Therapeutic antibody gene transfer

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AAV vectors containing the combined furin-site and 2A 'self-cleaving' peptide facilitate high-level expression of monoclonal antibodies *in vivo*.

The field of human antibody engineering has seen important recent advances, including *de novo* methods for isolating high-affinity human antibodies and the creation of transgenic mice expressing human antibodies. Yet serious bottlenecks in the development process remain, related primarily to the costs and time involved in producing and manufacturing human monoclonal antibodies (mAbs) both at the preclinical and clinical scales. In this issue, Fang *et al.*[1] provide convincing evidence that *in vivo* therapeutic antibody gene transfer is indeed possible—at least at the preclinical level, potentially accelerating the translation of therapeutic mAbs from bench to bedside.

There are now around 20 US Food and Drug Administration (FDA)–approved therapeutic mAbs on the market today for the treatment of cancer and of autoimmune, inflammatory and infectious diseases, and many more mAbs are in preclinical and clinical development. It has become increasingly evident over the last several years that human mAb therapies are here to stay. And why shouldn't they be? They have a long track record of safety in human trials, the FDA is quite familiar with them and specific manufacturing guidelines are established. In addition, as the humanization[2] of immunoglobulins (IgG) increases—for example, by conversion of rodent IgGs to chimeric IgGs to CDR-grafted IgGs to 'fully human' IgGs—they show reduced immunogenicity and improved therapeutic efficacy.

The trick in mAb production has been to find ways of generating stoichiometric amounts of both the heavy and light chains in the mAb-producing cells, especially because an imbalance in chain production can be toxic to the cells or can result in nonfunctional IgG chains in the supernatant that can complicate the purification process (**Fig. 1**). Fang *et al.* hit upon their simple yet elegant technological advance while trying to find a solution to the limited cloning space that exists in recombinant adeno-associated virus (rAAV) vectors, an attractive vector system for achieving long-term gene transfer *in vivo*[3]. Many investigators have relied on the use of internal ribosomal entry sites (IRES) to allow cap-independent expression of the second gene of interest in a bicistronic cassette. However, as is well known, this method is somewhat unpredictable, is both cell and gene dependent and can often result in lower expression of the gene encoded by the second cistron[4].

In the current study, Fang *et al.* employed a modification of the 2A self-processing sequence derived from the foot-and-mouth disease virus to express a full-length mAb from a single open reading frame driven by a single promoter. The 2A sequence mediates enzyme-independent 'cleavage' to separate polypeptides during the post-translation process[5]. The particular

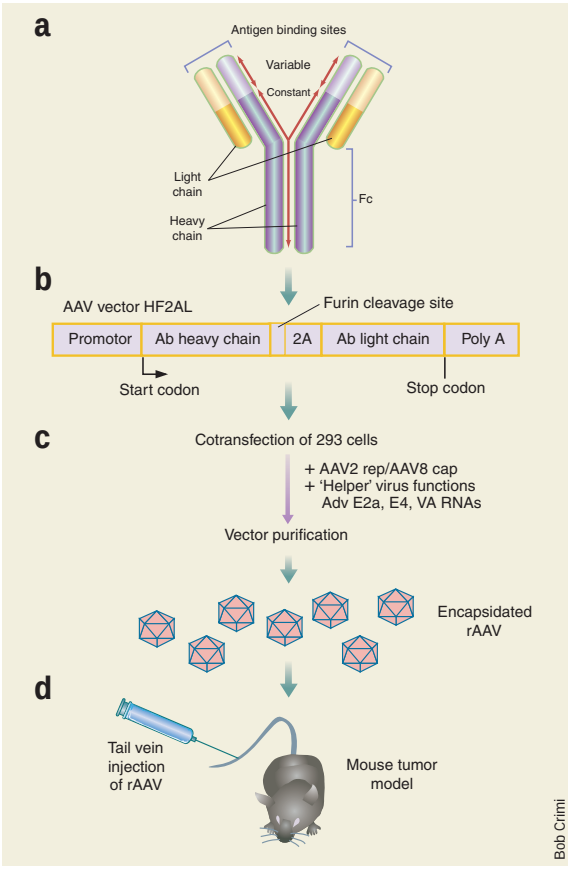

Figure 1 Pathway to therapeutic antibody gene transfer. (**a**) The complexity of monoclonal antibody production lies in the fact that immunoglobulins of the gamma family, the most commonly used in the clinical setting, are composed of four chains: two identical heavy and two identical light chains, which heterodimerize and homodimerize in the endoplasmic reticulum to form the mature four-chain molecule of IgG. Each heavy and light chain contains an N-terminal variable region and a C-terminal constant region. (**b**) The rAAV8-HF2AL mAb expression system can accommodate new variable region genes for different mAbs through PCR cloning with specific PCR primers. (**c**) Cotransfection of 293 cells with AAV-HF2AL vector and plasmids encoding AAV structural genes and adenovirus helper functions is followed by purification steps to produce rAAV particles. (**d**) rAAV is injected intravenously into mice bearing subcutaneous human or mouse tumors, and therapeutic levels of mAbs are produced.

