

AI-16 INTEGRATION OF BCR, TLR, BAFF RECEPTOR AND TACI SIGNALS PROMOTES AUTOANTIBODY PRODUCTION BY TRANSITIONAL B CELLS IN BAFF TRANSGENIC MICE

¹Du Samuel, ¹Jacobs Holly M, ¹Arkatkar Tanvi, ¹Scharping Nicole, ^{1,2,3}Rawlings David J, ^{1,2}Jackson Shaun W*. ¹Seattle Children's Research Institute, University of Washington School of Medicine, Seattle, WA, USA; ²Departments of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA; ³Immunology, University of Washington School of Medicine, Seattle, WA, USA

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Background The B cell survival cytokine BAFF has been linked with the pathogenesis of SLE. BAFF binds distinct B cell surface receptors, including the BAFF receptor (BAFF-R) and Transmembrane Activator and CAML Interactor (TACI). Although originally characterized as a negative regulator of B cell activation, TACI signals are critical for class-switched autoantibody production in BAFF transgenic (Tg) mice. Notably, while surface TACI expression is usually limited to mature B cells, we showed that excess BAFF promotes the expansion of TACI-expressing transitional B cells, with these cells representing an important source for class-switched autoantibodies in BAFF-Tg mice (Jacobs, et al. *J Immunol*, 2016). In the current study, we interrogate the signals required for transitional B cell TACI expression and BAFF-driven autoantibody production.

Methods We first used a new 'fate-mapping' strategy to confirm that activated TACI⁺ B cells falling within CD21^{lo} transitional flow cytometry gates are bone fide transitional B cells. Subsequently, we interrogated the B cell signals required for transitional B cell TACI expression and antibody production using relevant murine genetic models crossed on the BAFF-Tg background.

Results To confirm that immature, transitional B cells are a prominent source for class-switched autoantibodies in BAFF-Tg mice, we developed a CD21^{Cre}.ROSA-YFP^{fl/fl} reporter strategy. By irreversibly labeling B cells that have expressed CD21, we confirmed that transitional B cells that had not yet differentiated beyond the T2 stage spontaneously produce class-switched autoantibodies in BAFF-Tg animals. We next determined the signals required for TACI upregulation on T1 transitional B cells. Surprisingly, signals downstream of B cell (BCR) and Toll-like (TLR) receptors exerted distinct impacts on transitional B cells. Whereas loss of BCR signals in *Btk*^{-/-}.BAFF-Tg mice prevented transitional B cell TACI expression and resulted in loss of serum autoantibodies across immunoglobulin isotypes, lack of Myd88/TLR7 signals exerted a limited impact on autoantibody class switch recombination without impacting transitional B cell TACI expression. Moreover, in parallel with the protective effect of TACI deletion, loss of BAFF-R activation signals protected against BAFF-driven autoimmunity. Notably, integration of all these signalling cascades (BCR, TACI, TLR7/MyD88, and BAFF-R) is required for production of pathogenic class-switched autoantibodies in BAFF-Tg mice, and for development of BAFF-driven lupus nephritis.

Conclusions In summary, we highlight how distinct signaling pathways integrate to promote class-switched autoantibody production by transitional B cells, findings with implications to the understanding of SLE pathogenesis and other humoral autoimmune diseases characterized by elevated serum BAFF.

AI-17 RESPONSE GENE TO COMPLEMENT-32 EXPRESSION IS UPREGULATED IN LUPUS T CELLS AND PROMOTES IL-17A EXPRESSION

^{1,2}Anamaria Talpos-Caia, ^{2,3}Alexandru Tatmir, ^{2,3}Vinh Nguyen, ⁴Cornelia D Cudrici, ¹Simona Rednic, ^{2,3}Horea G Rus, ^{2,3}Violeta Rus*. ¹University of Medicine and Pharmacy 'Iuliu Hatieganu', Cluj-Napoca, Romania; ²University of Maryland School of Medicine, Baltimore, USA; ³Veterans Administration Maryland Health Care System, Baltimore, USA; ⁴NIAMS, National Institutes of Health, Bethesda, USA

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Background RGC (Response Gene to Complement)-32 is a cell cycle regulator widely expressed in normal tissues including brain, kidney, spleen, thymus, multiple tumors and in a variety of cell lines. RGC-32 is localized in the cytoplasm and translocates to the nucleus upon upregulation by complement activation, growth factors and cytokines. RGC-32 is induced by TGFβ in fibroblasts, astrocytes and human renal proximal tubular cells and mediates TGFβ dependent profibrotic pathways. RGC-32 is preferentially upregulated in murine Th17 cells and promotes their differentiation. Patients with Systemic Lupus Erythematosus (SLE) display increased serum levels, expanded frequency of IL-17 producing cells. Whether RGC-32 plays a role in human Th17 differentiation pathway and in Th17 abnormalities in lupus patients has not yet been investigated.

Methods RGC-32 expression in naïve CD4⁺ T cells from normal controls stimulated with cytokines alone or under Th0, Th1, Th2, Th17 and Treg conditions was determined by flow cytometry and RT-PCR. RGC-32 mRNA expression in PBMCs of lupus patients was assessed with the Autoimmune Disease Profiling cDNA Array spotted with cDNA from CD3⁺, CD19⁺ and CD14⁺ cells and by RT-PCR and flow cytometry. RGC-32 nuclear translocation after stimulation under Th17 conditions was assessed by Western blotting. RGC-32 overexpression and silencing was performed by nucleofection and the effect on IL-17A mRNA levels in CD4⁺ T cells under Th17 conditions was determined by RT-PCR.

Results RGC-32 mRNA expression was upregulated by TCR stimulation and TGFβ and was more robust under Th17 (3.2 ± fold) and Treg (2.6 ± 0.8 fold) vs Th1 (1.3 ± 0.4 fold) and Th2 (1.8 ± 0.1 fold) conditions. Moreover, upon stimulation with anti-CD3/CD28 and TGFβ, RGC-32 was translocated into the nucleus. Other cytokines such as IFNα, IL-1β, TNFα did not upregulate RGC-32 mRNA either alone or in combination with TCR stimulation. Overexpression or silencing of RGC-32 in CD4⁺ T cells upregulated, respectively downregulated IL-17A transcript levels and protein secretion. RGC-32 mRNA and protein level were significantly increased in CD19⁺ B cells and CD3⁺ T from lupus patients compared to controls.

Conclusions These results suggest that RGC-32 promotes the differentiation of human Th17 cells. Furthermore, T cells from patients with SLE exhibit increased expression of RGC-32 compared to controls. These data support the idea that RGC-32 signaling may enhance disease expression in SLE by promoting abnormalities in the Th17 pathway and provide a compelling rationale for further investigating the therapeutic potential of blocking RGC-32 in SLE.