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TLR7 Signalling: A Central Nexus in Autoimmunity and cGVHD

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TLR7 Signaling: A Central Nexus in Autoimmunity and cGVHD

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TO THE EDITOR:

Conclusive evidence supports the hypothesis that dysregulation in the intrinsic endosomal toll-like receptor (TLR) signaling of antigen-presenting cells, particularly RNA-sensing TLRs, plays a pivotal role in initiating and exacerbating autoimmune conditions, as well as instigating chronic graft-versus-host disease (cGVHD)[1]. B cells play a crucial role in the initiation and exacerbation of GVHD. Schultz et al. (1995) demonstrated that depleting B cells prevents the onset of GVHD in recipient mice[2]. Subsequent studies have delved into the molecular mechanisms and deciphered the role of TLR in cGVHD. The CpG-primed B cells express elevated levels of CD86, heightening the risk of GVHD[3]. Moreover, cell-free mitochondrial DNA, an endogenous TLR9 ligand, is significantly associated with GVHD risk[4]. Certain mutations in TLR4, a sensor for bacterial lipopolysaccharide, can mitigate GVHD, while excessive antigen-presenting cell activation leads to bacteremia. Additionally, polymorphisms in TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are strongly linked to GVHD[5]. These preliminary findings strongly suggest that intrinsic TLR signaling in B cells and antigen-presenting cells plays a crucial role in GVHD[2-5]. B-cell activation is initiated upon antigen recognition by the B-cell receptor (BCR). However, heightened responsiveness of the BCR has the potential of disrupting both central and peripheral self-tolerance mechanism, contributing to onset and exacerbation of cGVHD. TLR7's ability to recognize both pathogenic and self-single-stranded RNA (sRNA) moieties, its escape from X-chromosome inactivation, and its heightened intrinsic signaling in B cells contribute to its significance in autoimmune conditions. Targeting TLR7 signaling presents a promising approach for therapeutic advancement and containment of autoimmune responses. In their recent investigation conducted by Bracken *et al*., it was shown that BCR activated B cells from patients with cGVHD have aberrant response to TLR7 signalling compared with no cGVHD controls. The heightened TLR7 signalling primes BCR activated B cells in

CGVHD, characterized by the presence of various RNA binding antigenic proteins, activation of TLR7 downstream interferon regulatory factor (IRF)5, and production of anti-RNA/RNP autoantibodies[1]. The authors have provided insights into the molecular interaction, revealing that the binding of cGVHD-specific IgG to tripartite motif containing-21 (TRIM21; hereafter Ro52) relies on RNA moieties[1]. This observation supports the debatable hypothesis regarding whether Ro52 is an RNA-binding protein. Nevertheless, the direct binding of Ro52 to RNA remains a subject of debate. This debate will persist until a comprehensive delineation is achieved regarding the specific RNA molecules that bind with Ro52 and the domains within Ro52 that are implicated in these interactions. Are these RNA depending interactions between Ro52 and anti-Ro52 IgG are mediated by other RNA binding autoantigens? Ro60 is a known RNA binding protein that sequesters small RNA moieties, forming a ribonucleoprotein complex for intracellular transportation and metabolism. Anti-Ro52 and anti-Ro60 antibodies are common diagnostic markers in systemic lupus erythematosus (SLE) and Sjogren's syndrome. Does the work presented by Bracken *et al*. support the hypothesis that Ro52 is part of the Ro60-containing RNP complex, or does Ro52 form an independent complex with RNA moieties?[6] The observed heightened TLR7 expression in tissue lesions of cGVHD patients aligns with an increased TLR7 expression in peribronchiolar B cells, suggesting an elevated expression of TLR7 in circulating B cells among cGVHD patients. How does enhanced TLR7 expression modulate the ubiquitously expressed E3 ubiquitin ligase Ro52 and its antigenic presentation to IgGs in extra cellular space? Do Ro52 binding antibodies specifically recognize Ro52 in the extracellular space, or can these antibodies also recognize Ro52 intracellularly? Beyond cGVHD, anti-Ro52 antibodies are established diagnostic markers for various autoimmune disease including Sjogren syndrome, SLE, etc. Evidently, *in-vitro* stimulation of TLR7 in salivary gland epithelial cells (SGECs) leads to increased expression of Ro52, major histocompatibility complex (MHC-I), and proteins of

