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TLR7 Signalling: A Central Nexus in Autoimmunity and cGVHD

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17 TO THE EDITOR:

Conclusive evidence supports the hypothesis that dysregulation in the intrinsic 18 endosomal toll-like receptor (TLR) signaling of antigen-presenting cells, particularly RNA-19 20 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 21 the initiation and exacerbation of GVHD. Schultz et al. (1995) demonstrated that depleting B 22 cells prevents the onset of GVHD in recipient mice². Subsequent studies have delved into the 23 molecular mechanisms and deciphered the role of TLR in cGVHD. The CpG-primed B cells 24 express elevated levels of CD86, heightening the risk of GVHD³. Moreover, cell-free 25 26 mitochondrial DNA, an endogenous TLR9 ligand, is significantly associated with GVHD 27 risk⁴. Certain mutations in TLR4, a sensor for bacterial lipopolysaccharide, can mitigate 28 GVHD, while excessive antigen-presenting cell activation leads to bacteremia. Additionally, polymorphisms in TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are strongly linked to 29 GVHD⁵. These preliminary findings strongly suggest that intrinsic TLR signaling in B cells 30 and antigen-presenting cells plays a crucial role in GVHD²⁻⁵. B-cell activation is initiated 31 upon antigen recognition by the B-cell receptor (BCR). However, heightened responsiveness 32 of the BCR has the potential of disrupting both central and peripheral self-tolerance 33 34 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-35 36 chromosome inactivation, and its heightened intrinsic signaling in B cells contribute to its significance in autoimmune conditions. Targeting TLR7 signaling presents a promising 37 approach for therapeutic advancement and containment of autoimmune responses. In their 38 recent investigation conducted by Bracken et al., it was shown that BCR activated B cells 39 from patients with cGVHD have aberrant response to TLR7 signalling compared with no 40 cGVHD controls. The heightened TLR7 signalling primes BCR activated B cells in 41

CGVHD, characterized by the presence of various RNA binding antigenic proteins, activation 42 of TLR7 downstream interferon regulatory factor (IRF)5, and production of anti-RNA/RNP 43 autoantibodies¹. The authors have provided insights into the molecular interaction, revealing 44 45 that the binding of cGVHD-specific IgG to tripartite motif containing-21 (TRIM21; hereafter Ro52) relies on RNA moieties¹. This observation supports the debatable hypothesis regarding 46 47 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 48 49 achieved regarding the specific RNA molecules that bind with Ro52 and the domains within 50 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 51 52 a known RNA binding protein that sequesters small RNA moieties, forming a 53 ribonucleoprotein complex for intracellular transportation and metabolism. Anti-Ro52 and 54 anti-Ro60 antibodies are common diagnostic markers in systemic lupus erythematosus (SLE) 55 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 56 complex with RNA moieties?⁶ The observed heightened TLR7 expression in tissue lesions of 57 cGVHD patients aligns with an increased TLR7 expression in peribronchiolar B cells, 58 59 suggesting an elevated expression of TLR7 in circulating B cells among cGVHD patients. 60 How does enhanced TLR7 expression modulate the ubiquitously expressed E3 ubiquitin 61 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 62 recognize Ro52 intracellularly? Beyond cGVHD, anti-Ro52 antibodies are established 63 diagnostic markers for various autoimmune disease including Sjogren syndrome, SLE, etc. 64 Evidently, in-vitro stimulation of TLR7 in salivary gland epithelial cells (SGECs) leads to 65 increased expression of Ro52, major histocompatibility complex (MHC-I), and proteins of 66

