Development of a Robust ELISA Kit to Detect Human APRIL (TNFSF13) Homotrimers and APRIL-BAFF Heterotrimers in Human Serum, Plasma, and Other Biological Samples

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Abstract

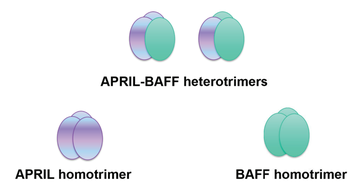
APRIL (TNFSF13) is an important secreted protein that stimulates cell proliferation. As with most other TNF family members, APRIL exists as a functional homotrimer. It can bind to two cell-surface receptors: BCMA and TACI, which it shares with BAFF (BLyS or TNFSF13B), to exert downstream T- and B-cell regulatory effects. APRIL is most well known for its tumor proliferation effects. It is a potential biomarker, with serum levels elevated in certain cancers and autoimmune diseases. In fact, recombinant TACI is currently in clinical trials as a neutralization drug against APRIL and BAFF for the treatment of SLE.

Besides forming homotrimers, APRIL can also form functional heterotrimers with BAFF. The stoichiometric relationship of the protomeric units is still unclear, however it appears that these heterotrimers are significant since they are elevated in serum of certain autoimmune patients[1, 2]. Both the APRIL and BAFF pathways are currently hot targets for therapeutic intervention.

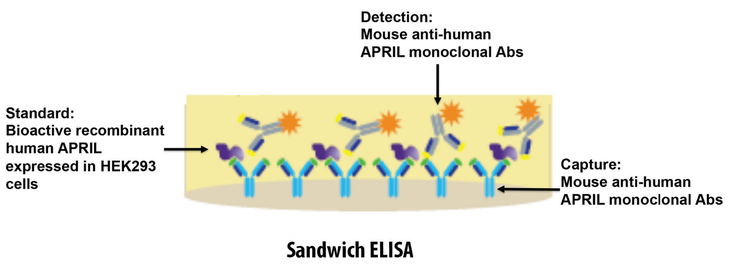
We developed a sandwich ELISA kit to detect human APRIL, including its various multimeric forms, in biological samples. The assay uses two mouse monoclonal antibodies specific against human APRIL, which together recognize APRIL homotrimers, as well as APRIL-BAFF-BAFF and BAFF-APRIL-APRIL heterotrimers, with no cross-reactivity with BAFF itself. Other validation data include recovery, linearity, specificity, sensitivity, interference, and precision. This assay was validated using human serum and plasma samples, as well as stimulated cell culture supernatant. It provides a unique and sensitive tool for measuring the various multimeric forms of human APRIL, which have useful clinical applications in oncology and autoimmunity.

Introduction

APRIL and BAFF belong to the TNF superfamily of cytokines, and both can exist in soluble forms. Besides forming their respective homotrimers, they can also make APRIL-BAFF heterotrimers in either 2:1 or 1:2 protomeric ratios. These heterotrimers are biologically active, and seem to correlate to certain autoimmune conditions. Our aim was to create an assay to quantify not only APRIL in its homotrimeric form, but also including APRIL-BAFF heterotrimers.



Methods



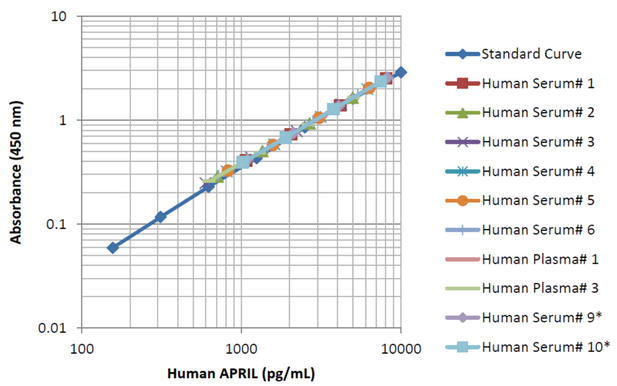
Results

Table 1. Recovery averaged 94% in human serum and plasma.

