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GENE THERAPY

Comment on Alba et al, page 965

Mutagenesis of hexon "*FX*" hepatic tropism

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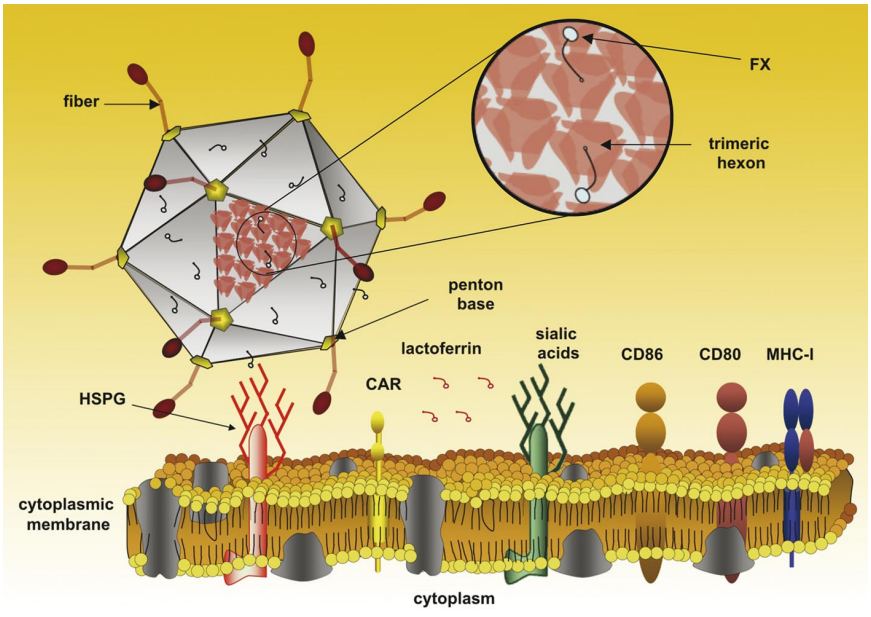
In this issue of *Blood,* Alba and colleagues identify key amino acids in adenovirus hexon hypervariable regions that interact with coagulation factor X. By mutating these residues on the adenovirus's major capsid protein in Ad-based vectors, the authors succeed in retargeting gene transfer away from hepatocytes. The advances achieved in this study may create a vector platform that can be used to develop Ad-based therapeutics for nonliver-targeted diseases.

Occam's razor is the principle of parsimony. Paraphrased, it means "if several hypotheses are possible, it is likely the simplest that is correct." What does the philosophy of a 14th century logician and Franciscan friar have to do with Alba et al and 21st century hematology?

This story starts approximately 25 years ago when adenoviruses were initially developed as gene transfer vectors. Adenoviridae are double-stranded nonenveloped DNA viruses with icosahedral symmetry, and a homotrimeric fiber projecting from each vertex of the approximately 90-nm capsid (see figure). Early on, it was found that human adenovirus type 5–derived vectors injected intravenously into mice efficiently transduced hepatocytes. CAR (Coxsackie and adenovirus receptor), the principle in vitro receptor for almost half of the human adenovirus serotypes, was identified in 1997.[1,2] When it was quickly shown that many tissues, including liver, expressed high levels of CAR, most if not all of the labs working in this domain made the Occamian leap and assumed that the CAR-tropic Ads (most serotypes from species A, C, D, E, and F) were using this cell-adhesion molecule to infect hepatocytes.



The icosahedral adenovirus capsid contains projecting fibers that can be flexible in some serotypes. Previously, most receptors were believed to interact with the fiber knob (red globular structure). FX interacts with the trimeric hexon hypervariable regions and forms a bridge to bind heparan sulfates proteoglycans (HSPG).

The fly in the ointment came when one tried to retarget Ad vectors to other tissues.[3] The CAR-binding residues in the fiber knob were initially identified by Roelvink and colleagues via cocrystallization of CAR and the fiber knob.[4] Mutations were introduced into the knob to generate recombinant proteins that no longer bound CAR, and then "CAR-ablated" vectors were created. Although the CAR-ablated vectors could not use CAR to infect cells in vitro, the vectors still efficiently transduced liver cells, to the surprise of many. It wasn't until 2008, when Waddington et al untangled the mountain of conflicting data, that it was found that hexon, the major protein of the adenovirus capsid, was binding coagulation factor X (FX) when adenovirus vectors were injected intravenously into mice.[5] This hexon-FX interaction formed a bridge to heparin sulfates on hepatocytes that, in turn, mediated transduction.

