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Surrobodies with Functional Tails

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Surrobodies[2] are a unique type of binding protein based on the pre-B-cell receptor (pre-BCR). The pre-BCR is transiently expressed during development of the antibody repertoire. Unlike heterotetrameric canonical antibodies that are composed of identical pairs of heavy and light chains, the pre-BCR is a heterohexameric complex composed of identical pairs of heavy chains that are each paired with a two-subunit surrogate light chain (SLC). The SLC contains nonimmunoglobulin-like peptide extensions on each of the two SLC components. This arrangement provides unique opportunities for protein engineering by functional derivatization of these nonimmunoglobulin-like tails. Here we report recombinant fusions to these tails with either a fully active cytokine or with single-chain variable fragment (scFv) domains to generate Surrobodies with unique functions or Surrobodies that are bispecific with respect to targeted binding.

Keywords: Surrobody; surrogate light chain; bispecific; combinatorial libraries

Introduction

The development of combinatorial antibody libraries has essentially solved the problem of generating human antibodies in the test tube.[1] Nowadays, human libraries that have diversities exceeding that of natural repertoires by many orders of magnitude have been prepared in academic and commercial laboratories. The antibodies isolated from these libraries have affinities that are at least as good as those obtained by immunization and have the advantage that their isolation is not subject to the constraints imposed by immunological tolerance.[2–5] When synthetic complementarity-determining regions (CDRs) are used, the time-honored practice of harvesting antibodies from living organisms, and indeed the process of immunization itself, is no longer necessary. The general experience with these fully human antibodies is one of rapid clinical development time lines and high levels of safety and efficacy in the clinic, as compared to small-molecule approaches.[1] The considerable productivity and value of antibodies generated from such combinatorial collections are now being appreciated in therapeutic areas such as oncology and inflammation where one fully human antibody is approved (Humira), and others have completed successful late-stage clinical trials (e.g., Raxibacumab, Benlysta, and ABT-874).[1] Nevertheless, for technical and logistical reasons, attention has now turned to the use of libraries to explore how alternative protein scaffolds or modification of canonical antibodies might be used to produce therapeutic binding proteins with properties that, in some aspects, may be superior to those of canonical immunoglobulins.[6–12] Furthermore, the modular construction of proteins that are bispecific is considered a major goal because such molecules could have enhanced therapeutic utility, specificity, and affinity compared to monospecific antibodies or monospecific scaffolds.

Our approach to the problem of constructing a binding protein platform with desirable modular properties centers on the exploitation of a molecule that appears during development of the antibody repertoire, the surrogate light chain (SLC).[13] The SLC

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Abbreviations used: Cdr, complementarity-determining region; SLC, surrogate light chain; scFv, single-chain variable fragment; SEC, size-exclusion chromatography; HA, hemagglutinin; HGF, hepatocyte growth factor; PBS, phosphate-buffered saline; HRP, horseradish peroxidase; CHO-K1, Chinese hamster ovary K1.

pairs with a rearranged heavy chain prior to light-chain V-J rearrangement and functions largely as a quality control checkpoint to eliminate nonfunctional heavy chains from the repertoire.[14–21] The SLCs differ physically from normal antibody light chains in two important ways. First, unlike mature antibody light chains where the V_L and C_L domains are fused following recombinant gene rearrangement to encode a single polypeptide, the comparable regions of the SLCs contain two separate molecules termed VpreB and λ5, respectively (Fig. 1a), that are encoded by separate genes. Second, VpreB and λ5 both have long nonimmunoglobulin-like peptide extensions. For VpreB, the peptide extension is present on the C-terminus of the mature protein in an area that coincides structurally where one would expect to find the starting point of a CDR3 loop in a classic antibody light chain. In the case of λ5, the peptide extension is present at the N-terminus of the mature λ5 protein in an area where one would expect to find the termination of a CDR3 loop in a classic antibody light chain. Since the extensions are present in areas that form loop structures in antibodies, it was not surprising to see that these peptide extensions are not directly involved with pairing of the SLC components with the heavy chains.[22,23] Consequently, these peptide extensions offer the opportunity to add a wide variety of potentially useful functions to the Surrobody without interfering with the primary binding pockets formed by the heterodimeric pairing between heavy-chain variable domains and the VpreB protein. In previous work, we have shown that large combinatorial libraries composed of heavy chains paired with SLCs can be constructed and molecules that bind strongly to targets can be harvested by, for example,

