DECREASED INTRACELLULAR CALCIUM FLUX IN FOLLICULAR HELPER T CELLS AFTER T CELL RECEPTOR STIMULATION

AI-15

Department of Internal Medicine (Rheumatology)

Abstract AI-16

THE ROLE OF FC IN THE BINDING OF ANTI-DNA ANTIBODIES TO DNA

Background Antibodies to DNA (anti-DNA) are the serological hallmark of systemic lupus erythematosus (SLE) and mediate pathogenesis via the formation of immune complexes. While the avidity of these antibodies is high, it depends on monogamous bivalency, a mode of antibody binding in which both IgG combining sites interact with an extended piece of DNA. In the current study, we investigated this interaction further by assessing the activity of Fab and F(ab’2) preparations of IgG from plasmas of SLE patients.

Materials and methods Using purified IgG, Fab fragments were generated by papain digestion while F(ab’2) fragments were prepared with pepsin. The binding to native calf thymus (CT) DNA was assessed by ELISA using an anti-human IgG (Fab specific) peroxidase reagent. In these experiments, the concentrations of IgG and fragments were determined on the basis of an equivalent number of binding sites. Control antibodies were tetrana and an EBV antigen preparation. IgG and fragments from normal human subjects were used as controls for binding to foreign antigens.

Results For each of the SLE IgG preparations studied, Fab and F(ab’2) fragments failed to bind significantly to DNA in the ELISA (Figure 1). In contrast, the Fab and F(ab’2) fragments were active against the tetrana and EBV antigens. The binding of the fragments from SLE patients to the foreign antigens was similar to that of normal human subjects.

Conclusions These results define a new pattern of anti-DNA binding. Since a Fab fragment can bind monovalently, a lack of MAP kinases following TCR stimulation, suggesting a specific alteration in the ability of Tfh cells to handle calcium.

Conclusion Our results demonstrate that Tfh cells have a selective defect in calcium mobilisation upon TCR stimulation. The altered calcium handling profile of Tfh cells likely contributes to the unique molecular program of these specialised cells. These results have important implications for designing therapeutic strategies to selectively target Tfh cells in autoimmune disease.

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BAFF PROMOTES SYSTEMIC AUTOIMMUNITY VIA TACI-DEPENDENT ACTIVATION OF TRANSITIONAL B CELLS

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Background Although excess levels of B cell activating factor of the TNF family (BAFF, also known as BlyS) have been implicated in the pathogenesis of SLE, how excess BAFF promotes breaks in B cell tolerance is not completely understood. Transgenic mice (Tg) overexpressing BAFF develop an autoimmune disease resembling human SLE. BAFF binds to distinct receptors on B cells, the BAFF receptor (BAFF-R) and transmembrane activator and C AML interactor (TACI). Since BAFF-R deletion results in loss of mature B cells, BAFF-R-dependent signals are presumed to explain BAFF-mediated autoimmunity. However, potential important roles for TACI in lupus pathogenesis have not been addressed.

Materials and methods After crossing BAFF-Tg and Tac1−/− mice, we used standard immunologic techniques to test the impact of TACI on BAFF-driven autoimmune.

Results Despite prior evidence of a negative role for TACI in B cell activation, we discovered that TACI deletion resulted in a striking loss of class-switched serum autoantibodies. Loss of serum autoantibodies also correlated with protection from immune-complex glomerulonephritis in Tac1−/−:BAFF-Tg mice. Importantly, lack of autoimmunity was not explained by alterations in peripheral B cell development, since both BAFF-Tg and Tac1−/−:BAFF-Tg mice exhibited similar B cell hyperplasia, with equivalent expansion of the follicular (FM) and marginal zone (MZ) compartments. Rather, whereas surface TACI expression is usually limited to mature B cells, we discovered that excess BAFF integrates with dual B cell receptor (BCR)- and MyD88-dependent signals to promote TACI upregulation on transitional B cells. The novel Tac1m subpopulation of transitional B cells from BAFF-Tg mice are characterised by an activated, cycling phenotype and expressed activation-induced cytideine deaminase (AID) and T-bet. Single-cell cloning of B cell receptors from Tac1m vs Tac1+ transitional B cells demonstrated that the Tac1m cell subset is specifically enriched for autoreactivity and exhibits evidence of somatic hypermutation. Finally, consistent with a direct role in autoimmunopathogenesis, Tac1m transitional B cells from BAFF-Tg mice spontaneously produce class-switched autoantibodies ex vivo.

Conclusion Our combined findings highlight a novel mechanism whereby BAFF promotes humoral autoimmunity via TACI-dependent activation of transitional B cells. In addition to SLE and other autoimmune disorders characterised by elevated BAFF, dysregulated transitional B cell activation is likely to be relevant a range of other clinical scenarios, including: autoimmune disease relapse after treatment with B cell-depletion therapies; de novo humoral autoimmunity following stem cell transplantation; and, rapid IgM- and IgG-mediated antibody responses during pathogen challenges.

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B CELL IFN-γ RECEPTOR SIGNALLING PROMOTES AUTOIMMUNE GERMINAL CENTRES VIA CELL-INTRINSIC INDUCTION OF BCL-6

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Background Dysregulated germinal centre (GC) responses are implicated in the pathogenesis of human autoimmune diseases, including systemic lupus erythematosus (SLE). Although type 1 interferons (IFNs) are most frequently associated with lupus pathogenesis, type 2 interferon (IFN-γ) has also been shown to promote SLE. However, the respective impacts of these cytokines in promoting B cell activation during humoral autoimmunity have not been addressed.

Materials and methods We recently developed a chimeric murine lupus model in which Wiskott-Aldrich syndrome protein (WAS)-deficient B cells promote spontaneous humoral autoimmunity (Jackson, et al. J Immunol 2014). An important advantage of the WAS chimaera model is that dysregulated immune responses are limited to the B cell compartment, allowing genetic manipulation in a cell-intrinsic fashion. In the current study, we contrast the impact B cell-intrinsic type 1 IFN vs. IFN-γ signals on autoimmune GC formation and the pathogenesis of SLE.

Results Although type 1 IFN prominently enhanced B cell responses in vitro, B cell-intrinsic IFNAR deletion exerted surprisingly minimal impacts on class-switched autoantibody titers and spontaneous GC formation in vivo. This finding suggested that other cytokines promote B cell activation in the WAS chimaera model. Notably, B cells directly initiated CD4+ T cell activation and T follicular helper cell formation via MHCClass II (MHC-II)-dependent antigen presentation. In addition, activated T cells exhibited prominent IFN-γ production that was lost following B cell-intrinsic MHC-II deletion, suggesting a direct role for IFN-γ in promoting autoimmune GC formation. Strikingly, B cell-intrinsic deletion of the IFN-γ receptor was sufficient to abrogate spontaneous GCs, class-switched autoantibodies and systemic autoimmunity. Mechanistically, although IFN-γ receptor signals increased B cell T-bet expression, B cell-intrinsic deletion of T-bet exerted an isolated impact on class-switch recombination to pathogenic IgG2c autoantibody subclasses without impacting GC development. Rather, in both murine and human B cells, IFN-γ synergized with BCR, TLR and/or CD40 activation signals to promote cell-intrinsic BCL-6 expression. Finally, IFN-γ driven BCL-6 expression in B cells was blocked using clinically-relevant Janus kinase inhibitors, ruxolitinib and tofacitinib.

Abstracts