Engineered antibody therapies to counteract mutant huntingtin and related toxic intracellular proteins

David C. Butler [a], Julie A. McLear [c], Anne Messer [a,b,*]

[a] Wadsworth Center, New York State Dept. of Health, Albany, NY 12208, United States
[b] Department of Biomedical Sciences, School of Public Health, University at Albany, SUNY, NY 12201, United States
[c] Biology Department, Utica College, Utica, NY 13502, United States

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ABSTRACT

The engineered antibody approach to Huntington's disease (HD) therapeutics is based on the premise that significantly lowering the levels of the primary misfolded mutant protein will reduce abnormal protein interactions and direct toxic effects of the misfolded huntingtin (HTT). This will in turn reduce the pathologic stress on cells, and normalize intrinsic proteostasis. Intracellular antibodies (intrabodies) are single-chain (scFv) and single-domain (dAb; nanobody) variable fragments that can retain the affinity and specificity of full-length antibodies, but can be selected and engineered as genes. Functionally, they represent a protein-based approach to the problem of aberrant mutant protein folding, post-translational modifications, protein–protein interactions, and aggregation. Several intrabodies that bind on either side of the expanded polyglutamine tract of mutant HTT have been reported to improve the mutant phenotype in cell and organotypic cultures, fruit flies, and mice. Further refinements to the difficult challenges of intraneuronal delivery, cytoplasmic folding, and long-term efficacy are in progress. This review covers published studies and emerging approaches on the choice of targets, selection and engineering methods, gene and protein delivery options, and testing of candidates in cell and animal models. The resultant antibody fragments can be used as direct therapeutics and as target validation/drug discovery tools for HD, while the technology is also applicable to a wide range of neurodegenerative and other diseases that are triggered by toxic proteins.

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1. Introduction to intrabodies

Intracellular antibodies (intrabodies) provide a protein-based approach to neutralizing the pathogenic characteristics of toxic misfolding proteins. Intrabodies are small, recombinant antibody fragments that target antigens intracellularly via the Fv variable regions that are responsible for antibody specificity. Fig. 1 diagrams the immunoglobulin protein and the binding regions that are used in intrabodies. These constructs exploit many of the advantages of conventional antibodies, including their high specificity and affinity for target epitopes. However, they are much smaller than full-length antibodies, they lack the potentially inflammatory Fc region, and they can be manipulated and delivered as genes or as proteins. This makes them powerful tools with which to target a wide range of pathways affected by pathogenic intracellular proteins (Fig. 2). Intrabodies were first reported in 1988 (Carlson, 1988). They have been studied extensively as potential therapeutics for infectious diseases (Aires da Silva et al., 2004; Doorbar and Griffin, 2007; Marasco et al., 1998; Mukhtar et al., 2009); and cancer (Groot et al., 2008; Lo et al., 2008; Tanaka et al., 2007). Our work and that of others have recently exploited the specificity and affinity characteristics of intrabodies to combat neurodegenerative diseases that share the cellular and molecular features of protein misfolding and aggregation (Cardinale and Biocca, 2008; Lynch et al., 2008; Messer et al., 2009; Messer and McLear, 2006; Miller et al., 2003; Zhou and Przedborski, 2008). In this article, we will discuss and update how intrabodies can provide novel therapeutics and target validations for neurodegeneration, with a focus on Huntington's disease (HD) and related polyglutamine (polyQ) diseases.

To create single-chain Fv (scFv; sometimes also abbreviated as sFv) intrabodies, the genes encoding the variable heavy (V$_H$) and light chain (V$_L$) binding domains of an antibody are cloned using reverse transcription. Antigen binding sites were initially thought to reside in the pocket between the V$_H$ and V$_L$ chains; however, examples of antigen binding that is exclusively on one or the other
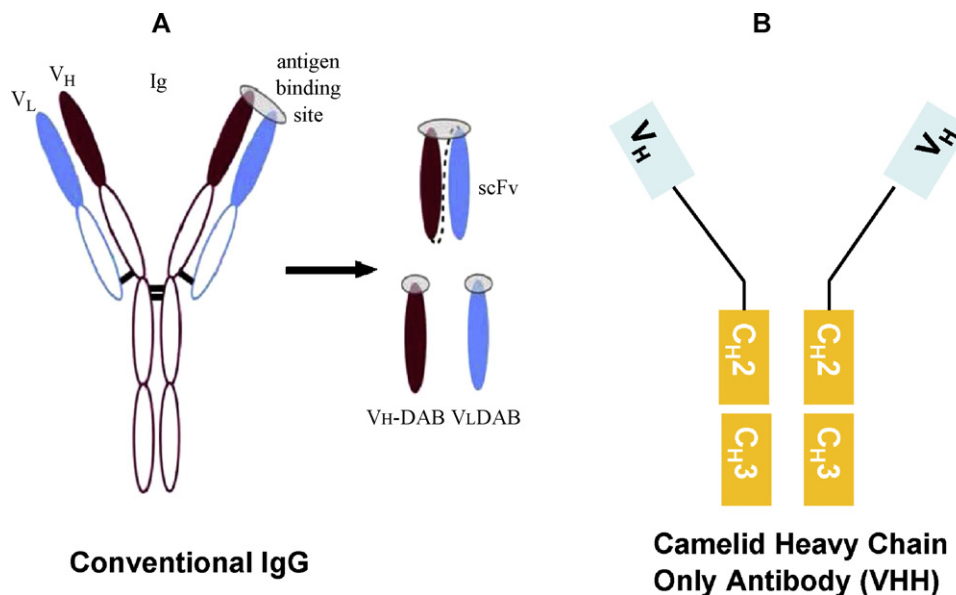

Fig. 1. Diagram of antibody fragments. (a) Classic immunoglobulin and variable fragments; (b) camelid heavy-chain only antibody, and variable fragments.