Sample	Recovery of 5 ng/mL	Recovery of 1.25 ng/mL	Recovery of 0.31 ng/mL	Overall Recovery
Human Serum # 1	91%	105%	150%	98%
Human Serum # 2	85%	91%	95%	92%
Human Serum# 3	80%	82%	85%	84%
Human Serum# 4	103%	113%	157%	105%
Human Serum# 5	86%	91%	100%	96%
Human Serum# 6	95%	94%	101%	98%
Human Serum# 7	76%	90%	110%	93%
Human Serum# 8	74%	66%	37%	87%
Human Plasma# 1	81%	90%	100%	90%
Human Plasma# 2	98%	100%	95%	98%

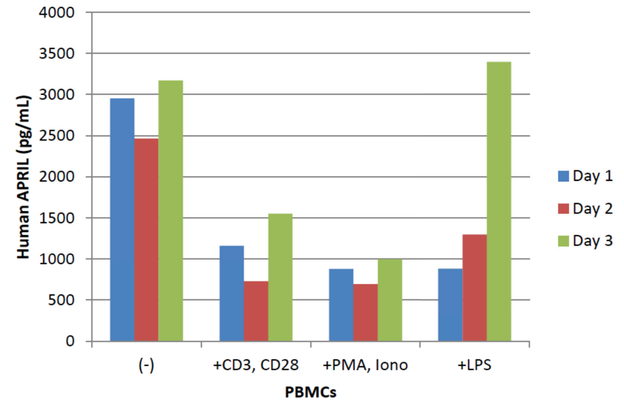
Recombinant human APRIL at various concentrations was spiked into human serum and plasma samples, then analyzed with the LEGEND MAX™ Human APRIL/TNFSF13 ELISA Kit. On average, 94% recovery of spiked APRIL was observed. Normal range of measured APRIL in human serum and plasma samples was 0.04-9.4 ng/mL. Sensitivity of the assay is typically ~0.038 ng/mL.

Figure 1. Linearity averaged 107% in human serum and plasma.



Recombinant human APRIL was spiked into human serum and plasma samples, diluted with Assay Buffer A to produce samples with values within the dynamic range, then analyzed with the LEGEND MAX™ Human APRIL/TNFSF13 ELISA Kit. Alternatively, naturally high endogenous samples* were diluted without spiking. On average, 107% linearity of dilution was observed.

Figure 2. Measured concentrations of APRIL in stimulated PBMCs agree with the literature[3].



Human PBMCs were stimulated with CD3+ CD28, PMA+ Ionomycin, or LPS, and supernatants were removed at 1-3 days after stimulation and assayed for human APRIL.

Table 2. Assay specifically recognizes APRIL homotrimers & APRIL-BAFF heterotrimers, but not BAFF homotrimers.

Sample	Signal Strength (O.D.)
Recombinant human APRIL homotrimer from Source# 1	+++
Recombinant human APRIL homotrimer from Source# 2	+++
Recombinant human APRIL-BAFF-BAFF heterotrimer from Source# 3	+
Recombinant human BAFF-APRIL-APRIL heterotrimer from Source# 3	+++
Recombinant human APRIL/BAFF heterotrimer from Source# 4	+++
Recombinant human BAFF-BAFF-BAFF homotrimer from Source# 3	-
Recombinant human BAFF homotrimer from Source# 1	-

Conclusions:

We successfully developed an ELISA kit for quantification of human APRIL, including the homotrimer form, and heterotrimer forms with BAFF.

The assay is analytically validated for robustness (specificity, accuracy, sensitivity, reproducibility, stability, biological relevance, etc.) for use in biomedical research.

References:

1. Roschke, *et al. J of Immunol.* 2002 (169): 4314.

2. Dillon, *et al. Arthritis Res Ther.* 2010 (12): R48.

3. Pradet-Balade, *et al. EMBO J.* 2002 (21): 5711.

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