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Ann Rheum Dis 2009;68;836-843; originally published online 15 Jul 2008; doi:10.1136/ard.2008.091405

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Increased serum levels of B cell activating factor (BAFF) in subsets of patients with idiopathic inflammatory myopathies

O Kryštůfková,¹ T Valterskog,² S Barbasso Helmers,² H Mann,¹ I Půtová,¹ J Běláček,³ V Malmström,² C Trollmo,² J Vencovský,¹ I E Lundberg²

ABSTRACT

Objective: To investigate serum levels of B cell activating factor (BAFF) in patients with myositis and correlate these to autoantibody profile, clinical phenotype and treatment. Methods: BAFF levels in sera from 49 patients with dermatomyositis, 44 with polymyositis, 8 with inclusion body myositis and 30 matched controls were measured by ELISA. Specific autoantibodies were detected by line blot and western blot assays.

Results: Serum levels of BAFF were significantly higher in patients compared to healthy controls (p = 0.003). Patients with anti-Jo-1 autoantibodies had higher BAFF levels than control individuals (p < 0.003) or patients without any specific autoantibodies (p < 0.05). Patients with dermatomyositis had higher BAFF levels compared to polymyositis (p < 0.05). Patients with interstitial lung disease (ILD) had higher BAFF levels than patients without ILD (p < 0.05) or controls (p < 0.01) but this could be explained by presence of anti-Jo-1 autoantibodies. BAFF levels correlated with serum creatine kinase (CK) (rs = 0.365, p = 0.0005) but not with C-reactive protein (CRP) levels. A negative correlation of BAFF levels with glucocorticoid daily dose for serum creatine kinase (CK) and a negative correlation with dose of glucocorticoids, indicate that BAFF could be a potential therapeutic target in such cases.

Conclusion: Our finding of elevated serum levels of BAFF in patients with myositis with described phenotypes together with the correlations between levels of BAFF and C reactive protein (CRP) levels. A negative correlation of BAFF levels with glucocorticoid daily dose for serum creatine kinase (CK) and a negative correlation with dose of glucocorticoids, indicate that BAFF could be a potential therapeutic target in such cases.

The idiopathic inflammatory myopathies, polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM), collectively named myositis, are characterised by muscle weakness and by inflammatory infiltrates in skeletal muscle tissue.¹ Other organs are frequently involved such as skin in dermatomyositis and lungs in polymyositis and dermatomyositis. Autoantibodies are frequently present in polymyositis and dermatomyositis sera, but are less frequent in IBM. Some autoantibodies are considered myositis-specific autoantibodies (MSAs), and are present in up to 40% of patients with polymyositis and dermatomyositis and in up to 18% of patients with IBM.² The anti-histidyl-tRNA synthetase (anti-Jo-1) autoantibody is the most frequent and is associated with a distinctive clinical phenotype, the so called antisynthetase syndrome, which is characterised by myositis, Raynaud phenomenon, interstitial lung disease (ILD), arthritis and skin changes of the hands (mechanic’s hands).³ Some autoantibodies are also present in other autoimmune diseases and thus called myositis-associated autoantibodies (MAAs), such as anti-Sjögren syndrome antigen A (SSA)/Ro (Ro52, Ro60), anti-Sjögren syndrome antigen B (SSB)/La, anti-PM-Scl, anti-Ku and anti-ribonucleoprotein (RNP) autoantibodies.⁴ The frequent presence of autoantibodies in polymyositis and dermatomyositis indicates a role of B cells in these diseases. In addition, in dermatomyositis, B cells have been observed in the peripheral infiltrates of the muscle tissue.⁵ More recently, plasma cells were reported in muscle tissue of polymyositis and patients with IBM.⁶ A further support for a pathogenic role of B cells in myositis is the beneficial effect of B cell depletion therapy with rituximab in resistant cases of patients with dermatomyositis and polymyositis as described in one open pilot study and several case reports.⁶–¹³