67 the peptide loading complex (PLC), suggesting a potential role of TLR signaling in peptide loading and antigenic presentation of Ro52⁷. However, TLR7 stimulation does not modulate 68 Ro60 expression. Ro60 is a cognate RNA-binding protein, whereas Ro52 is not. In that 69 70 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 71 72 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 73 inducible gene, and its expression is tightly regulated by IRFs^{8,9}, however, Brackon et al did 74 75 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 inflammation¹⁰. The 76 77 latter function is executed through ubiquitination, leading to the proteasomal degradation of 78 IRFs and pro-inflammatory cytokines, including IL6. Ro52 ubiquities IRF5 and regulates its stability in isoform specific manner¹¹. Therefore, transcriptional, and functional dysregulation 79 of TRIM21 can potentially have a profound impact on dysregulation of TLR7-IRF-IL6 80 axis^{1,12}. Imperatively, TLR7-IRF5 signaling can instigate transcription of various pro-81 inflammatory cytokines, type-I interferons, and interferon-stimulated genes (ISGs)¹³. 82 Therefore, TLR7-IRF5 signaling should not be narrowly construed solely within the TLR7-83 84 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 85 cGVHD patients than comparator groups. This effect is further heightened by TLR7 86 activation, demonstrating synergism between BCR activation and TLR7 signaling in IL6 87 production. However, blockade of IRF5 (downstream to TLR7) does not completely abrogate 88 IL6 production, suggesting involvement of alternative signalling pathways. The synergism 89 between BCR and TLR signaling is known to activate both canonical and non-canonical NF-90 kB pathways, initiating activation-induced cytidine deaminase-mediated class switch 91

recombination¹⁴. Heightened expression of TLR7 in SLE patients finds association with 92 newly formed transitional B cells (CD19⁺CD24⁺⁺CD38⁺⁺) and production of anti-RNA/RNP 93 autoantibodies, predominantly targeting antigens of nuclear compartment¹⁵. Like in SLE, in 94 cGVHD, a large proportion of identified cGVHD autoantibodies predominantly target nucleic 95 acid (RNA/DNA)-bound proteins, including aldehyde dehydrogenase 7 (ALDH7A1), 96 pyruvate dehydrogenase E1 component alpha subunit (PDHA1), and protein kinase D3 97 (PRKD3)¹. However, Ro52 is primarily located in the cytoplasm, and the discussion 98 surrounding its RNA binding properties stems from interactions with the PRY/SPRY domain. 99 100 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 101 responsiveness in cGVHD patients¹⁶. Given that both BAFF and TLR7 play roles in BCR 102 responsiveness, elucidating the intricacies of TLR7 responsiveness within the context of IgG-103 secreting cells, both pre-and post-germinal centre phase, is essential¹⁷. Moreover, the 104 105 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 106 lineage commitment, warrant comprehensive elucidation in the context of cGVHD^{9,18}. This 107 understanding is pivotal for unravelling the regulatory mechanisms governing the interplay 108 109 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 110 et al. did not elucidate the basal level of BAFF in cGVHD, heightened BAFF levels in the 111 112 given situation are associated with the onset of cGVHD. The post-transplantation BAFF/B cells ratio is a significant predictive measure for cGVHD prognosis²⁰. Furthermore, 113 heightened BAFF levels find its association with anti-Ro52 autoantibodies producing cells²¹. 114 In autoimmune conditions such as Sjogren's syndrome and SLE, the escape of X-115 chromosome inactivation for TLR7 gene is frequently proposed as a potential mechanism 116

contributing to the increased prevalence of autoimmune diseases in females²². Alongside 117 118 dysregulated TLR7 signaling, the co-occurrence of anti-Ro52 and anti-Ro60 autoantibodies is 119 common diagnostic markers in various autoimmune diseases. Nevertheless, it is noteworthy 120 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 121 mediate RNA interactions, facilitating IgG binding with Ro52, remains inadequately defined 122 in their investigation. In summary, Bracken et al. revealed the synergy between BCR and 123 124 TLR7 signaling, emphasizing on centrality of TLR7-IRF5-IL6 axis in cGVHD. This is 125 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 126 antibodies remain unclear. The mode of TLR7 sensing remains enigmatic, with uncertainties 127 128 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 129 130 complexes are presented as RNP complexes, RNP-autoantibody complexes, or are enclosed 131 within membranous structures like extracellular vesicles. Unraveling questions raised in this commentary could significantly enhance our understanding of cGVHD and autoimmune 132 conditions in general. 133

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