

Letter to the editor,

Buffy's, B-cells and membrane BAFF.

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In their recent article in *Arthritis and Rheumatism* (1), Chu et al. reported the aberrant production of BAFF by overactivated B cells in patients suffering from systemic lupus erythematosus. This report followed a previous one from the same group, showing that B cells start to produce BAFF upon activation (2). The purpose of this letter is to correct one point of the report of Chu et al regarding the reactivity of an anti-BAFF antibody that they have used. This correction doesn't change the overall conclusion of the study, but may help avoiding false statements in future studies investigating BAFF-producing cells. BAFF is produced as a transmembrane protein, mostly by myeloid cells, and released as a soluble cytokine after cleavage by furin-like proteases at arginine 133(3). Of note, B cells avidly sink soluble BAFF by binding it to BAFF-R, so that soluble BAFF can be detected at the B-cell surface (4). When looking at BAFF-producing cells, it is therefore important to use antibodies reacting with portions of the BAFF molecule, such as the stalk region, that are not contained in the soluble product. This approach proved successful with the closely related ligand APRIL (5). Chu et al. used the Buffy-2 mAb to detect BAFF-producing cells, arguing that Buffy-2 reacts with the stalk of BAFF because it didn't stain BAFF-R⁺ cells pre-incubated with soluble BAFF (2). Buffy's are rat monoclonal antibodies raised against a long form of recombinant human soluble BAFF (aa 83-285) containing part of the stalk fragment. Hence, in theory, Buffy-2 may have been directed against the stalk of BAFF, but this is actually not the case. Indeed, Buffy-2 not only recognized long forms of BAFF containing the stalk region, but also shorter forms of BAFF devoid of the stalk region, including soluble, naturally processed BAFF (Fig. 1A and B). In contrast, Buffy-1 only recognized long forms of BAFF, including full-length BAFF, but not naturally released BAFF or other short forms lacking the stalk region (Fig. 1A and B). We further observed by flow cytometry that Buffy-1 stained stable cell lines expressing either full-length BAFF or a fusion protein in which the C-terminal portion of BAFF was replaced by the corresponding region of APRIL (Fig. 1C). These results indicate that Buffy-1, but not Buffy-2, is directed against the stalk region (Fig 1D). In conclusion, Buffy-1 may be used to identify BAFF-producing cells, but not Buffy-2.

References:

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2. Chu VT, Enghard P, Riemekasten G, Berek C. In vitro and in vivo activation induces BAFF and APRIL expression in B cells. *J Immunol* 2007;179(9):5947-57.
3. Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 1999;189(11):1747-56.
4. Carter RH, Zhao H, Liu X, Pelletier M, Chatham W, Kimberly R, et al. Expression and occupancy of BAFF-R on B cells in systemic lupus erythematosus. *Arthritis Rheum* 2005;52(12):3943-54.
5. Schwaller J, Schneider P, Mhawech-Fauceglia P, McKee T, Myit S, Matthes T, et al. Neutrophil-derived APRIL concentrated in tumor lesions by proteoglycans correlates with human B-cell lymphoma aggressiveness. *Blood* 2007;109(1):331-8.

Legend to figure 1

A) Long (aa 83-285) or short (aa 136-285) BAFF at 1 µg/ml were coated in an ELISA plate. Binding of Buffy-1 (rat IgG2a) and Buffy-2 (rat IgM) (0.2 µg/ml) was revealed with peroxidase-coupled anti-rat IgM (H+L) secondary antibody. B) 293T cells were transiently transfected with a plasmid encoding full-length BAFF and thereafter cultured in serum-free medium. Five days later, various dilutions of cell lysate (left panels), concentrated supernatant (middle panels) were tested by Western-blot analysis with Buffy-1 (top panels) and Buffy-2 (bottom panels) at 1 µg/ml. Purified long BAFF was used as positive control (right panels). C) Stable clones of HEK-293 cells expressing full length human BAFF, or a fusion protein in which the C-terminal TNF-homology domain of BAFF was replaced by the corresponding region of human APRIL, were stained with Buffy-1 at 2 µg/ml, and analyzed by flow cytometry. Non-transfected HEK-293 cells were used as a negative control. D) Schematic representation of the proteins used in panels A-C. Epitopes recognized by Buffy-1 and -2 are indicated. Black rectangles represent a Flag tag. ID: intracellular domain. TMD: transmembrane domain.