the peptide loading complex (PLC), suggesting a potential role of TLR signaling in peptide loading and antigenic presentation of Ro52[7]. However, TLR7 stimulation does not modulate Ro60 expression. Ro60 is a cognate RNA-binding protein, whereas Ro52 is not. In that scenario, how do RNA moieties mediate the binding of anti-Ro52 IgGs to Ro52 in cGVHD? Brackon *et al*. have identified that both untouched and in-vitro stimulated B cells from cGVHD patients have increased expression of IRF5 and interleukin (IL)-6. How does Ro52 modulate the TLR7-IRF-IL6 signaling axis, or is it modulated by it? Ro52 is an IFN inducible gene, and its expression is tightly regulated by IRFs[8,9], however, Brackon *et al* did not elucidate the status of interferons and interferon signalling in cGVHD. Ro52 is defined to exhibit dual functionality, serving both as an inducer and suppressor of inflammation[10]. The latter function is executed through ubiquitination, leading to the proteasomal degradation of IRFs and pro-inflammatory cytokines, including IL6. Ro52 ubiquities IRF5 and regulates its stability in isoform specific manner[11]. Therefore, transcriptional, and functional dysregulation of TRIM21 can potentially have a profound impact on dysregulation of TLR7-IRF-IL6 axis[1,12]. Imperatively, TLR7-IRF5 signaling can instigate transcription of various pro-inflammatory cytokines, type-I interferons, and interferon-stimulated genes (ISGs)[13]. Therefore, TLR7-IRF5 signaling should not be narrowly construed solely within the TLR7-IRF5-IL6 axis. The cGVHD patients exhibit heightened basal level of IL6. In-vitro BCR stimulation (by low-dose anti-IgM) results in increased IL6 production in B cells from cGVHD patients than comparator groups. This effect is further heightened by TLR7 activation, demonstrating synergism between BCR activation and TLR7 signaling in IL6 production. However, blockade of IRF5 (downstream to TLR7) does not completely abrogate IL6 production, suggesting involvement of alternative signalling pathways. The synergism between BCR and TLR signaling is known to activate both canonical and non-canonical NF-kB pathways, initiating activation-induced cytidine deaminase-mediated class switch

recombination[14]. Heightened expression of TLR7 in SLE patients finds association with newly formed transitional B cells ($CD19^{+}CD24^{++}CD38^{++}$) and production of anti-RNA/RNP autoantibodies, predominantly targeting antigens of nuclear compartment[15]. Like in SLE, in cGVHD, a large proportion of identified cGVHD autoantibodies predominantly target nucleic acid (RNA/DNA)-bound proteins, including aldehyde dehydrogenase 7 (ALDH7A1), pyruvate dehydrogenase E1 component alpha subunit (PDHA1), and protein kinase D3 (PRKD3)[1]. However, Ro52 is primarily located in the cytoplasm, and the discussion surrounding its RNA binding properties stems from interactions with the PRY/SPRY domain. B-cell activating factor (BAFF) is crucial for survival, maturation, and class switch recombination of B cells. At the same time BAFF also contributes to heightened BCR responsiveness in cGVHD patients[16]. Given that both BAFF and TLR7 play roles in BCR responsiveness, elucidating the intricacies of TLR7 responsiveness within the context of IgG-secreting cells, both pre-and post-germinal centre phase, is essential[17]. Moreover, the regulatory role of IRF4 and IRF8 in Ro52 expression (as IRF4 and IRF8 bind to TRIM21 promoter ISREs) and their involvement in central and peripheral B cell tolerance, as well as lineage commitment, warrant comprehensive elucidation in the context of cGVHD[9,18]. This understanding is pivotal for unravelling the regulatory mechanisms governing the interplay between BAFF, TLR7, and BCR signaling pathways in the dynamic processes of B-cell activation and antibody production in autoimmune diseases and cGVHD[1,19]. While Bracken *et al*. did not elucidate the basal level of BAFF in cGVHD, heightened BAFF levels in the given situation are associated with the onset of cGVHD. The post-transplantation BAFF/B cells ratio is a significant predictive measure for cGVHD prognosis[20]. Furthermore, heightened BAFF levels find its association with anti-Ro52 autoantibodies producing cells[21]. In autoimmune conditions such as Sjogren's syndrome and SLE, the escape of X-chromosome inactivation for TLR7 gene is frequently proposed as a potential mechanism

contributing to the increased prevalence of autoimmune diseases in females[22]. Alongside dysregulated TLR7 signaling, the co-occurrence of anti-Ro52 and anti-Ro60 autoantibodies is common diagnostic markers in various autoimmune diseases. Nevertheless, it is noteworthy to highlight that in the study conducted by Bracken *et al*., Ro60 was not identified as a target for anti-IgG antibodies. The precise elucidation of which IgG antigen targets potentially mediate RNA interactions, facilitating IgG binding with Ro52, remains inadequately defined in their investigation. In summary, Bracken *et al*. revealed the synergy between BCR and TLR7 signaling, emphasizing on centrality of TLR7-IRF5-IL6 axis in cGVHD. This is associated with the production of anti-RNA/RNP antibodies, including those against Ro52. Nevertheless, the specific RNA moieties mediating interactions between Ro52 and anti-Ro52 antibodies remain unclear. The mode of TLR7 sensing remains enigmatic, with uncertainties about whether RNA moieties are recognized as standalone small fragments or as components of tissue damage-associated RNA-protein complexes. Additionally, it is unclear if these complexes are presented as RNP complexes, RNP-autoantibody complexes, or are enclosed within membranous structures like extracellular vesicles. Unraveling questions raised in this commentary could significantly enhance our understanding of cGVHD and autoimmune conditions in general.

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