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The unique and immunoglobulin-like regions of surrogate light chain component λ5 differentially interrogate immunoglobulin heavy-chain structure

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ABSTRACT

PreBCR signaling is critical for B cell development and normally depends on the association of a nascent, component Ig H chain with the surrogate L chain (SLC), which helps ensure that only B cells that synthesize structurally sound antibody can develop. How the invariant and λ-like SLC vets billions of unique V_H domains for compatibility with polymorphic κ and λ L chains is unclear, because the SLC is composed of not only the Ig domains of VpreB and λ5, but also the unique regions (URs) that reside at what would be the L chain CDR3. We evaluated the contribution of the Ig and UR domains of λ5 to H chain screening by evaluating the preBCR-forming capability of λ5 mutants with a diverse panel of H chains. Using transformed mouse B cells, we demonstrate that the Ig domain of λ5 was sufficient and its UR dispensable for the rejection of V_HQ52 and V_H10 SLC-incompatible H chains. In contrast, the λ5 UR was necessary to discriminate between SLC-incompatible and -compatible V_H81X H chains. Substituting the Ig domains of λ5 with equivalent κ sequences impaired the SLC's ability to escort all H chains to the surface. Two SLC-incompatible H chains were able to form surface BCRs with two κ L chains, indicating that the SLC's ability to predict the L chain compatibility of a H chain is not absolute. In sum, λ5 differentially relies on the λ-like Ig and UR to probe H chain structure to best accommodate diversity among H chains.

1. Introduction

Effective humoral immune responses depend upon a highly polymorphic and anticipatory antibody repertoire. The process of creating such a repertoire must balance structural integrity with antigen binding diversity. The gene encoding one of the two antibody components, the H chain, is created first in B cell development by V(D)J recombination (Chen and Alt, 1993). Although millions of genetically unique H chain genes can be made, the nature of the recombination process does not guarantee that all of them encode for a functional, structurally sound protein. Testing the functionality and structural integrity of H chains occurs first at the pre-B cell receptor (preBCR) checkpoint (Melchers, 2005). Generally speaking, the ability of H chains to form surface, signaling-competent preBCRs with a surrogate L chain (SLC) ensures the positive selection and developmental progression of B cell clones that created such H chain genes (Martensson et al., 2007; Vettermann et al., 2006). This is because, with few exceptions (so-called "SLC-independent" H chains; e.g., Minegishi and Conley, 2001; Shimizu et al., 2002), H chains cannot leave the endoplasmic reticulum (ER) to form signaling competent receptors without associating with SLC (or L chain). PreBCR signaling then activates V(D)J recombination-mediated assembly of the gene encoding L chain. The L chain gene is deemed functional if the L chain product, in turn, can form a surface, signaling-competent BCR with the H chain. The preBCR checkpoint, therefore, is one of the several checkpoints that ensure the development of a B cell repertoire that produces structurally sound antibodies: a prerequisite to adaptive humoral immunity.

Approximately 50% of functionally rearranged H chain genes encode for proteins that are SLC-incompatible but not SLC-independent, and, consequently, B cells expressing these H chains are never incorporated into the antibody repertoire (ten Boekel et al., 1997). The structural flaw of such H chains resides within their variable domains, and, in particular, within their HCDR3 loops, an antigen binding motif created by V(D)J recombination and responsible in large part for antigen binding specificity (Decker et al., 1995; Jemmerson et al., 1993; Martin et al., 1994; Xu and Davis, 2000). Since many of these H chains prove to be incompatible with a variety of L chains, the model extrapolated is that the SLC screens H chains for future L chain compatibility and shapes the antibody

Abbreviations: preBCR, precursor-BCR; SLC, surrogate L chain.

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repertoire so as to insure its functionality (Kline et al., 1998). Since SLC-compatible and -incompatible H chains can differ by as few as one residue in their HCDR3 loops (Kawano et al., 2005), it is not intuitive how HCDR3 loop structure regulates SLC–H chain pairing. Extensive structural analyses of antibodies suggest that the HCDR3 loop interacts with several L chain structures, including the LCDR3 loop (Padlan, 1994; Schroeder and Kirkham, 2000). Interactions between HCDR3 and LCDR3 structures may regulate H chain–L chain pairing, just as LCDR3–LCDR3 interactions are known to regulate L chain dimerization (Aburatani et al., 2002; Stevens et al., 1980).

The SLC, however, has no LCDR3; rather its absence highlights two key structural differences between the SLC and all conventional L chains. First, the SLC is composed of two germline-encoded peptides, VpreB and λ5, that associate non-covalently to form the canonical two Ig domain structure seen with all L chains. Second, the C-terminus of VpreB and the N-terminus of λ5 consist of highly charged, non-Ig, unique regions (URs) that collectively occupy the location where the LCDR3 would reside (reviewed in Melchers, 1999, 2005). Indeed, a recent crystallographic analysis of the human preBCR has shown that HCDR3 structures and the URs physically interact (Bankovich et al., 2007). Nevertheless, how the SLC's URs contribute to the interpretation of H chain structure as L chain-compatible or -incompatible based on what appear to be subtle single amino acid differences is not clear. Moreover, it is peculiar that the SLC's C_L domain is virtually identical to λ, since the SLC screens H chains for compatibility with what would be mostly κ L chains in the mouse.