B cell activating factor (BAFF, also known as B lymphocyte stimulator (BLyS)) of the tumour necrosis factor (TNF) family is crucial for B cell maturation and survival. BAFF is also believed to play a role in autoantibody production as well as in T cell activation and differentiation.¹⁴ The related cytokine, a proliferation-inducing ligand (APRIL), is also important for B cell development and function.¹⁴ High serum levels of BAFF and APRIL have been reported in patients with various autoimmune diseases, such as Sjögren syndrome (SS), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).¹⁵–²⁷ In addition, abnormal levels of BAFF have been correlated with disease activity or autoantibodies.¹⁶–¹⁹ 22–25 These observations have made BAFF a new potential target for therapy in these autoimmune diseases, reviewed by Sutherland et al.²⁸ In a small group of patients with dermatomyositis serving as a disease control for systemic sclerosis (SSc), BAFF levels were found to be elevated, but with this exception no information is available on the role of BAFF in patients with myositis.²⁹

Owing to the suggested role of BAFF and APRIL in autoantibody production and their correlation to disease activity in other autoimmune disorders, we investigated their serum levels in different subsets of patients with myositis and correlated these to autoantibody profile, clinical phenotype and treatment.

PATIENTS AND METHODS

Patients and controls
Sera from 99 Caucasian patients with myositis, 28 from the Rheumatology Clinic at Karolinska
University Hospital in Solna, Stockholm, Sweden and 71 from Institute of Rheumatology Prague, Czech Republic, identified from the National Registry for Rheumatic diseases, were investigated. To an initial pilot study, performed in the Swedish cohort, patients were recruited based on information captured from patient records including presence or absence of anti-Jo-1 and anti-Ro52/60 autoantibodies. After having received the preliminary results, a second cohort was recruited from the Prague Institute of Rheumatology. The Czech patients were also recruited based on presence or absence of anti-Jo-1 autoantibodies and, in addition, patients who were positive for anti-Mi-2 or anti-PM-Scl autoantibodies were included. The Swedish and Czech cohorts had comparable demographic and clinical characteristics, except that the Swedish patients were on average older and had longer disease duration. As these factors did not appear to correlate with serum levels of BAFF, the cohorts were analysed together.

Altogether, 44 patients were diagnosed with polymyositis and 49 with dermatomyositis defined by Bohan and Peter criteria, and 6 as IBM. Clinical data were retrieved from patient records or from the myositis registry in Prague.ILD was defined as described previously. Other myositis-associated manifestations including skin rash, Raynaud phenomenon, mechanic’s hands, Sjögren syndrome and arthritis, were registered when present at any time of the disease history.

The serum levels of creatine kinase (CK), C reactive protein (CRP), IgG and IgA were retrieved from the patients’ records at the time point corresponding to BAFF analysis. The laboratory analyses were performed as routine tests in certified Clinical Chemistry Laboratories at Prague Institute of Rheumatology or Karolinska University Hospital. At both centres the same method was performed for CK analysis, and reference levels were comparable. Treatment of the patients at serum sampling is shown in table 1. There were 12 untreated patients, 4 were early cases sampled before treatment was introduced. In patients with short disease duration (<6 months, n = 27) we calculated the cumulative dose of glucocorticoids taken up to the time of serum sampling. A total of 30 healthy individuals were included as controls. This study was performed after human ethics approval (Karolinska Hospital Nord, Stockholm and Institute of Rheumatology, Prague) and informed consent was obtained from all contributing individuals.

### Measurement of BAFF and APRIL levels

Serum levels of BAFF (R&D Systems, Minneapolis, Minnesota, USA) and APRIL (Bender MedSystems GmbH, Vienna, Austria) were measured by ELISA. Samples were analysed in duplicate. Serum APRIL levels were tested in the pilot study of 28 Swedish
Table 2  Demographic characteristics, clinical and laboratory data of patients and controls

