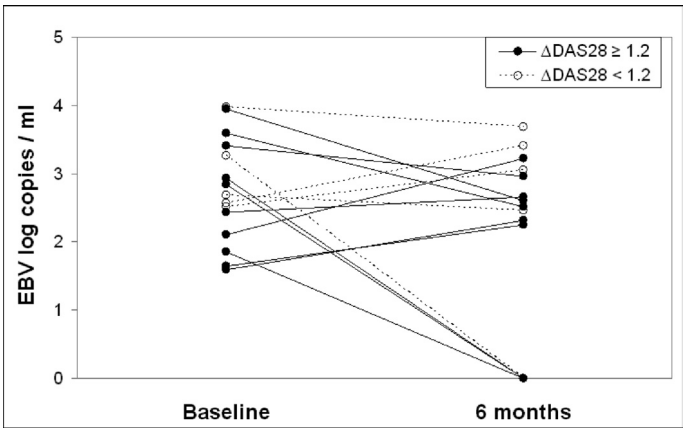

Fig. 1. EBV viral load at baseline and at six months in 15 RA patients treated with leflunomide.

Undetectable CMV viral load in WB contrasts to the result in a prior study in which CMV DNA was found in PBMCs in 25 per cent of RA patients (1). Although latent (intracellular) CMV infection is common in healthy persons who have previously been exposed to the virus, the proportion of infected cells is so low that it is often undetectable even when sensitive DNA amplification methods are used (9). In line with previous reports (1, 10) EBV DNA was detected in 68% of our patients. The EBV load in these 15 patients was relatively stable during the 6-month follow-up period and was not affected by leflunomide therapy. Only 5 out of the 15 patients that were EBV-PCR-positive at baseline completed the entire 6 months of leflunomide therapy, 13 out of these 15, however, took leflunomide for at least 2 months. In the study by John *et al*. clearance of CMV DNAemia took place in the median of 1.5 months after the start of leflunomide (7). Thus, it can be speculated that if leflunomide would exert antiviral effect on EBV *in vivo*, our study would probably have been able to demonstrate it.

In the present study, no association was observed with EBV DNA prevalence in WB and response to leflunomide. Interestingly, the presence of EBV genome in the bone marrow of RA patients was shown to be associated with clinical response to anti-CD20 treatment (11). In the clinical setting it is more practicable to measure EBV DNA in WB samples compared to bone marrow. Thus, it would be worthwhile to study if EBV DNA load in WB could serve as a biomarker to treatment responsiveness to B-cell depleting therapy in RA.

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