The two mRNA transcription variants of the B-cell activating factor are differentially expressed, but in a stable ratio

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The B-cell activating factor (BAFF) is essential for maturation and survival of B-cells. This protein has a significant anti-apoptotic effect on B-lymphocytes, including auto-reactive clones that play substantial role in autoimmune disorders. The strong association between the level of BAFF protein in serum and number of B-cells in peripheral blood has been described (1). Besides, studies in humans show positive correlation of higher serum BAFF levels and severity of some autoimmune disorders and/or correlation with auto-antibodies presence (2-7).

The human BAFF gene contains six exons with several known transcriptional variants. Two were described by Gavin *et al*. (8): the ΔBAFF lacking 3rd exon and the full-length BAFF transcript. Peptides derived from the full-length transcripts are considered to stimulate B-cells, the alternatively spliced ΔBAFF variants could suppress the BAFF mediated B-cell stimulation (8).

Since the increased levels of BAFF protein were described in patients with idiopathic inflammatory myopathies (IIMs), we aimed to detect any abnormalities in BAFF expression at the mRNA level that could potentially contribute to the high BAFF levels. We have therefore analysed the expression of the full-length BAFF and ΔBAFF in a cohort of 18 healthy controls compared with 26 patients with IIMs. The expression levels of two mRNA variants of the BAFF gene were assessed by qRT-PCR method. For the amplification, we have used following primers 5´-AGAAATAAGCGTGCCGTTCAG-3´, 5´-GAGAAGCCATGGAACAAATGTG-3´ for both BAFF transcripts. The transcription variants were identified using specific MGB probes (Life Technologies, USA): 5´-CAGAAGAAACAGGATCTT-3´ - VIC for full-length BAFF transcript and 5´-AGTCACTCAAGACTGCTT-3´ - 6-FAM for ΔBAFF transcript.

Our results show, that the two mRNA splice variants are differentially expressed. The expression of the full length transcript is significantly higher in comparison to the ΔBAFF (p<0.0001). This difference in expression was found in both healthy controls (Fig. 1A) and in patients with IIMs (Fig. 1B). The expression of ΔBAFF was 3.1 times higher in patients than in controls and the expression of full-length BAFF was 2.8 times higher in patients than in controls. However, the difference in the expression regulation of ΔBAFF and full-length BAFF did not reach statistical significance (Fig. 1C).

The expression ratio of BAFF *vs*. ΔBAFF was found to be 3.5 in IIM (Fig. 1D) which is not consistent with reported ratio of BAFF *vs*. ΔBAFF 15.2 in PBMCs of IIM patients (9). Finally, at the moment, it is impossible to distinguish the two BAFF peptides translated from these two transcription variants, although they may have different functional effects. Therefore, in studies considering involvement of the BAFF cytokine in the immune response, the analysis of mRNA expression of BAFF transcriptional variants could be of high significance beside the analysis of BAFF at the protein level. Recent study shows, that BAFF and particularly ΔBAFF expression

