PNAS COMMENTARY

# Joined at the hip: The role of light chain complementarity determining region 2 in antibody self-association

Paul R. Sargunas[a] and Jamie B. Spangler[a,b,c,d,e,f,g,h,1]

The amplification of weak transient protein–protein interactions to elicit robust immune responses is a recurring theme in immune processes (1) (Fig. 1). Given the often highly repetitive nature of pathogenic targets, antibodies have evolved architectures to allow for avidity-driven augmentation of immunological mechanisms: for instance, increased-valency antibody geometries such as the dimeric immunoglobulin A (IgA) (2) and the pentameric IgM (3). More rarely, variable domain swaps in broadly neutralizing antibodies (bnAbs) can drive the formation of nontraditional IgG dimers with greatly enhanced, multivalent engagement of the target antigen (4).

There is increased interest in understanding transient mechanisms that utilize weak homotypic interactions between antibody molecules to increase avidity via self-association. Known examples of antibodies leveraging homotypic interactions include hexamerization of antibody fragment crystallizable (Fc) regions to activate the complement cascade (5) and interactions between antibody fragment antigen-binding (Fab) regions to form Fab-dimerized glycan-reactive bnAbs (6). Delineating the molecular mechanisms driving homotypic interactions could enable their exploitation for rational design of agonist antibodies that mediate a variety of biological functions. Moreover, from a drug discovery and biomanufacturing point of view, antibody self-association via homotypic interactions finds further importance through its impact on biophysical properties, presenting a largely untapped lever for developing therapeutics with superior drug-like qualities. In PNAS, Leonard et al. (7) investigated receptor agonism systems that are sensitive to oligomerization to study antibody homotypic interactions and identified genetic determinants of self-association, pinpointing the light chain complementarity determining region 2 (CDRL2) as a key player in dictating homotypic avidity properties.

## Germline CDRL2 Dictates Self-Association

To interrogate antibody homotypic interactions, Leonard et al. (7) used an antibody (KMTR2) that agonizes its ligand, death receptor 5 (DR5), via receptor clustering without the need for extrinsic cross-linking. Through structure–activity relationship (SAR) mapping of KMTR2 point mutants to DR5 binding affinity, the authors functionally decoupled antigen binding from Fab–Fab interactions for the KMTR2 light chain, implicating several residues within and proximal to CDRL2 as important for stabilization of the Fab–Fab interface. Until now, the role of CDRL2 in antibody function was largely unknown given that this motif rarely contributes to antigen binding when compared with other CDR loops (8).

A salient feature of KMTR2 is that its sequence is strikingly similar to germline, including a light chain variable region that is 100% identical to the germline immunoglobulin kappa variable 3-11 (IGKV3-11) sequence. To investigate whether CDRL2 homotypic interfaces are characteristic of IGKV3-11 light chains, Leonard et al. (7) identified another antibody (the anti-4–1BB antibody urelumab) with almost 100% similarity to the IGKV3-11 germline sequence, which also promotes clustering of its target antigen. SAR mapping again identified CDRL2 as a main driver of Fab–Fab interactions between antibody molecules (Fig. 1). Interestingly, despite a common germline sequence and CDRL2-driven interface, KMTR2 and urelumab displayed different self-association binding modes.

As many biological systems require higher-order clustering for signaling, tunable determinants of self-association would be a powerful tool in the antibody engineering toolbox. To test whether self-association of the IGKV3-11 CDRL2 was a transferrable property, Leonard et al. (7) grafted this loop into a panel of anti-OX40 antibodies in place of their native CDRL2 loops. A majority of the grafted clones showed strong antigen clustering and agonism, and this effect was independent of both affinity and epitope. Further still, the authors showed that Fab–Fab interactions induced by IGKV3-11 CDRL2 engraftment were synergistic with other engineered homotypic interactions, promoting higher levels of receptor agonism when engraftment was paired with mutations that promote IgG Fc hexamer formation. Thus, transplantation of the IGKV3-11 CDRL2 sequence into other antibodies represents a promising approach for increasing antibody avidity effects.

## Homotypic Interactions Are Broadly Encoded by CDRL2

Although the nature of antibody–antigen interactions is random and combinatorial, it is not completely chaotic; antigens often bind at the site where all six CDRs spatially

Author affiliations: [a]Department of Chemical & Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218; [b]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218; [c]Translational Tissue Engineering Center, Johns Hopkins University, Baltimore, MD 21231; [d]Department of Oncology, Johns Hopkins University, Baltimore, MD 21231; [e]Bloomberg–Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University, Baltimore, MD 21231; [f]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, Baltimore, MD 21231; [g]Department of Ophthalmology, Johns Hopkins University, Baltimore, MD 21231; and [h]Department of Molecular Microbiology & Immunology, Johns Hopkins University, Baltimore, MD 21231

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[1]To whom correspondence may be addressed. Email: jamie.spangler@jhu.edu.