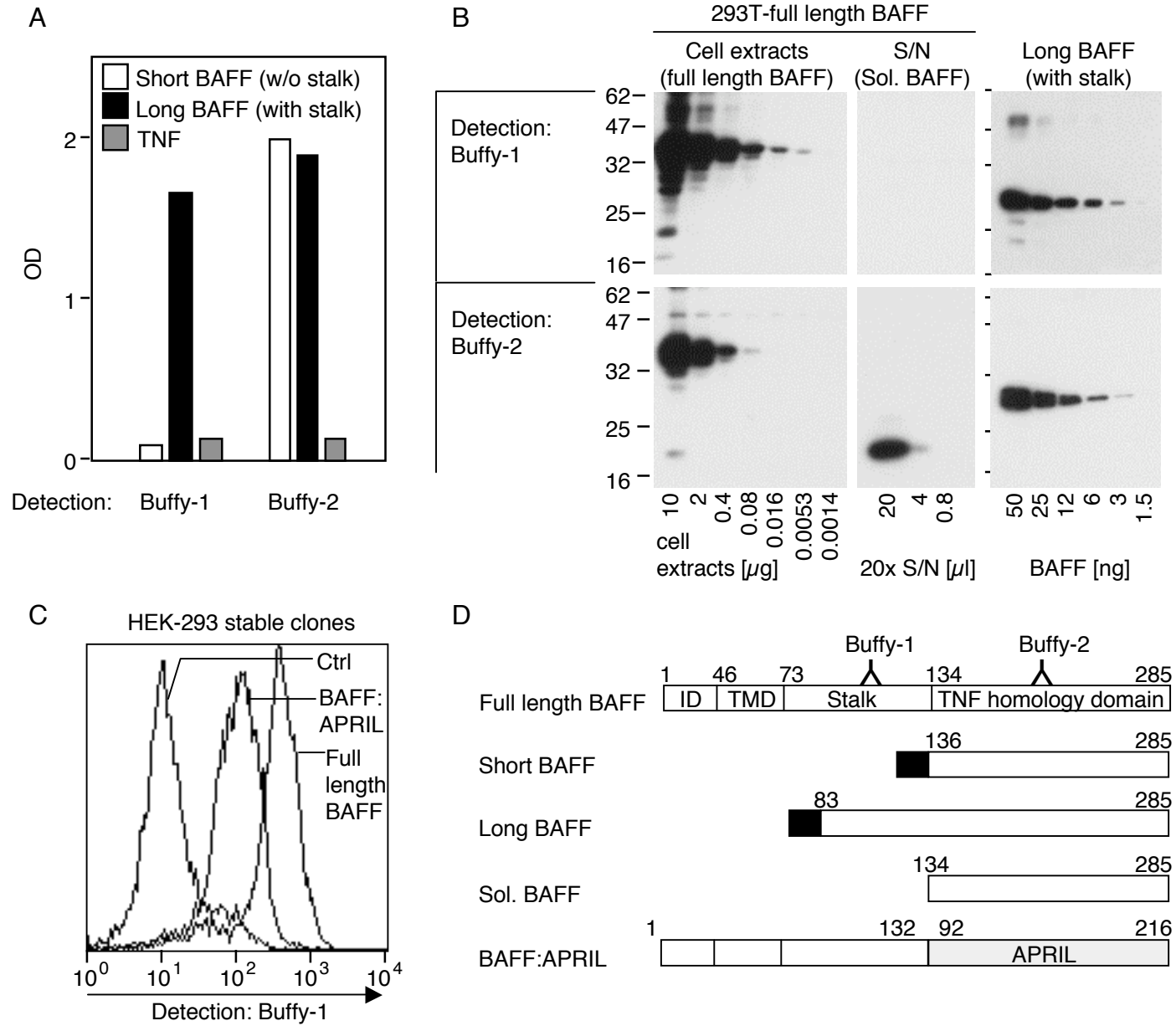


Figure 1