In this issue of *Blood,* Andy Baker's latest study identified key residues in the hexon hypervariable regions (the 7 protruding loops that harbor antigenic epitopes that, in most cases, define the adenovirus serotype) that are involved in FX binding.[6] Alba et al use a collection of approaches based on modeling, cryoEM, swaps and substitutions of amino acids in hexon hypervariable regions 5 and 7, and intravenous gene transfer in mice to not only pinpoint the amino acids involved, but also to formally demonstrate that Ad vectors could be engineered to bypass liver transduction.

Where do we go from here? While these paradigm-shifting results remind us that William of Occam never worked with an adenovirus, we have quite a way to go before we can harness

the power of adenovirus vectors for clinical gene transfer via the systemic circulation. Even in children, one finds cross-reacting anti-adenovirus Abs that can opsonize or neutralize vectors. Add to this the fact that erythrocytes have CAR on their membranes,[7,8] platelets bind some adenovirus types, and that the fenestra size in human liver may be near the limit to allow the approximately 90-nm adenovirus particle to enter. We see that we still have work to do before one can master the fate of adenovirus vectors in the circulation. Nonetheless, the field now has one less hurdle to cross.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● CLINICAL TRIALS

Comment on Witt et al, page 952

Avoiding "sticker" shock

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In this issue of *Blood,* Witt and colleagues show that it is safe to alter the frequency of laboratory monitoring from every 4 weeks to every 8 weeks in stable patients on long-term anticoagulant therapy.[1]

Long-term administration of a coumarin drug is currently the only option for chronic oral anticoagulation therapy in the United States. Coumarins are effective and inexpensive, but close monitoring and frequent dose adjustments are usually required to achieve the desired antithrombotic effect and avoid bleeding. Monitoring requires venipuncture, performing a prothrombin time, and calculating the International Normalized Ratio (INR). Studies have established that an INR of 2 to 3 (or 2.5 to 3.5 in patients with mechanical heart valves) best balances safety and efficacy. The task of the clinician is to determine the dose of drug that consistently produces an INR in the therapeutic range. Current recommendations call for testing to begin 2 to 3 days after therapy is initiated. Once the INR is therapeutic, the monitoring frequency is decreased to 2 to 3 times weekly for 1 to 2 weeks, and if the INR remains in the therapeutic range, eventually to once every 4 weeks for as long as the patient is on the drug.[2] Patients whose INRs remain in the therapeutic range on repeated testing are considered to have achieved stable anticoagulation.

Can the frequency of monitoring be decreased in patients on stable anticoagulation? This question has been addressed by Witt et al in this issue of *Blood*. They conducted an observational study of more than 6000 patients followed by a centralized clinical pharmacy anticoagulation service. Forty-one percent had all INR values within the therapeutic reference interval for a continuous 6-month interval (stable patients), and the remainder (59%) served as comparator patients. Patients had at least 1 INR determination every 8 weeks. In the comparator group, only 47% of INR values were in the therapeutic range, and this group had a significantly higher rate of bleeding (2.8% vs 0.8%; $P < .001$). Stable patients were less likely to have a goal INR ≥ 3.0 (OR = 0.48, 95% CI 0.38-0.61). Other predictors of a stable INR were age older than 70, and absence of diabetes, heart failure, or other chronic disease. Thus, the authors have identified and characterized patients potentially requiring less frequent anticoagulant monitoring. To confirm that such patients can be safely managed with less testing, they suggest a prospective trial of stably anticoagulated patients in whom the interval between INR determinations is progressively increased to 8 or even 12 weeks.

Table 1. Explanations to patients taking coumarins (5)

1.	Why treatment is necessary
2.	How the drug works
3.	Why monitoring is needed
4.	Why it is important to take the drug at a fixed time daily
5.	How alcohol may affect anticoagulation
6.	How dietary changes affect therapy
7.	How drug-drug interactions might affect treatment
8.	Why clinic personnel should be notified of changes in medications
9.	What precautions should be taken to avoid bleeding
10.	How to recognize signs and symptoms of bleeding

While such a suggestion is reasonable, the dismal track record of coumarins with respect to bleeding must be recognized. Despite careful monitoring of anticoagulant intensity, the risk of major hemorrhage in several large clinical trials has ranged from 0.3% to 0.5% per year as compared with controls,[3] and during long-term anticoagulation, 1.1% per patient-year.[4] Some of the hemorrhages might have occurred because of unpredictable events such as intercurrent illnesses or trauma. Changes in patient medications and use of nonprescription drugs and alcohol might have precipitated other bleeding events. Perhaps the best approach to limit these complications is a well-informed patient. In this regard, the 10 key issues for patient explanation (see table) are as valid today as they were when published 25 years ago by Errichetti et al.[5] These issues should be continually reinforced while patients are taking these drugs. Well-educated patients who have achieved stable anticoagulation are candidates for less frequent monitoring and fewer needle sticks.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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