Multivalent virus-like epitope display amplifies BCR signaling independent of avidity

We show that multivalent epitope display on the surface of viral-sized particles functions as a 'stand-alone' danger signal by evading inhibitory pathways to trigger a unique mode of B cell receptor signaling. The activation, survival and proliferation of B cells stimulated with particulate antigen is highly enhanced compared with those stimulated with soluble antigen, and does not require co-stimulation from T cells.

This is a summary of:
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The project

The repetitive and ordered display of antigenic epitopes on viruses and virus-like particles induces rapid, robust antibody responses by rare and low affinity B cells even without help from T cells. The potency of such T cell-independent humoral responses to particulate antigens is thought to reflect extensive cross-linking of B cell receptors (BCRs) due to high-avidity interactions. These observations contributed to the development of HIV nanoparticle immunogens in clinical trials. However, the structural and molecular basis for BCR triggering by monovalent and multivalent antigens is unclear and might involve mechanisms other than avidity[1,2]. We sought to decipher how B cells sense and interpret multivalent epitope displays on particles of viral size, which might inform the design of future vaccine candidates.

The discovery

To test how B cells respond to particulate antigens, we engineered a library of synthetic virus-like structures (SVLS) consisting of liposomes decorated at a programmable density with the model antigen hen egg lysozyme (HEL)[3]. We used MD4 transgenic mice as a source of monoclonal B cells expressing a BCR that binds HEL with high affinity. To study physiological B cell–antigen contacts, we introduced mutations to HEL that reduced the affinity of this interaction, and generated SVLS with a broad spectrum of epitope densities that mimicked naturally occurring viruses. Coupled with cellular and molecular methods for studying B cell activation and signaling, this approach enabled us to interrogate how B cells interpret epitope affinity and density on viral-sized particles expressing HEL (pHEL) compared with matched affinity mutants of monovalent, soluble HEL (sHEL).

B cells responded to low-affinity mutants of pHEL at concentrations that were orders of magnitude lower than for sHEL (Fig. 1a). BCR signaling was amplified at several distal nodes in response to pHEL but not sHEL (for example, MAPK signaling, calcium signaling and NFκB activation were enhanced), leading to improved B cell survival and proliferation (as well as antibody production in wild-type mice immunized with pHEL[4]). Surprisingly, although key enzymes upstream of these amplified signal nodes (namely SYK, BTK and PI3K) were required for this response, they were only weakly engaged by pHEL, which suggests that extensive BCR cross-linking – which would be predicted to strongly engage these mediators – does not account for amplified signaling by pHEL. Instead, even a small number of particles (in contrast to soluble stimuli) could fully activate a B cell by selectively evading inhibitory co-receptors and their effector phosphatases downstream of the Src family kinase LYN[5] (Fig. 1b). Particulate antigen triggered a unique amplification step to produce maximal 'all-or-none' signaling downstream of BCRs, distinct from the response to soluble antigen and independent of BCR cross-linking and the nucleic acid cargo of bona fide viruses. This finding revealed an unexpected mechanism for the potency of virus-like antigen display.

The implications

We have identified unique signal amplification initiated by particulate antigen, which mimics the co-stimulation typically delivered by helper T cells. This finding explains how particulate antigens (but not soluble antigens) induce rapid B cell responses independently of T cells. Our data challenge the assumption that the potency of multivalent antigens is exclusively due to avidity and extensive cross-linking of antigen receptors on B cells. Instead, B cells can respond to particles of viral size as a 'stand-alone' danger signal by amplifying the BCR signaling cascade via evasion of inhibitory pathways. This mechanism enables even anergic, self-reactive B cells to respond to self-mimicking epitopes on a virus, and should inform the design and optimization of vaccines with a particulate platform.

Future work will identify the LYN-dependent inhibitory co-receptors and phosphatases evaded by particulate antigen and the structural basis for such evasion. We provide circumstantial biochemical evidence that signals are amplified by the accumulation of membrane PIP3, but this theory needs further testing. Not all signaling differences between sHEL and pHEL were accounted for by the evasion of LYN (including the upregulation of NFκB signaling), which indicates that other mechanisms exist. Our study suggests that even a small number of particles can trigger a maximal B cell signal; future work will define the minimal number of particles and BCRs required to activate a B cell for each BCR isotype.