<table>
<thead>
<tr>
<th></th>
<th>DM</th>
<th>PM</th>
<th>IBM</th>
<th>All patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>49</td>
<td>44</td>
<td>6</td>
<td>99</td>
<td>30</td>
</tr>
<tr>
<td>Ratio female: male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age*, years</td>
<td>50 (10 to 78)</td>
<td>52 (22 to 79)</td>
<td>72 (64 to 79)</td>
<td>52 (10 to 79)</td>
<td>53.5 (15 to 80)</td>
</tr>
<tr>
<td>Disease duration*, years</td>
<td>4 (0 to 29)</td>
<td>2 (0 to 30)</td>
<td>6 (9 to 19)</td>
<td>3 (0 to 14)</td>
<td>NA</td>
</tr>
<tr>
<td>Early cases*, months</td>
<td>13 (0 to 6)</td>
<td>13 (0 to 3)</td>
<td>1 (0 to 0)</td>
<td>27 (0 to 6)</td>
<td>NA</td>
</tr>
<tr>
<td>Medication:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucocorticoids†</td>
<td>38 (78%)</td>
<td>42 (95%)</td>
<td>2 (33%)</td>
<td>82 (83%)</td>
<td>NA</td>
</tr>
<tr>
<td>DMARDs†</td>
<td>21 (43%)</td>
<td>29 (64%)</td>
<td>3 (50%)</td>
<td>52 (53%)</td>
<td>NA</td>
</tr>
<tr>
<td>No therapy</td>
<td>1 (2%)</td>
<td>8 (18%)</td>
<td>1 (2%)</td>
<td>13 (13%)</td>
<td>NA</td>
</tr>
<tr>
<td>Extramuscular manifestations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILD†</td>
<td>16 (33%)</td>
<td>27 (61%)</td>
<td>0</td>
<td>43 (43%)</td>
<td>NA</td>
</tr>
<tr>
<td>Sjögren syndrome†</td>
<td>3 (6%)</td>
<td>2 (4.5%)</td>
<td>1 (17%)</td>
<td>6 (6%)</td>
<td>NA</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td>27 (55%)</td>
<td>31 (70%)</td>
<td>2 (33%)</td>
<td>60 (61%)</td>
<td>ND</td>
</tr>
<tr>
<td>- anti-Jo-1†</td>
<td>15 (31%)</td>
<td>19 (43%)</td>
<td>0</td>
<td>34 (34%)</td>
<td>ND</td>
</tr>
<tr>
<td>- anti-Mi-2†</td>
<td>8 (16%)</td>
<td>3 (7%)</td>
<td>0</td>
<td>11 (11%)</td>
<td>ND</td>
</tr>
<tr>
<td>- anti-Pm-Scl†</td>
<td>5 (10%)</td>
<td>6 (14%)</td>
<td>0</td>
<td>11 (11%)</td>
<td>ND</td>
</tr>
<tr>
<td>- anti-Ro52†</td>
<td>13 (27%)</td>
<td>11 (25%)</td>
<td>2 (33%)</td>
<td>26 (26%)</td>
<td>ND</td>
</tr>
<tr>
<td>- anti-Ku72/86†</td>
<td>8 (16%)</td>
<td>5 (11%)</td>
<td>0</td>
<td>13 (13%)</td>
<td>ND</td>
</tr>
<tr>
<td>ANA1 positivities†</td>
<td>32 (65%)</td>
<td>25 (57%)</td>
<td>4 (67%)</td>
<td>61 (62%)</td>
<td>ND</td>
</tr>
<tr>
<td>CK (mcat/litre)*</td>
<td></td>
<td></td>
<td>2.0 (0.6 to 64.4)</td>
<td>2.3 (0.3 to 32.0)</td>
<td>5.4 (1.9 to 14.1)</td>
</tr>
<tr>
<td>CRP (mg/litre)*</td>
<td>1.8 (1.0 to 60.0)</td>
<td>5.1 (1.1 to 85.5)</td>
<td>7.0 (4.0 to 47.0)</td>
<td>4.8 (0.8 to 85.5)</td>
<td>ND</td>
</tr>
<tr>
<td>IgG (g/litre)*</td>
<td></td>
<td></td>
<td>11.0 (3.0 to 24.0)</td>
<td>10.7 (4.6 to 17.3)</td>
<td>12.8 (11.1 to 18.1)</td>
</tr>
<tr>
<td>IgA (g/litre)*</td>
<td>1.8 (1.0 to 4.7)</td>
<td>2.0 (0.4 to 14.5)</td>
<td>3.0 (0.9 to 3.4)</td>
<td>2.1 (0.4 to 14.5)</td>
<td>ND</td>
</tr>
<tr>
<td>BAFF (ng/ml)*</td>
<td>1.3 (0.3 to 12.8)</td>
<td>0.9 (0.2 to 8.7)</td>
<td>1.1 (0.9 to 3.5)</td>
<td>1.1 (0.2 to 12.8)</td>
<td>0.9 (0.3 to 1.6)</td>
</tr>
</tbody>
</table>

