Bispecific antibodies with native chain structure

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Two new methods overcome a major hurdle that blocked easy and efficient design and expression of human bispecific antibodies.

Human monoclonal antibodies can display exquisite specificity for a single target antigen. However, for many therapeutic applications, single antibodies with specificity for two different target antigens—often called bispecific antibodies—are preferred[1]. For example, a bispecific antibody that binds to a T-lymphocyte surface protein and a protein expressed on tumor cells can, unlike two separate monoclonal antibodies, act like a bridge and connect or recruit killer T cells to tumor cells. In another scenario, if a tumor expresses two hyperactive growth factor receptors, a bispecific antibody that binds to both receptors can simultaneously antagonize both. Unfortunately, generating bispecific antibodies, even in cases where one is in possession of monoclonal antibodies with the desired antigen specificities, is not a trivial task. In papers published in *Nature Biotechnology*, Spiess *et al.*[2] and Lewis *et al.*[3] present two new methods that overcome one of the major obstacles hindering the use of bispeific reagents, namely the expression of bispeific antibodies with native chain structure.

Bispecific antibody production is complicated because human antibodies of the IgG isotype—commonly used for therapeutic applications—are themselves complex multidomain proteins. Each IgG molecule consists of a total of four polypeptides (two heavy and two light chains each) linked by multiple disulfide bonds, and each polypeptide contains multiple types of immunoglobulin domains (designated according to variable (V) or constant (C) and heavy (H) or light (L); e.g., V_H, C_H1, C_H2, C_H3, V_L and C_L) (**Fig. 1**). These polypeptides are normally carefully assembled into monoclonal antibodies by the eukaryotic protein export machinery, in such a way that the final product contains two antigen-binding regions (designated Fab); this bivalent nature allows each antibody to bind to two copies of the same antigen molecule. Because in natural monoclonal antibodies both Fab regions bind the same antigen, it does not matter which heavy chain pairs with which light chain. However, in the case of a bispecific antibody, the heavy chain specific for one antigen must pair with the light chain specific for the same antigen; if a heavy chain pairs with the light chain specific for a different antigen,

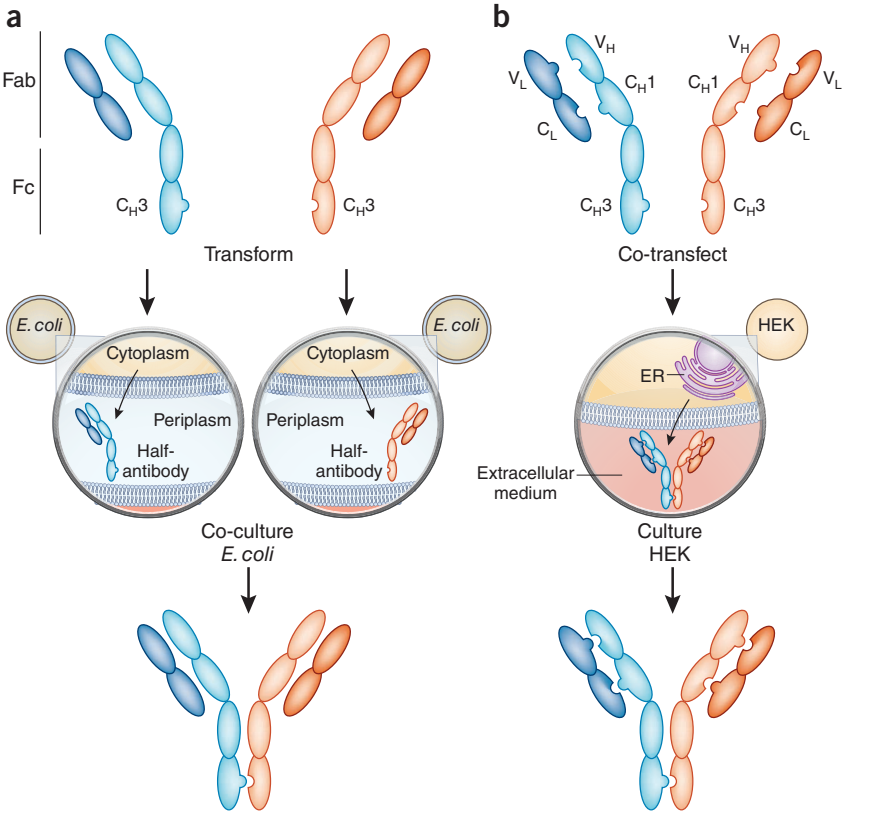

Figure 1 Expression of human bispecific antibodies. Heterodimerization of antibody heavy chains specific for different antigens can be enforced through knobs-into-holes mutations in C_H3 domains; however, the problem of incorrect heavy-light chain pairings remained. Two new methods seek to address this problem. (**a**) In the bacterial co-culture strategy developed by Spiess *et al.*[2], *E. coli* are transformed with constructs encoding half-antibodies; whole intact bispecific antibodies can be purified from co-cultures seeded with *E. coli* expressing two different half-antibodies. (**b**) In the mammalian cell-expression strategy reported by Lewis *et al.*[3], heavy and light chains of one half-antibody contain complementary mutations in V_H-V_L and C_H1-C_L interfaces that result in preferential pairing between these two chains rather than between these chains and chains from the other half-antibody. As a result, correctly assembled bispecific antibodies can be expressed in mammalian cells (e.g., human embryonic kidney (HEK) cells) co-transfected with constructs encoding all four chains.

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the intended antigen specificities will be destroyed. As it is thus far not possible to control which heavy chain pairs with which light chain during assembly of an IgG of native chain structure, efforts to introduce additional antibody specificities into a single antibody often result in the secretion of misassembled species.