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sequence used leads to cleavage between the last two amino acids at its C terminus. One of the tested configurations (heavy chain–2A sequence–light chain (H2AL)) yielded equivalent amounts of both heavy and light chains, with a C-terminal 21-amino-acid 'tail' on the heavy chain, as expected, and the secreted antibodies were functional.

Extending the work of others who have used furin cleavage sites to process heterologous polypeptides[6], the investigators showed that addition of a four-amino-acid furin cleavage site immediately proximal to the 2A sequence (HF2AL) resulted in a second cleavage event, so that the final mature heavy chain contained only two extra amino acids at its C terminus both *in vitro* and *in vivo*. Impressively, this configuration allowed higher levels of secretion of functional antibodies.

These experimental findings alone provide a useful advance in the field of antibody engineering. However, Fang *et al*. went one important step further by incorporating their single mAb expression cassette into a newly reported rAAV serotype 8 vector. Recently isolated from rhesus macaques, this vector has the capacity to transduce hepatocytes and skeletal cells with very high efficiency when delivered directly by portal vein injection or intravenous infusion[7,8]. By combining these experimental systems, the investigators achieved remarkably high levels (>1,000 ug/ml) and long-term expression (>140 days) of an anti-VEGFR2 mAb in mice and demonstrated therapeutic efficacy against two tumor cell lines in two mouse tumor models.

The significance of this study for the field of therapeutic mAb development is manifold. First, the rAAV8-HF2AL mAb expression system should provide investigators with a rapid and relatively straightforward way to evaluate potential therapeutic mAbs with less cost and labor than are required to produce sufficient mAbs *in vitro* for eventual *in vivo* testing in animal models of disease, at least at the early stages of the discovery process. This applies most readily to mAbs that have attained lead-candidate status through *in vitro* biological studies. However, it is easy to imagine that the system could be adapted to accept new variable-region genes from human single-chain antibody (scFv) and Fab libraries that have been selected against a target protein of interest.

Second, this system could be adapted to evaluate combination mAb therapies—an area of active investigation, particularly for cancer and infectious diseases. In this experimental setting, one could test important and underexplored questions concerning the initial timing and relative dosing of two or more mAbs.

Third, as the authors imply, the system may facilitate the manufacture of therapeutic mAbs by providing a way of generating cell lines that produce high-titer, stable antibodies.

Before this elegant work can advance the cause of *in vivo* therapeutic antibody gene transfer, however, several important obstacles must be overcome. First, chronic diseases such as cancer and HIV-1/AIDS, for which mAb therapies hold great promise, are characterized by genetic instability, and it is well established that immune pressure can lead to phenotypically altered tumors or viral escape mutants, respectively. Whether high levels of circulating mAbs *in vivo* would prevent or accelerate this process remains to be determined.

A second concern is the immune response to the vector. Heterologous antisera raised against AAV serotypes 1–6 are not neutralizing against AAV8, and therefore prior immunity in humans to AAV serotypes 1–6 is unlikely to interfere with *in vivo* gene transfer[9]. Nevertheless, the high promiscuity of AAV8 gene transfer *in vivo* suggests that the consequences of potentially transducing unintended populations of cells must be completely evaluated from the safety standpoint. Furthermore, the use of tissue-specific promoters to restrict gene expression should be explored.

Finally, the 23-amino-acid 2A self-processing peptide, although cleaved, is a foreign sequence. Processing and presentation of this sequence by major histocompatibility complexes class I and II could still occur and prove detrimental to the host.

Nevertheless, although many questions remain, this study offers new tools and avenues of investigation that should accelerate the process of therapeutic mAb discovery and shorten the time needed to reach the clinic.

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Bringing amyloid into focus

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Amyloid deposits can be rapidly detected in the brains of living mice using a novel ligand and near-infrared fluorescence imaging.

Until recently using clinical imaging technologies such as positron emission tomography (PET) and magnetic resonance imaging (MRI), the amyloid plaques that accumulate in the brains of patients with Alzheimer disease have been difficult, if not impossible, to detect *in vivo*. In this issue Hintersteiner *et al.*[1] describe a different approach to imaging amyloid. Using a near infrared (NIR) fluorescence probe that crosses the blood-brain barrier and binds amyloid plaques in the brains of mice, the amount of amyloid can be cost-effectively estimated using near infrared fluorescence imaging. Eventually such an approach may be adapted to visualize amyloid in humans.

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The ability to image disease processes in living humans is one of the major technologic advances of modern medicine. In the context of disease management, *in vivo* imaging is one of the many, and often most informative, modalities that can be used to diagnose diseases and evaluate treatment outcomes. Largely because of costs, imaging is less commonly used to predict risk for the development of disease in asymptomatic individuals. Although not always thought of as such, tests that rely on imaging are fundamentally biomarker studies. As with any biomarker assay, the utility of such tests depends on the sensitivity and specificity of the biomarker and on the sensitivity and specificity of the test used to measure that biomarker. The former issue is extremely important to recognize. No matter how good the assay, its predictive ability is only as good as the predictive ability of the biomarker being studied.

Deposition of the amyloid β-peptide into a fibrillar β-sheet structure referred to as amyloid is a diagnostic hallmark of the post-mortem Alzheimer-disease brain. In Alzheimer disease, amyloid β deposits as amyloid in senile plaques