*Median (range); †Number (percentage from DM, PM, IBM or from total number of patients). 1ANA nuclear or/and cytoplasmic Hep2 cell immunofluorescence, titre>1:80. BAFF, B cell activating factor; CK, creatine kinase (factor by 60 to U/litre; normal levels for Swedish cohort: <2.85 mcat/litre for men and <2.0 mcat/litre for women; for Czech cohort: <2.85 mcat/litre and <2.42 mcat/litre; CRP, C-reactive protein (normal levels <10 mg/litre); DM, dermatomyositis; DMARD, disease modifying antirheumatic drug; IBM, inclusion body myositis; IgA, immunoglobulin A (normal levels 0.7–4.5 g/litre); IgG, immunoglobulin G (normal levels 6–16 g/litre); IId, interstitial lung disease; NA, not applicable; ND, not determined; PM, polymyositis.

patients, (14 with polymyositis, 8 with dermatomyositis, 6 with IBM, mean (SD) age 60.5 (12.8) years, 18 women and 10 men) and from 16 age and gender matched healthy individuals. Assays were performed in one laboratory (Rheumatology Unit, Centre of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden) under identical conditions.

Autoantibody detection

Three tests were used for detection of specific autoantibodies; two line blot assays and one western blot assay. The Inno-Lia ANA Update line blot assay (Innogenetics, Ghent, Belgium) was used for detection of antibodies to Jo-1/HRS, SmB, SmD, Ro70, RNP-A, RNP-C, Ro52/SSA, Ro60/SSA, La/SSB, centromere B (anti-centromere antibody (ACA)), topoisomerase-1/Scl70, ribosomal P antigen and histones. Myositis line immunosassay (LIA) (IMTEC, Berlin, Germany) was used for detection of antibodies to Jo-1, Mi-2, PSm-Scl100, U1 small nuclear ribonucleoprotein (antiU1 snRNP), Ku72/86. Myositis western blot was performed for detection of antibodies to Jo-1, Mi-2, PM-Scl, Ku, PL-7 and PL-12 using an anti-myositis antigen EUROLINE-WB kit (Euroimmun, Lubeck, Germany).

Patients who had negative results in all three specific tests were considered to be negative. In most serum samples the data were congruent but there were some discrepant results for anti-Mi-2 (n = 7), anti-FM-Scl (n = 2) and anti-Ku72/86 (n = 12) autoantibodies between the EUROLINE-WB kit and Myositis LIA. The positive results were confirmed by positive antinuclear antibody (ANA) staining with corresponding fluorescence pattern on Hep2 cells, detected by indirect immunofluorescence kit ANA–IIF (Immunoconcepts, Sacramento, California USA), for all but one with anti-Ku72/86 antibodies. As a result, patients who had positive results in one of the specific methods were considered to be positive. In addition the Swedish cohort was analysed by Inno LIA ANA Update twice, in Sweden and in Prague, with exactly the same results. Assays with the EUROLINE-WB kit and Myositis LIA and ANA testing were performed in all serum samples in Prague (Immunology laboratory, Institute of Rheumatology).

Statistical analysis

Statistical analysis was performed using GraphPad Prism V. 3.03 (GraphPad Software, San Diego, California, USA) and SPSS V. 14.0 (SPSS, Chicago, Illinois, USA). For analysis of differences between groups, Kruskal–Wallis (multiway analyses of variance (ANOVA)) with the Dunn post hoc test and non-parametric Mann–Whitney U test were performed. The Spearman rank order test (rs) was used for correlations of parameters. A p value equal or less than 0.05 was considered as statistically significant. Multivariate ANOVA was used for analysis of logarithmically transformed serum Baff levels. For assessment of the formal hierarchy of independent variables significantly influencing serum Baff levels, the simultaneous linear regression ANOVA model for logarithmically transformed Baff levels as dependent variable was created and sequentially reduced by one step.


