Withhold or discontinue AZA/MTX had been made at consultant level, despite previous reports that transaminase elevations may be found in myositis [2–4] and other muscle diseases [4–6]. Transaminases are important intracellular components of metabolic function in many cells, including those of muscle, and elevated ALT levels may reflect transaminase leakage into the bloodstream, due to myofibrillar damage, in a manner analogous to lactate dehydrogenase and creatine phosphokinase (CPK) leakage [7]. The purpose of this ethically approved study was to examine the relationship between serum ALT and CPK levels in myositis patients, in order to establish whether ALT levels could be predicted from CPK levels.

From the Salford adult-onset myositis (disease onset after 18 yr of age) database, 61 patients were identified with probable or definite myositis, according to Bohan and Peter criteria [8, 9]. Of these patients, 18 had DM, 22 PM and 21 PM as part of a connective tissue disease overlap. In a retrospective analysis of these 61 patients’ data, 208 occasions were identified when their ALT, CPK and alkaline phosphatase (ALP) levels were measured together in the same laboratory, and where all the results were available. As this study was retrospective, the results of other hepatic transaminases or γ-glutamyl transferase were not available, so ALP was used instead as a surrogate ‘other’ marker of hepatic function. Correlations between the log-converted values of ALT, CPK and ALP were made using linear regression analysis and scatter-graph plots were constructed. The results demonstrated a strong correlation between serum CPK and ALT (r coefficient = 0.78, R² = 0.59, P < 0.0001; Fig. 1), but no correlations between ALP and ALT (r = 0.04, P = 0.7), or between ALP and CPK (r = -0.1, P = 0.5). An equation of the line can be derived from the Fig. 1, describing the correlation between log CPK/ALT values \( \log_{\text{CPK}} = 1.47 \times (\log_{\text{ALT}} + 0.18) \), and allows prediction of ALT levels from measured CPK levels. For instance, a myositis-induced CPK rise to double the upper normal limit, i.e. to 390 U/l (log value 2.59), would be associated with an ALT level still just within the laboratory normal upper limit, of 50 U/l (log value 1.7), while a myositis-induced ALT rise to 100 U/l would not be expected until CPK levels had risen to around 1000 U/l.

That not all rheumatologists appreciate that ALT rises occur in active myositis was the stimulus for this brief study. The knowledge that CPK and ALT correlate so well and that ALT levels can be predicted from CPK levels should reassure those treating myositis patients that ALT elevations are probably of muscle origin, and remind that elevated ALT values do not necessarily require exhaustive or invasive investigations. As ALP does not rise despite obvious ALT rises should further reassure. If, following the initiation of corticosteroids in new-onset disease, CPK and ALT levels remain elevated but their log values correlate in a fashion similar to that seen in the Fig. 1, and ALP is normal, then it appears safe to add AZA/MTX if required. Similarly, during disease relapses requiring AZA/MTX dose increments, these appear safe if the log CPK/ALT values clearly correlate and ALP remains normal. Rigorous monitoring would, of course, still be required. Three of the tertiary patients stimulating this study were from hospitals routinely measuring transaminases other than ALT, e.g. aspartate aminotransaminase (AST). A formal correlation between AST and CPK was not possible due to the small patient numbers and the fact that AST had been measured in three laboratories, but high AST levels were also noted when CPK levels were high, in keeping with previous reports [3, 4]. If transaminase levels do not fall as CPK levels fall with treatment, or if ALT levels are inappropriately high for the measured CPK in stable disease, investigations of hepatic function should then be prompted.

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including rheumatoid arthritis (RA) [1]. RAGE binding of ligands up-regulated in RA synovial tissue, fluid and serum can lead to increased cell activation, including migration, hyperplasia and increased cytokine production. Several animal studies have described a possible role for RAGE in the onset and severity of arthritis. In these animal studies, blockade of the receptor showed suppression of arthritis, while administration of RAGE ligands induced arthritis in healthy mice [2,3]. In addition, increased levels of RAGE ligands have been found in RA patients and correlate with disease severity [4–6].

Previous studies have indicated that a gain-of-function mutation of RAGE correlates with RA. Linkage of RAGE with the HLA-DRB1-DQ region, a region known to associate with RA susceptibility and severity, could account for this correlation. To dissect the possible confounding effects of the HLA-DRB1 region in the possible association of RAGE with RA, we investigated the correlation of a gain-of-function mutation in RAGE to HLA-DRB1 alleles and RA.

Three hundred and seventy-seven consecutive RA patients of the Leiden Early Arthritis Cohort, an inception cohort for patients with recent-onset arthritis [7] (mean ± s.d. age 48 ± 17 yr, 55% female) and 535 non-RA controls of the same cohort (57 ± 16 yr, 67% female) were included in the analysis (Table 1). All RA patients fulfilled the 1987 criteria of the American College of Rheumatology. The study was approved by the local ethics committee and written informed consent was obtained from all patients and controls according to the Declaration of Helsinki. HLA genotyping was available for all patients and controls. In addition, to type the participants for the RAGE G82S polymorphism we used PCR followed by overnight digestion with Alu I. The 82S polymorphism results in the formation of an extra Alu I restriction site.