Several solutions to the misassembly problem have been developed, including use of a common light chain, Fab arm exchange and domain crossovers[1]. However, these methods generally rely on non-native chain arrangements (which can potentially be immunogenic) or some degree of post-expression processing (which can be time consuming).

Strategies that maintain the native chain structure of the antibody molecule were pioneered by Paul Carter and co-workers at Genentech (S. San Francisco, CA, USA) in the mid-1990s; these focused on the introduction of specific residue pairs into the C_H3 domain of the heavy chain[4]. This 'knobs-into-holes' approach, which forces the heterodimerization of antibody heavy chains specific for different antigens, is well validated and can be further optimized by increasing charge complementarity and the number of disulfide linkages between the chains[5,6]. However, this approach does not eliminate the problem of potentially incorrect heavy-light chain pairings. It is this problem that the strategies presented by Spiess *et al.*[2] and Lewis *et al.*[3] address.

In a previous issue of *Nature Biotechnology*, Spiess *et al.*[2] circumvent the heavy-light chain-pairing problem by expressing 'half-antibodies', each comprising the heavy and light chain specific for one of the two antigens recognized by the bispecific antibody, in the periplasm of bacteria (**Fig. 1a**)[2]. As each heavy-light chain pair is expressed within a different cell, the approach results in the 'compartmentalization' of heavy-light chain assembly, thereby effectively preventing mispairing (whereas heterodimerization of heavy chain is controlled by the knobs-into-holes approach). *Escherichia coli* cells were used for expression because the protein quality-control machinery of human and other eukaryotic cells would likely prevent the export and secretion of half-antibodies.

Lewis *et al.*[3], in contrast, sought a strategy for efficient bispecific antibody production in eukaryotic cells. Knowing that in the absence of the compartmentalization facilitated by the bacterial periplasm, heavy-light chain mispairing must be prevented by other means, the team used an elegant combination of computational design, site-directed mutagenesis and X-ray crystallography to identify mutations. These mutations, when introduced into V_H-V_L and C_H1-C_L heavy-light chain interfaces, function like knobs-into-holes type mutations in that they encourage a heavy chain to preferentially pair with a light chain bearing a set of complementary mutations. When expressed in the same mammalian cell, heavy and light chains bearing these mutations assemble into bispecific antibodies with minimal heavy-light chain mispairing compared to bispecific antibodies made with wild-type versions of the same heavy and light chains (**Fig. 1b**).

The strategies developed by Spiess *et al.*[2] and Lewis *et al.*[3] thereby represent new strategies for the production of human bispecific antibodies. In many respects the methods produce material with comparable characteristics. For instance, both groups report similar expression yields (often around 50 mg/liter, but variable, depending on the identity of the component half-antibodies), as well as pharmacokinetics and binding characteristics expected for bivalent IgG molecules. However, each strategy has unique situation-specific advantages and disadvantages.

Because antibody chain assembly is driven by compartmentalization within the periplasmic space of bacteria, the co-expression method of Spiess *et al.*[2] does not require introduction of mutations into antibody domains other than C_H3; this allows assembly of IgG molecules with native-like protein sequences. However, expression in bacterial cells also results in the loss of key glycosylation modifications. Although unlikely to affect target antigen binding, absence of glycosylation does abolish antibody effector functions, and in particular antibody-dependent cellular cytotoxicity mediated through carbohydrate-dependent binding to Fcγ receptors. This has the potential to limit the use of bispecific antibodies produced by this method in oncology applications, which frequently rely on such tumor killing mechanisms.

In contrast, the extended knobs-into-holes strategy of Lewis *et al.*[3] is compatible with common eukaryotic cell expression systems and therefore facilitates normal eukaryotic post-translational modifications, including glycosylation, of IgG. However, it requires relatively extensive mutation of conserved regions of the antibody molecule; in total, 15–20 mutations are necessary. Further experiments and clinical studies in humans are needed to determine if these mutations increase the immunogenicity of the final bispecific antibodies, through introduction of new B- and/or T-cell epitopes. Reliance on extensive mutation may also restrict the use of this method to certain variable domain families. More specifically, with regard to light chains, this method is currently applicable to kappa but not lambda variable domains. In terms of heavy chains, the authors present data validating the use of the method for V_H3 and V_H1 variable domain families. As these are commonly used in human antibody therapeutics, the method may have broad applicability in the therapeutic antibody arena. However, it should also be noted that these variable domain families have relatively high thermodynamic stability, which greatly simplifies their expression and engineering[7]. Caution is necessary when extrapolating results to other variable-domain families, which are common in the human repertoire and in many commercial phage display libraries, but are generally much less stable[8]. An early focus on antibody stability during preclinical development is therefore warranted and of particular importance when combining variable domains of different genetic origins and stabilities, as the biophysical properties of the resulting bispecific antibody molecule are likely to be determined by its 'weakest link'. Fortunately, the thermodynamic stability of human antibodies can be increased through well-established grafting and consensus strategies[7]. Protein aggregation (colloidal stability) remains a more persistent problem affecting many different antibody therapeutics and variable domain families (including the commonly used V_H3 subtype)[7]. However, strategies for increasing the colloidal stability of human antibody V_H and V_L domains have recently become available[9]. As the above methods for stabilization are compatible with antigen binding, it is likely that they can be used to further expand the scope of the Lewis *et al.*[3] method.

In summary, the strategies outlined by Spiess *et al.*[2] and Lewis *et al.*[3] provide novel and elegant means for the production of a variety of human bispecific antibodies. Although the use of bacterial expression and the requirement for extensive mutation of conserved antibody regions may ultimately limit their use in drug production, the methods are likely to be highly suitable for discovery applications in academia and preclinical research.

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The authors declare no competing financial interests.

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