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*Median (range); †Number (percentage from DM, PM, IBM or from total number of patients). 1ANA nuclear or/and cytoplasmic Hep2 cell immunofluorescence, titre>1:80. BAFF, B cell activating factor; CK, creatine kinase (factor by 60 to U/litre; normal levels for Swedish cohort: <2.85 mcat/litre for men and <2.0 mcat/litre for women; for Czech cohort: <2.85 mcat/litre and <2.42 mcat/litre; CRP, C-reactive protein (normal levels <10 mg/litre); DM, dermatomyositis; DMARD, disease modifying antirheumatic drug; IBM, inclusion body myositis; IgA, immunoglobulin A (normal levels 0.7–4.5 g/litre); IgG, immunoglobulin G (normal levels 6–16 g/litre); IId, interstitial lung disease; NA, not applicable; ND, not determined; PM, polymyositis.
RESULTS

Clinical, laboratory and serological data

Clinical and laboratory data are presented in table 2. There were no differences in extramuscular manifestations between patients with DM, PM and IBM, except the presence of ILD, which was twice as frequent in patients with PM than in patients with DM. Six patients had SS. Autoantibodies were present in 60 out of 99 patients and positive ANA were seen in 61 cases. Most patients had more than one autoantibody specificity. Certain combinations were more common, as seen in fig 1. We used this for subclassification of patients into 5 major groups: (1) 34 patients with anti-Jo-1 (including 2 patients also positive for anti-Mi-2, but who were confined to the anti-Jo-1 group for the statistical analyses), (2) 9 with anti-Mi-2 (including 2 patients positive also for PM-Scl who were confined to the anti-Mi-2 group for the statistics), (3) 9 with anti-PM-Scl, (4) 5 with anti-Ro52 and (5) 3 with anti-Ku72/86 autoantibodies. Two patients had anti-PL-7 autoantibodies in combination with other MSA autoantibodies (anti-Jo-1 + anti-Mi-2 or anti-Mi-2 + anti-PM-Scl) and therefore they were included in the aforementioned major groups.

Serum levels of BAFF

Serum levels of BAFF were significantly higher in patients with myositis compared to healthy controls ($p = 0.003$) (fig 2A, table 2). Among patients with serum BAFF levels higher than cut-off (1.47 ng/ml; defined as mean +2SD of healthy subjects), patients with autoantibodies predominated. From 25 patients with elevated serum BAFF (range 1.5 to 4 ng/ml; fig 2B), 15 (65%) were autoantibody positive and 10 of them had anti-Jo-1 autoantibodies. All 11 patients with high level of BAFF in serum (>4 ng/ml) had autoantibodies and 8 of them (73%) had anti-Jo-1 antibodies (fig 2C).

When we grouped patients according to autoantibody profiles and used multiple comparison analysis, patients with anti-Jo-1 autoantibodies had significantly higher serum levels of BAFF (median 1.64, range 0.4 to 12.8 ng/ml) than control individuals (0.85, range 0.3 to 1.6 ng/ml; $p<0.01$) or patients without any specific autoantibodies (1.1, range 0.2 to 3.5 ng/ml; $p<0.01$) (fig 3A). This was not seen in patients with anti-Mi-2 (0.95, range 0.2 to 4.97 ng/ml), anti-PM-Scl (0.97, range 0.3 to 11.5 ng/ml), anti-Ro52 (1.09, range 0.8 to 2.7 ng/ml) or anti-Ku72/86 (1.7, range 0.5 to 2.2 ng/ml) autoantibodies (fig 3A). Patients with ILD had significantly higher levels of BAFF than patients without ILD ($p<0.05$) or controls ($p<0.01$) (fig 3B). This difference could be explained by anti-Jo-1 autoantibodies, as patients with ILD, but without anti-Jo-1 autoantibodies did not have higher BAFF levels when compared with patients without ILD or controls.

When patients were subgrouped according to clinical phenotypes we found that patients with dermatomyositis had significantly higher BAFF levels compared to polymyositis or controls (fig 3C, table 2). Serum levels of BAFF in patients with IBM did not differ significantly from other patient groups or controls (fig 3C, table 2). Within the group of patients without anti-Jo-1 autoantibodies and ILD, patients with dermatomyositis ($n=27$) had significantly higher BAFF levels compared to patients with polymyositis ($n=15$) (fig 3D). The latter had significantly lower BAFF levels compared to patients with polymyositis who had anti-Jo-1 autoantibodies and ILD ($n=17$) (fig 3D).