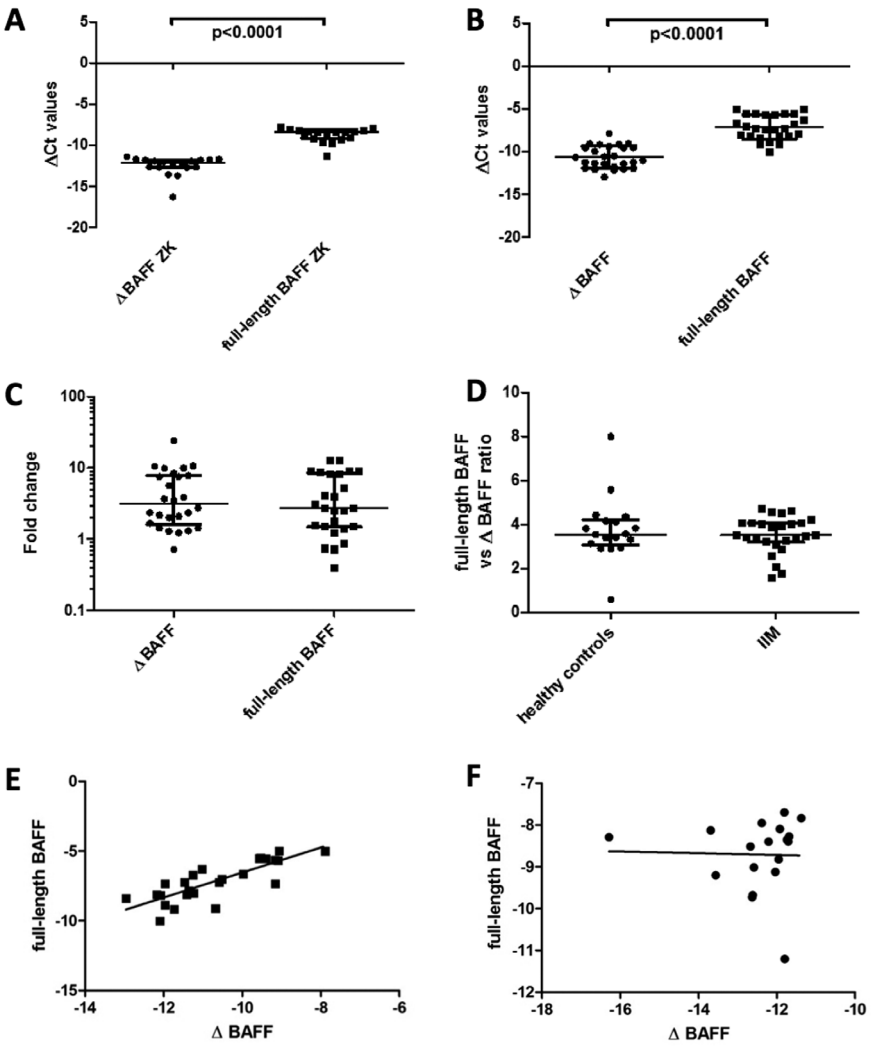

Fig. 1. Expression level of two mRNA transcription variants of BAFF gene in (**A**) 18 healthy controls and (**B**) 26 patients suffering from IIM, expression fold change of BAFF gene transcripts in patients (**C**) and expression ratio of BAFF transcripts in patients and controls (**D**). All subjects in the study were Caucasians, coming from the region of Czech Republic. Total RNA was extracted from the whole blood using the RNeasy Mini Kit (Qiagen) extraction procedure, with on-column DNA digestion. The detection of mRNA expression was carried out in ABI 7900 real-time thermal cycler using the SYBR® Green chemistry according to the manufacturers recommendations (Life Technologies, USA). For the expression normalisation and for further calculation of expression levels, two housekeeping genes were used (B2M, GAPDH). The statistical analysis was done using the GraphPad Prism v5 software (GraphPad Software, USA). Based on the normal distribution of expression values, we have used the paired two tailed t-test for analysis of the statistical significance of the splice variant's expression differences. The expression level of both groups of BAFF mRNA transcription variants are shown as delta Ct value normalised to the expression of housekeeping genes as medians with interquartile range.

The expression of both, full-length BAFF and ΔBAFF was higher in patients (median value -7.2 and -10.9) in comparison with controls (median value -8.4 and -12.1) (**A**, **B**). However, this difference did not reach statistical significance. The expression of full-length BAFF was found to be higher in both groups of samples (p<0.0001; **A**, **B**). The expression of ΔBAFF was 3.1 times higher in patients (**C**) than in controls; the expression of full-length BAFF was 2.8 times higher in patients than in controls (p-value not significant for both transcripts). The ratio of full-length BAFF / ΔBAFF expression (delta Ct values) was found to be 3.5 in patients and 3.8 in controls. The difference in this ratio did not reach statistical significance (**D**). Furthermore, the mRNA levels of BAFF or ΔBAFF did not correlate with disease activity, sex, age and no significant differences were found between the IIM subtypes dermatomyositis and polymyositis. We have found a positive correlation between the levels of ΔBAFF and full-length BAFF in patients suffering from myositis (r=0.81, p<0.0001, **E**). However, there was no statistically significant correlation found in the cohort of healthy controls (r=0.30, NS, **F**).

correlated with disease activity (9). We did not see such a correlation with basic disease activity parameters in our cohort.

To sum up, we present here that the two groups of BAFF splice variants (full length and exon 3 lacking form) are differentially expressed, but in comparable ratio in healthy controls and in myositis patients. Moreover, we noticed that as no methods for distinguishing the full-length BAFF peptide and ΔBAFF derived peptide are available, and we know that these two peptides can have different role in BAFF signaling cascade, one has to be very careful in the presentation of any BAFF protein-disease association-study results in general.

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