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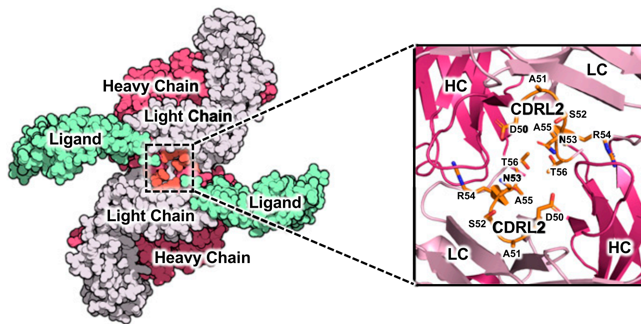


**Fig. 1.** Antibody light chain (LC) CDR2 drives antibody homotypic interactions. The Fab–Fab dimer interface of the anti-4–1BB antibody urelumab binding to its target ligand (Protein Data Bank ID code 6MHR) (1) reveals homotypic packing of antibody LCs (light pink) primarily mediated by CDRL2 loop residues (orange). Mutations to these residues did not affect antibody affinity for the target antigen but resulted in the loss of antibody self-association–mediated signaling activity, implicating them in the homotypic interface. HC, heavy chain.

converge, with the heavy chain CDR3 (CDRH3) loop most often driving antigen binding (8, 9). CDRL2 rarely participates in antigen binding and is mostly utilized only in the case of very large antigens (8). Due to the germline nature of the KMTR2 and urelumab CDRL2 sequences, Leonard et al. (7) hypothesized that CDRL2 has a natural propensity toward augmenting homotypic interactions between antibody Fabs across all germlines. To evaluate this theory, the authors grafted every known germline CDRL2 sequence onto an anti-OX40 antibody clone and assessed receptor agonism. In addition to IGKV3-11, a number of germline CDRL2 sequences exceeded the expected affinity–activity correlation. This result was further validated through the analysis of de novo–designed CDRL2 sequences, which biased antibodies toward improved self-association properties independent of their predicted target affinities.

Given the conserved function of CDRL2 in driving homotypic interactions, one would expect this region to be less involved in antibody–antigen binding and to have a lower rate of somatic hypermutation compared with other CDRs in order to maintain Fab–Fab association during B cell clonal selection. To this end, Leonard et al. (7) showed that, consistent with past reports (8, 10), CDRL2 was the least involved of the six CDRs with respect to antigen binding and had the lowest number of mutations from the nearest germline sequence, further emphasizing the conserved role of CDRL2 in mediating homotypic interfaces.

## Implications for Antibody Drug Discovery and Development

Therapeutic antibodies and antibody fusion proteins represent a prominent and rapidly growing class of pharmaceuticals, with monoclonal antibodies alone accounting for ∼20% of new Food and Drug Administration (FDA) approvals each year (11). The specificity, multitiered mechanistic activities, and extended serum persistence of antibodies enhance their value as drugs. A particularly valuable feature of antibodies is their bivalency, which allows for avidity-mediated enhancement of antigen binding, both to carry out natural functions in disease clearance and for use as targeted therapeutics. Previous work has shown evidence of higher-order avidity effects through antibody self-association or clustering, particularly in the instance of agonistic antibodies (12–14). The critical knowledge provided by Leonard et al. (7) with respect to encoded homotypic interactions will have important near- and long-term implications for the fields of antibody discovery and development.

There is revitalized interest in the generation of agonist antibodies, with emerging work focusing on high-throughput experimental and computational discovery workflows (14, 15). Agonist antibodies that activate costimulatory receptors such as CD28, ICOS, OX40, and 4–1BB offer a promising avenue to stimulate anticancer immune responses (12), and these approaches have great potential for synergy with other immunotherapeutic modalities, such as immune checkpoint blockade, cell therapies, and engineered cytokines. In addition, numerous studies have developed receptor-targeted antibodies that recapitulate the signaling activity of natural ligands, such as growth factors and cytokines (16–20), and there is growing interest in translation of this approach for biomedical applications. A recent study by Yen et al. (21) reported a general strategy to screen for antibody-based cytokine receptor agonists and exploited this strategy to enforce a nonnatural interaction between components of the interleukin-2 (IL-2) and IL-10 receptor complexes that led to functional signaling. It will be interesting to explore the extent to which clustering contributes to the activity of these previously reported agonistic antibodies and to correlate this with CDRL2 sequences. Moreover, the findings from Leonard et al. (7) can be applied to alter homotypic interactions between Fab regions in these systems in order to modulate the functional properties of these antibodies.

On the flip side, there are scenarios in which one would wish to avoid homotypic interactions, for instance in cases where such associations result in aggregation or other liabilities that could impede biomanufacturing and clinical development. There is tremendous interest in modifying antibodies early on in the development pipeline to increase specificity, reduce immunogenicity, and improve stability (22–24). Although Leonard et al. (7) did not observe detrimental effects on developability properties of a CDRL2-engrafted antibody in preliminary studies, more extensive investigation will be required to fully elucidate the effects of homotypic interactions on antibody developability. Understanding the relationship between CDRL2 sequences and antibody self-association will be critical to designing antibodies that avoid potential hurdles to clinical translation. Furthermore, with the meteoric rise of computational prediction and design approaches in antibody engineering (25), the bioinformatics insights from this work could be harnessed to fine-tune these algorithms. Collectively, Leonard et al.'s (7) fundamental discovery about CDRL2 regions in natural antibody repertoires promises to have significant and wide-ranging impacts on the thriving antibody therapeutic space.

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