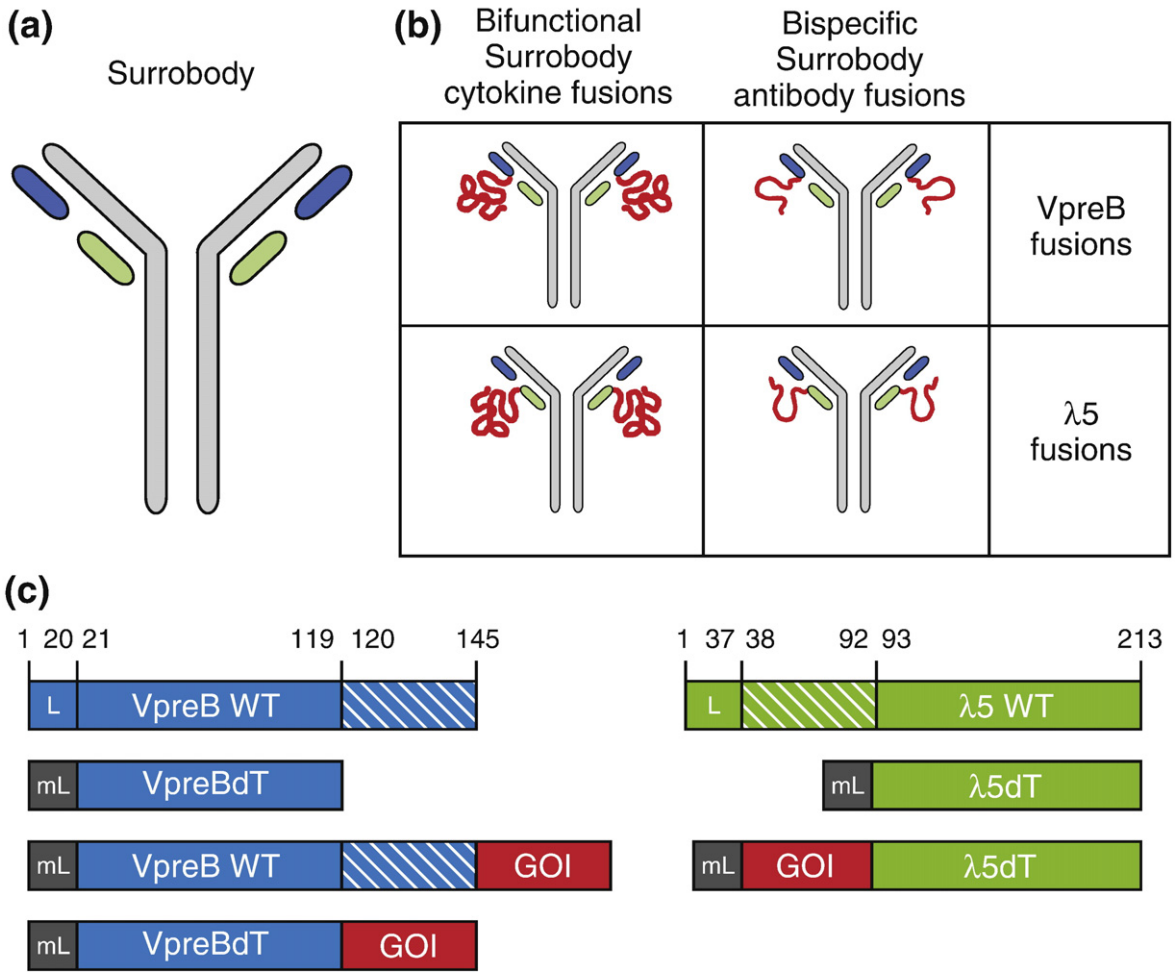

Fig. 1. General scheme for SLC genetic fusions for cytokines and antibodies. (a) Color-coded heteromeric Surrobody structure: light gray chains represent heavy chains, blue represents the SLC VpreB subunits, and green represents the SLC λ5 subunits. (b) Recombinant cytokine fusions are represented by red chains (left) and recombinant scFv antibody fusions are represented by red chains (middle). Subunit targets for fusions are indicated in right panels. (c) Color-coded SLC fusion cloning and the respective amino acids. Basic VpreB fusion constructs are outlined on the left in blue and λ5 constructs are outlined on the right in green; white diagonal hatched areas are used to represent the nonimmunoglobulin tail regions of VpreB (amino acids 120–145) and λ5 (amino acids 38–92). Endogenous leader sequences are indicated by an L, and dark gray mL-labeled regions indicate where synthetic murine kappa light chain leader sequence have been substituted for these endogenous leaders. Red regions indicate fusion sites for genes of interest (GOIs), namely, the cytokines or antibody fragments used in this report.