Δ4BAFF, an Alternate-Splice Isoform That Acts as a Transcription Factor To Enhance BAFF Production in Primary Sjögren's Syndrome.

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Background:
Elevated expression of 'B cell activating factor belonging to the tumor necrosis factor family' (BAFF), a potent B cell survival factor contributes to the expansion of low-affinity self-reactive B cells during the establishment of tolerance. However, mechanisms leading to BAFF over-expression in autoimmune settings, such as primary Sjögren's syndrome (SS) or rheumatoid arthritis (RA) and in lymphoproliferation such as chronic lymphocytic leukemia (CLL) are not understood. To date, 3 transcriptional variants of the BAFF gene have been described, including Δ3BAFF, a truncated form that negatively regulates BAFF, by forming non-functional heterotrimers with full-length (FL) BAFF. Herein, we report the discovery and the role of a new transcript for BAFF due to the fact that exon 4 is spliced out.
Methods and Results:
A 3' RACE-PCR was performed in B cells from SS patients and revealed another transcript with deletion of nucleotides 749 to 861 encoding the predicted exon 4. A new in-frame stop codon was artificially generated resulting in a truncated protein. A same alternate splice isoform was detected in mice. To assess the biochemical properties of the new variant of BAFF, RAMOS B cells were transiently transfected with pIRES2-EGFP-Δ4BAFF. Two bands at 21 and 17-kD prove to belong to Δ4BAFF, which is very telling of a post-translational modification. Incubation with PNGase F showed that Δ4BAFF was glycosylated and led to the predicted mobility of Δ4BAFF at 17kD. Interestingly, Δ4BAFF was located in the nucleus and, contrary to FL BAFF, absent from the cytoplasm. Because BAFF was up-regulated after Δ4BAFF transfection in RAMOS cells, we asked the question as to whether Δ4BAFF might function as a transcriptional regulator of BAFF. A ChIP analysis revealed that the Δ4BAFF protein bound to the BAFF promoter. To confirm the role of Δ4BAFF as a transcription factor that activates its own gene to increase BAFF production, we synthesized a digoxigenin-labeled consensus NFkB binding probe within the BAFF promoter and performed an EMSA. When nuclear extracts from Δ4BAFF transfected RAMOS B cells were incubated with this probe, a protein/DNA complex was seen. A supershift was only detected when the anti-BAFF mAb was added. Finally, we observed the presence of the Δ4BAFF protein in B cells from patients with CLL, in synoviocytes from patients with RA or in epithelial cells from patients with SS.
Conclusion: We thus expand the view of BAFF gene regulation, which should contribute a better understanding of complex physiologic mechanisms involved in normal B cell survival, as well as in pathophysiology of autoreactive B cells. Our work introduces for the first time an entirely novel concept in biology suggesting that a human cytokine gene can be transcriptionally regulated by the activity of one of its own splice variants.