Figure 2  Serum levels of B cell activating factor (BAFF) in patients with myositis compared to healthy subjects (A), their distribution (B) and the proportion of patients with or without different autoantibodies in subgroups (C) sorted by BAFF levels. $p$ Value from comparison of median values by Mann–Whitney test is presented. Marker for cut off level defined as mean +2SD of healthy subjects is displayed in (B). Horizontal bars indicate median levels.
Correlations between serum levels of BAFF and laboratory variables
BAFF levels in serum correlated with serum levels of CK ($r = 0.365$, $p < 0.001$) (fig 4A) and IgG ($r = 0.388$, $p < 0.001$) levels, but not with serum levels of CRP or IgA.

Effects of treatment on serum levels of BAFF
Untreated patients ($n = 12$) had significantly higher BAFF levels (2.0, 0.86–11 ng/ml) than patients treated with glucocorticoids or DMARDs ($n = 87$) (1.1, 0.2–13 ng/ml, $p = 0.01$). When all patients were analysed (treated and untreated) there was a negative correlation between levels of BAFF and glucocorticoid daily dose at the time of serum sampling ($r = -0.292$, $p = 0.003$) (fig 4B). In patients with early disease (less than 6 months), a negative correlation was seen between BAFF levels and cumulative doses of glucocorticoids ($r = -0.659$, $p < 0.001$) (fig 4C).

Hierarchy and interactions of ILD, autoantibodies, diagnosis or treatment on serum levels of BAFF
To analyse the relative influence of investigated variables on serum levels of BAFF, two pairs of alternative stochastically reliable linear regression models with ln(BAFF) as dependent variable were found with coefficients of determination ($R^2$) between 31.0% to 32.3%. In one pair of tests, the positivity of anti-Jo-1 autoantibodies as explaining variable was substituted by ILD (Kruskal–Wallis $p < 0.001$). Horizontal bars indicate median levels. Displayed $p$ values are results from the Dunn multiple comparison test, from one-way analysis of variance (ANOVA) test of logarithmically transformed data (*) and from the Mann–Whitney test (†). DM, dermatomyositis; PM, polymyositis.

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the two ANOVA models where the diagnosis dermatomyositis was indicated, while in the other two models with polymyositis the main influencing factor was the presence of anti-Jo-1 autoantibodies (9.5% of PES). The second important explaining factor was CK irrespective from indication dermatomyositis or polymyositis (8.7% of PES).

### Serum levels of APRIL

Serum levels of APRIL were only tested in the first cohort of 28 patients as a pilot study. There was no significant difference between patients (1.2 ng/ml, range 0.0–132.7) and healthy controls (0.08 ng/ml, range 0–40) or between subgroups of patients with (2.5 ng/ml, range 0–132.7) or without (1.3 ng/ml, range 0–54.7) autoantibodies and with (0.0 ng/ml, range 0–132.7) or without ILD (1.7 ng/ml, range 0–105.3). APRIL levels correlated with IgG serum levels ($r = 0.503$, $p = 0.005$), but not with IgA levels. There was no correlation between APRIL levels and CK, CRP or levels of BAFF in serum.

### Discussion

In this large myositis cohort we found increased serum levels of BAFF in patients compared to healthy individuals. Grouping according to autoantibody profiles and clinical phenotype, revealed that this was particularly true for patients with polymyositis with anti-Jo-1 autoantibodies and ILD. In addition, there was also a group of dermatomyositis patients without these phenotypes in which high BAFF levels were observed. Moreover, levels of BAFF correlated positively with serum CK levels and negatively with glucocorticoid treatment.

For this study, patients positive for anti-Jo-1, anti-Mi-2, anti-PM/Scl and anti-SSA, autoantibodies or patients who were autoantibody negative were selected. This explains the skewed prevalence of autoantibody profile in our cohort compared to others. All sera were tested with three different methods for a wide spectrum of additional myositis specific and myositis associated autoantibodies. In a few cases with discrepant results, one positive test was considered sufficient to regard a patient as positive for autoantibodies tested. In all these cases, the immunofluorescence pattern in ANA test corresponded to detected autoantibody type, which provided support for this decision. Still we cannot exclude that some patients might have other autoantibodies not included in our tests such as other more rare anti-synthetase autoantibodies. This is a possible explanation for the high serum levels of BAFF in two patients who were autoantibody negative.