Given the pivotal role of the SLC in screening H chain structure and the importance of HCDR3 and LCDR3 loop structures in mediating antibody formation, we sought to clarify the roles of the λ5 Cλ-like Ig domain and its UR in vetting H chain structure. Our data suggest that the λ5 Ig domain vets for overall structural integrity, whereas the λ5UR screens the fine structure of the HCDR3. Moreover, the wild-type SLC consistently outperformed chimeric SLC molecules in which Cλ was substituted with Cκ at bringing functional H chains to the surface. This suggests that the λ-like sequences of the SLC enable the SLC to escort a broader spectrum of potentially functional H chains to the signaling-active compartments. Unexpectedly, certain SLC-incompatible H chains formed surface BCRs with functional κ L chains, indicating that the SLC's ability to predict a H chain's L chain compatibility is imperfect.

2. Materials and methods

2.1. Plasmids

The creation of cDNAs encoding mouse μH chain V_H186.2, λ5, and the UR-deleted JCλ5 have been described previously (Fang et al., 2001). The cDNAs encoding for the κ L chain constructs from MOPC21 and MPC11, the λ5UR–JC κ proteins, the JCκ proteins, and the various H chain constructs V_H186.2 (Cherayil et al., 1993), V_HQ52 (ten Boekel et al., 1997), V_H81X(H) (Kawano et al., 2005), V_H81X(Y) (Kawano et al., 2005), V_H12–10/G4 (Ye et al., 1996), V_H12–12/G3,7 (Ye et al., 1996), were created by standard PCR overlap extension strategies (Horton et al., 1990). The SLC/L chain cDNAs were subcloned into MSCViresGFP (MiG; Van Parijs et al., 1999), a murine retroviral vector that expresses a bicistronic message encoding the gene of interest followed by an internal ribosomal entry site (IRES) and a cDNA encoding Green Fluorescent Protein (GFP). The H chain cDNAs were subcloned into MiH chainD4Δ, a version of MiG in which the IRES–GFP sequence was replaced with an alternative IRES and a cDNA encoding for a mutated, trafficking-competent, signaling-inert form of human CD4 (H chainD4Δ), or MiG lacking any IRES-marker gene. The IRES-H chainD4Δ sequence was obtained from the pMACS 4.1 vector (Miltenyi Biotech). All constructs were confirmed by sequence analysis (Genewiz).

2.2. Animals

The C57BL/6J Rag1$^{-/-}$ and λ5$^{-/-}$ (λ5T) mice have been described (Kitamura et al., 1992; Mombaerts et al., 1992). All animals were maintained under specific pathogen-free conditions in the mouse barrier facility at SUNY Downstate Medical Center in Brooklyn, NY. All animal care and experiments were performed in accordance with guidelines approved by the Institutional Animal Care and Use Committee of SUNY Downstate Medical Center.

2.3. Cell lines

Human embryonic kidney epithelial 293 (HEK293) cells were grown in DMEM (Cellgro) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco-Invitrogen) and 1% penicillin–streptomycin and L-Glutamine mixture (PSG) (Cellgro). The v-abl transformed Rag1$^{-/-}$ λ5$^{-/-}$ pro-B cell line was created by infection of bone marrow of an adult Rag1$^{-/-}$ λ5$^{-/-}$ mouse with helper-free v-abl retrovirus (Pear et al., 1993) and selection of a clone by established methods (Alt et al., 1981).

2.4. Retroviral infections

Retrovirus-containing supernatants were produced by calcium phosphate-mediated cotransfection of HEK293 cells with retroviral constructs and pΨECO, a plasmid encoding ecotropic helper functions (Pear et al., 1993). V-abl transformed pro-B cells were spin-infected with harvested supernatants using standard methods (Pear et al., 1993). Briefly, supernatants and polybrene, at a concentration of 4–8 μg/ml of culture, were added to 0.5–2 × 10^6 cells per well in 12-well or 24-well plates; plates were spun at 2000 rpm at 25 °C for 1.5 h. Supernatants were replaced with fresh growth medium 16–24 h later. In the case of double infections, H chain infections were performed first and then SLC infections were performed 24–48 h later. In the case of v-abl pro-B cells, cells were analyzed via flow cytometry 24 h after the second infection and via western blotting 48–72 h after the second infection.

2.5. Flow cytometry

For surface staining, 2–5 × 10^4 cells were incubated on ice with 0.5 μl of antibody in 50 μl of FACS buffer (FB: 1× PBS/1% Normal Goat Serum/1% Fetal Bovine Serum; 0.2% w/v NaN_3) for 1 h, washed twice with FACS buffer, and resuspended in 50 μl of FACS buffer and diluted in 400 μl of 1× phosphate buffered saline (PBS) just before analysis. Acquisition of 10^4 events per sample was performed with FACScan (Becton Dickinson Biosciences) and acquired data were analyzed with Cell Quest software. Only cells in the high forward scatter and low side scatter ranges were considered viable and suitable for analysis. Of those, cells within uniformly set parameters for GFP and ΔCD4 expression were gated for analyses of preBCR component expression. For detection of intracellular epitopes, cells were fixed and then permeabilized: 1 × 10^6 cells were resuspended in 875 μl of 1× PBS and 125 μl of 2% paraformaldehyde, vortexed immediately, and incubated on ice in the dark for 1 h. Cells were pelleted at 4 °C at 400 g for 5 min. Cell pellets were resuspended in 1 ml of 0.4% (v/v) Tween-20 in 1× PBS solution and incubated at 37 °C for 15 min. After pelleting (as above), cells were washed once in FACS buffer and resuspended in an appropriate volume of FB/staining antibody mixture. The following antibodies were used to stain cells for flow cytometric analysis: