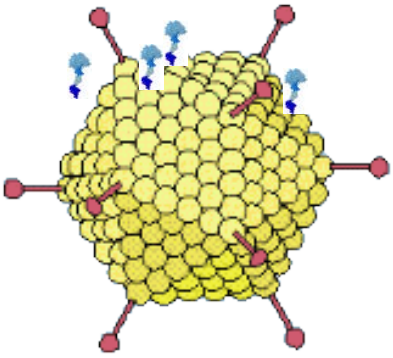
Advancing Regulatory Science

FDA discovery that Ad5 vector uses coagulation factor X to block complement proteins has major implications for gene therapy

Common wisdom has held that the gene transfer vector adenovirus 5 (Ad5) scavenges coagulation factor X (FX) from the bloodstream to use as a bridge for binding to hepatocytes. FDA scientists have shown that instead, Ad5 uses FX to ward off complement proteins that would otherwise inhibit binding to any target cell. This new insight into the critical role of FX could inform the design of a safe and effective vector used for gene therapy.



Coagulation factor X binding to adenovirus gene therapy vector

"Coagulation factor X shields adenovirus type 5 from attack by natural antibodies and complement"

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Coagulation Factor X (FX)

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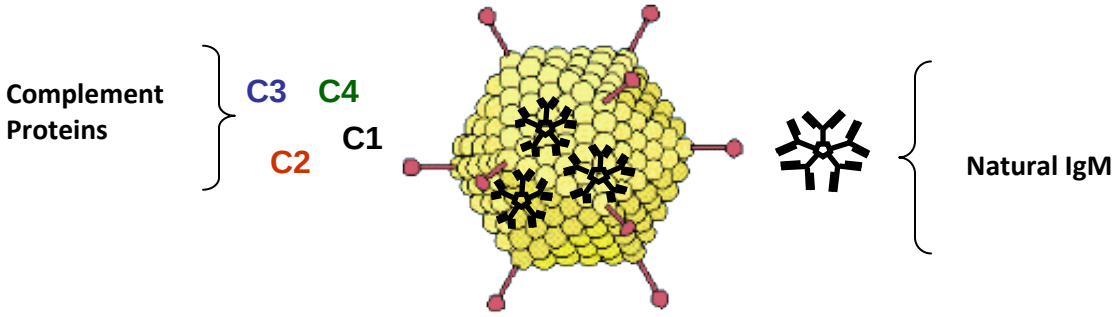
The immune system is a barrier to Ad5 gene transfer vectors

- Gene transfer vectors have had limited efficacy because of difficulties in reaching target cells.
- Natural IgM antibodies and complement proteins are always present in the bloodstream, even without a prior exposure of the immune system to viral vectors.
- IgM attaches to Ad5 (opsonization) following intravenous delivery of the gene transfer vector.
- Antibody-virus complexes activate the classical complement pathway, which triggers coverage of the vector by complement proteins that enhance its clearance from the circulation by the liver.
- This study was part of a long-term effort to determine the factors that limit the efficacy of this gene transfer vector.

Opsonization of Ad by the classical complement pathway



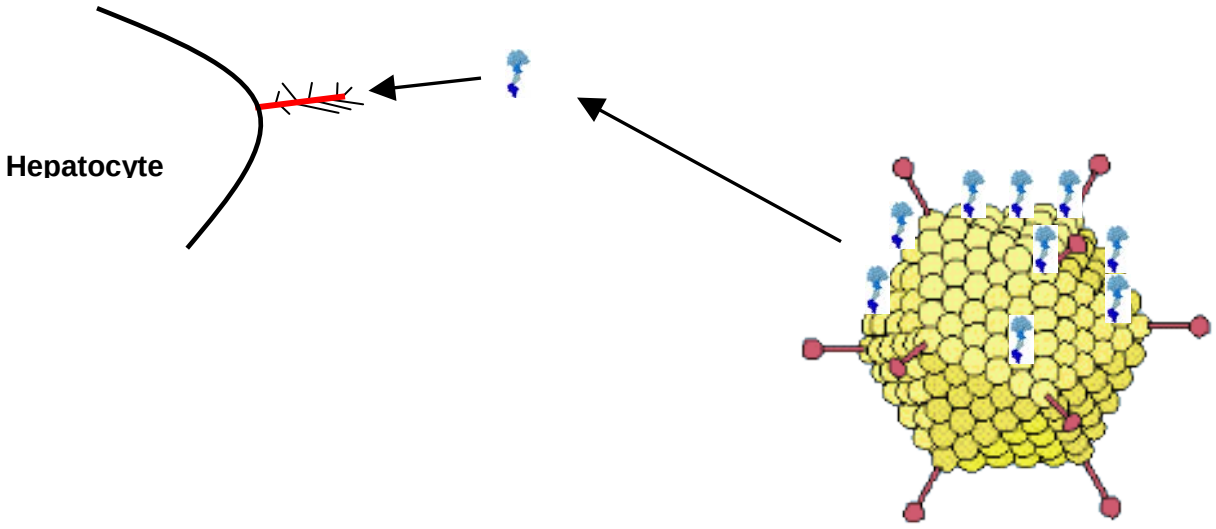
Previous research showed that adenoviruses recruit coagulation factors

Adenoviruses scavenge coagulation factors, and Ad5 specifically scavenges coagulation factor X (FX) at a rate of about 240 FX proteins per virion.

- Previous *in vitro* research showed FX is necessary for Ad5 attachment to hepatocytes (liver cells).
- "Common wisdom" suggested FX acts as a bridge that facilitates transduction of hepatocytes by Ad5 vectors *in vivo* as well, and that this information might inform design of more effective gene therapy vectors:
    - Preventing FX binding to Ad5 would block binding to hepatocytes.
    - Blocking Ad5 binding to hepatocytes might enable therapeutic gene therapy vector to target other cells and not be trapped by the liver.

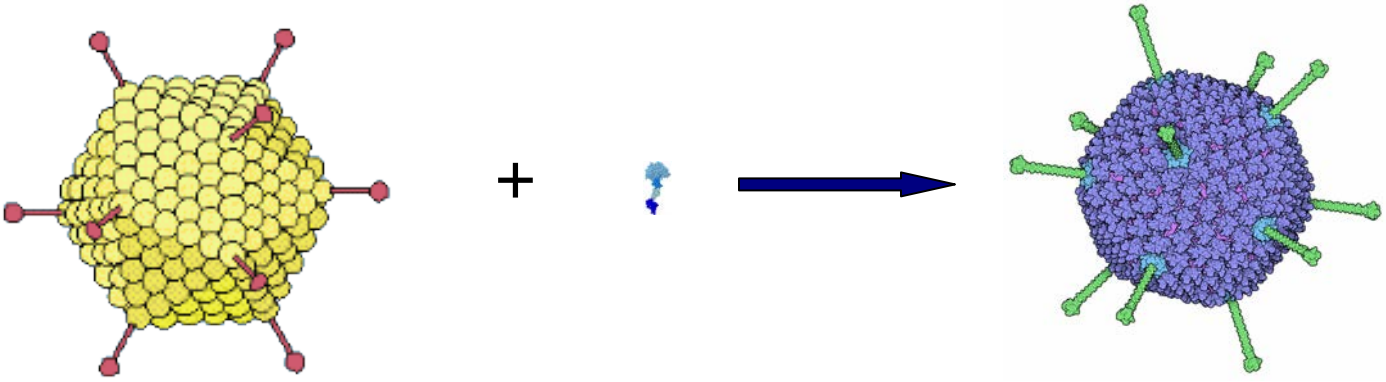
Old hypothesis

FX is a bridge that allows Ad5 to bind heparan sulfate on the hepatocyte



New FDA study finds unexpected role for FX in protecting Ad5

FX shields Ad5 from attack by complement



- FDA scientists extended previous *in vitro* findings of other researchers by conducting *in vivo* studies with mice.
- FDA studies showed that FX has a previously-unknown function as an inhibitor of Ad5-induced complement activation:
    - Ad5 vectors need coagulation factors for liver transduction only in mice that have an intact classical complement pathway.
    - Ad5 does not need to bind FX in knock-out mice lacking B and T cells.
    - Transduction of liver by Ad5 vector was significantly inhibited in mice treated so that either FX was depleted or prevented from binding to the vector.
    - Neutralization of Ad5 by complement impairs transduction in the liver as well as other organs (i.e., lung, kidney, spleen).
- Removing FX from therapeutic Ad5 vectors would leave the virus defenseless against complement proteins that then inactivate it.

The discovery that Ad5 uses FX to block attachment of complement proteins is important because this new information could save time, effort, and resources in the design of these gene therapy vectors.