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EXPERT OPINION

"The authors study B cell responses to antigens that are structurally organized to mimic a viral particle. They find that (low numbers of) high-avidity interactions of B cell receptors with a particular antigen can elicit a potent B cell response, which has several unique characteristics that explain its independence of T cells. This manuscript has interesting implications for our understanding of B cell responses to viral particles, vaccine design and loss of B cell tolerance." **Andrew Getahun, University of Colorado, Aurora, CO, USA.**

FIGURE

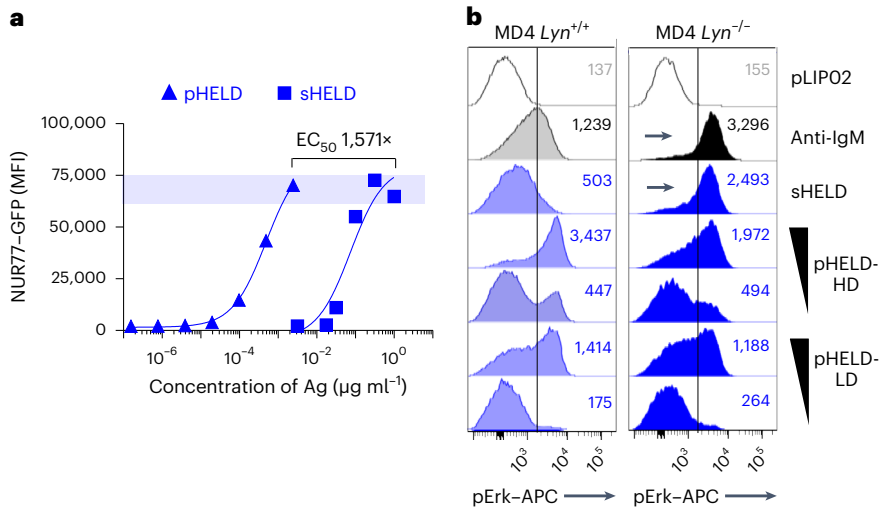

Fig. 1 | pHEL induces potent B cell signaling that evades LYN-dependent inhibitory pathways. **a**, Expression of the NUR77–GFP reporter of B cell activation in MD4 B cells expressing HEL-specific BCR revealed a large potency gap between pHELD and sHELD (HELD is a lower affinity mutant of HEL) when comparing broadly titrated doses of antigen (Ag) 24 h after stimulation. MFI, mean fluorescence intensity. **b**, Erk phosphorylation (measured via phosflow with antibody conjugated to allophycocyanin; pErk–APC) after 20 min BCR stimulation in LYN-deficient (MD4 *Lyn*$^{-/-}$) compared with LYN-sufficient (MD4 *Lyn*$^{+/+}$) B cells revealed that potency gaps are partly accounted for by evasion of inhibitory pathway activation by pHEL but not sHEL. HD, high density; LD, low density; IgM, anti-IgM isotype BCR antibody; pLIP02, naked liposome without Ag. © 2023, Brooks, J. F. et al.

BEHIND THE PAPER

Two essential attributes of SVLS enabled us to unravel the features of virus-like antigen display that influence B cell activation. First, SVLS are constructed from highly purified components, which ensures that they are nucleic acid-free in this study. Second, the affinity and density of the SVLS epitope display can be programmed[3]. Early on we discovered a notable potency gap between sHEL and pHEL, and found that B cells were remarkably sensitive to pHEL decorated with extremely low-affinity epitopes. Collaborative NIH grants funded a mechanistic study based on these observations; we were surprised by the scale of biochemical and functional differences between soluble and particulate antigens. Our 'Eureka' moment occurred when a pilot phosflow experiment showed that LYN-dependent inhibition was completely bypassed by pHEL but not sHEL. The overlap of our findings with fundamental B cell tolerance mechanisms was serendipitous given our long-standing interest in this area. **J.F.B., J.Z. & W.C.**

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FROM THE EDITOR

"While B cells have developed anergy as a method to safely retain self-reactive clones in the mature repertoire, here the authors show that antigen-loaded viral-sized liposomes (SVLS) can activate anergic B cells. These findings help to explain how self-reactive B cells remain inert in the face of soluble self-antigen, but responsive to viral display of the very same epitope since SVLS evade the precise signaling machinery that imposes anergy." **Laurie A. Dempsey, Senior Editor, *Nature Immunology*.**