Hofmann et al. [2] reported that the RAGE 82S polymorphism correlated with susceptibility to RA. However, RAGE is in strong linkage disequilibrium with HLA-DR4 [8], in particular HLA alleles encoding a common shared epitope within the HLA-DRB1 allele. In the population studied by Hofmann et al., linkage was found with DR1*0401, and after correction for this allele the correlation between the RAGE 82S polymorphism and susceptibility to RA was lost. These data were not conclusive, since the numbers of patients and controls positive for the RAGE 82S polymorphism were very low (5 out of 95 and 2 out of 134, respectively). Here, we identified 46 out of 377 RA patients and 36 out of 535 controls harbouring the RAGE 82S polymorphism. We found an association between the RAGE 82S polymorphism and RA without correction for HLA alleles. However, in patients, RAGE 82S was in linkage (defined as $P < 0.01$) with DRB1*0401 [odds ratio (OR) 6.5, $P < 0.0001$]. In controls RAGE 82S was in linkage with DRB1*0401 (OR 4.43, $P < 0.00001$) and 0901 (OR 5.05, $P = 0.002$). Correction for the presence or absence of these HLA alleles was done by the Svejgaard method [9]. When the association of RAGE 82S with RA was corrected for the absence or presence of DRB1*0901, the association between RAGE and RA remained present. Conversely, after correction for the presence/absence of HLA DRB1*0401, the association between RAGE 82S and RA was lost (Table 2), indicating that RAGE is not associated with RA independently of HLA DRB1*0401 in this cohort. Also in logistic regression

Table 1. Frequencies of patients and controls positive or negative for RAGE 82S or HLA-DRB1*0401

<table>
<thead>
<tr>
<th>RAGE 82S</th>
<th>DRB1*0401</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>78</td>
<td>60</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>253</td>
<td>439</td>
</tr>
</tbody>
</table>

Table 2. Statistical calculation by the method described by Svejgaard et al. [9]

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Entries of 2 × 2 table</th>
<th>Test</th>
<th>OR (CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAGE 82S vs RAGE 82S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>1.99 (1.19–3.21)</td>
<td>0.0095</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>2.47 (1.26–4.84)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>3.18 (1.57–6.43)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>1.08 (0.45–2.57)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>2.26 (1.53–3.22)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>11.68 (5.28–26.24)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>DRB1<em>0401+ vs DRB1</em>0401+</td>
<td>82.0401+ vs 82.0401+</td>
<td>9.15 (4.26–19.74)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Association between RAGE and DRB401 in patients

Association between RAGE and DRB401 in controls

Association between RAGE and DRB401 in controls

CI, confidence interval.
analysis with DRB1*0401 and RAGE as possible explanatory variables and the presence of RA as dependent variable, only DRB1*0401 was independently associated with RA (OR 2.5, \( P < 0.001 \)).

In conclusion, considering the size of our study, it is unlikely that the RAGE 82S polymorphism is associated with RA independently of HLA DRB1*0401. However, although this alternation in the receptor itself does not seem to play an important role in RA pathology, the correlation between RA severity and levels of RAGE ligands could still indicate that RAGE ligands do play an important role in RA pathology.

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Combination of immunoadsorption and CD20 antibody therapy in a patient with mixed connective tissue disease

Sirs, Mixed connective tissue disease (MCTD) is a multivariant syndrome first defined in 1972, characterized by overlapping clinical symptoms known from systemic lupus erythematosus (SLE), polymyositis/dermatomyositis and rheumatoid arthritis (RA). Clinically MCTD presents with Raynaud’s phenomenon, arthritis or polyarthritis, hand oedema, inflammatory muscle disease, oesophageal dysmotility and pulmonary hypertension [1–3]. MCTD is characterized by the presence of high titres of ANA of the speckled banner type and/or anti-U1-RNP (formerly known as anti-ENA) antibodies in addition to elevated ESR and diffuse hypergammaglobulinaemia. In contrast, anti-double-stranded DNA antibodies and the presence of LE cells are rare in the presentation of MCTD [4, 5].

In general, MCTD is extremely responsive to steroids or other immunosuppressive agents, such as methotrexate, cyclosporin, mycophenolate mofetil, azathioprine, chloroquine, immunoglobulin (Ig) and cytotoxic agents such as cyclophosphamide, but not in all patients [6, 7].

Extracorporeal therapy as a treatment option in MCTD has been used previously with little success but appears to be a valuable adjunct in the management of the disease, especially when it is combined with drugs that may inhibit the reappearance of pathogenic autoantibodies [8]. We reasoned that the combination of extracorporeal therapy with monoclonal antibodies (mAbs) to CD20 might exert additive effects on the modulation of disease activity [9, 10].

Here we report a 61-yr-old female Caucasian patient diagnosed with MCTD in 1992. Treatment was initiated with azathioprine and cyclosporin but had to be stopped in 1993 because of toxicity. Plasmapheresis was employed in 1993, 1994 and 1995 and was followed by high-dose intravenous (i.v.) Ig. From 1993 to 1997 the basic medication was methotrexate. In 1997, because of central nervous system involvement, the patient was treated with i.v. cyclophosphamide pulse therapy according to the Fauci protocol, followed by oral cyclophosphamide. In 2003 etanercept in combination with methotrexate was employed because of progredient necrosis of the left index finger, but had to be stopped due to toxicity, while infection and progredient necrosis of the finger tip required amputation. Cyclophosphamide pulse therapy was restarted followed by oral cyclophosphamide at 150 mg/day but results were unsatisfactory. In this situation, immunoadsorption was initiated together with anti-CD20 mAb treatment. The patient had elevated levels of antibodies to CENP-B and antibodies to cardiolipin were detected in 2001, whereas pANCA were always negative.


For the first five cycles of selective immunoadsorption with a column (Globaffin®) used repetitively for 4 days every 4 weeks, the patient was maintained on stable medication with low-dose steroids and oral cyclophosphamide. After the sixth cycle of immunoadsorption the CD20 antibody rituximab (375 mg/m2) was added on day 4 after the extracorporeal therapy. Corticosteroids (10 mg/day) were given continuously throughout the treatment. Oral cyclophosphamide was exchanged for mycophenolate mofetil in November 2004 after eight cycles of immunoadsorption Fig. 1.