BAFF levels varied considerably to autoantibody profile and presence of ILD, whereas traditional clinical subtypes, such as polymyositis and dermatomyositis, were less discriminatory in the multi regression analysis. The higher BAFF levels in patients with anti-Jo-1 autoantibodies compared to patients without autoantibodies support a role of B cells and BAFF in autoantibody production and disease manifestations in this subset of patients. However, this is not likely to be specific for anti-Jo-1 autoantibodies as not only patients who were anti-Jo-1 positive, but also some patients with anti-PM-Scl or anti-Mi2 autoantibodies, had high levels of BAFF (fig 3A). In the group analysis, serum BAFF levels in these groups were not significantly higher when compared to patients who were autoantibody negative or controls. The fact that patients with dermatomyositis had high basal levels of BAFF irrespective of presence of anti-Jo-1 and ILD, suggests additional B cell mechanisms, independent from production of the known autoantibodies, in this subset of patients. In this context a
novel observation is interesting, namely that patients with dermatomyositis regardless of autoantibody profile as well as patients with polymyositis with anti-Jo-1 or anti-SSA autoantibodies and ILD differed from other subsets of patients with myositis by a distinct type I IFN inducing capacity by their sera.11-12 IFNα can induce BAFF expression.36 The major producer of type I IFN, the plasmacytoid dendritic cells have been demonstrated in skin biopsies of patients with dermatomyositis37 and in muscle biopsies of patients with polymyositis and dermatomyositis.38-39 Thus, local induction of the endogenous type I IFN system in muscle and skin could be a possible explanation to elevated serum BAFF levels in the subset of patients with polymyositis with anti-Jo-1 autoantibodies and in dermatomyositis.

Comparable serum levels of BAFF to what we found in patients with myositis have been reported in patients with primary Sjögren syndrome (pSS) who had anti-SSA/Ro autoantibodies, in patients with SLE with anti-double-stranded (ds)DNA autoantibodies; and in patients with SSC with anti-histone autoantibodies.10-17 18 19 20 21 22 23 24 25 26 27 Serum levels of BAFF correlated with levels of CK, but not with CRP arguing against BAFF as an acute phase reactant in patients with myositis. Moreover, the position of “muscle enzyme” (CK in the hierarchy of factors detected by our ANOVA models for patients with dermatomyositis and polymyositis) indicates that BAFF levels are in close relation to local muscle involvement. In concordance with patients with SLE, the decreased serum levels of BAFF seen after treatment with glucocorticoids suggest that BAFF production is sensitive to this treatment. Recently a correlation between anti-Jo-1 autoantibody titres and disease activity was reported.46 In our study, we were not able to compare BAFF levels with levels of anti-Jo-1 antibodies due to the lack of quantitative data on anti-Jo-1 antibodies.

Although APRIL is a closely related cytokine to BAFF, with overlapping receptors, we did not find differences in APRIL levels between patients and controls. Notably our investigation was limited to a pilot study in the first investigated cohort. When the results were negative we decided not to test this variable in the second cohort. The difference between BAFF and APRIL could be explained by the fact that these cytokines are differently regulated in rheumatic diseases.47 Our results indicate that BAFF is more directly involved in disease mechanisms in myositis than APRIL.48

In conclusion, the finding of increased BAFF levels in association with particular autoantibodies or clinical presentation points to an important role of B cells in these patients and further support the current theory that different disease mechanisms are involved in different subsets of myositis. From this aspect, subclassification according to autoantibody profile could be particularly fruitful in future studies. The increased BAFF levels could be mediated through common and subset-specific mechanisms, as patients with dermatomyositis without autoantibodies also had increased BAFF levels. One common mechanism for BAFF induction might be through type I IFN. Together, our data point to BAFF and B cells as potential targets for therapy in anti-Jo-1 autoantibody positive patients with polymyositis with ILD and in patients with dermatomyositis regardless of autoantibodies.

Funding: This study was supported by the European Community’s FP6, AutoCure LSHB CT-2006-018661 funding, by institutional support MSM 0021620812 from Ministry of Education, Youth and Sports in the Czech Republic and by The Swedish Research Council (03642) K2005-74X-14045-00, The Swedish Rheumatism Association, King Gustaf V 80 year Foundation, Karolinska Institutet Foundation.

Competing interests: None.

Ethics approval: This study was performed after human ethics approval (Karolinska Hospital Nord, Stockholm and Institute of Rheumatology, Prague) and informed consent was obtained from all contributing individuals.

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