The Human Immunodeficiency Virus (HIV-1) is a pathogen that is on the rise worldwide. It is estimated that 37 million adults and 2.5 million children were living with HIV in 2003 (Worldwide 2003). HIV-1 is a retrovirus that causes fatal damage to the human immune system which eventually develops into the acquired immunodeficiency syndrome (AIDS). Worldwide campaigns to promote awareness and coalitions between the scientific and medical communities are desperately trying to impede the progress of the disease.

Treatments commonly used against HIV-1 cause harm not only to the virus, but also to the victim due to their toxicity. The fact that the virus frequently mutates also impedes the effectiveness of treatments. Commonly used treatments for HIV are reverse transcriptase (RT) and protease inhibitors. Reverse transcriptase inhibitors work by providing analogs of nucleotides to the cells that the RT might incorporate into the proviral DNA synthesis and thereby prevent further proviral DNA synthesis. Protease inhibitors mimic the protease substrate and bind to protease decreasing its chances of binding to the actual substrate. Unfortunately for the victims of HIV, resistant mutants can appear within six months for the reverse
transcriptase inhibitor and the protease inhibitors are highly toxic and easily out competed (Atkinson 2003).

Intramer therapy is a possible solution to the problems with the traditional treatment of HIV-1. Intramer therapy works through in the intracellular expression of nucleic acid molecules binding to specific HIV-1 target proteins. These nucleic acid molecules are generated through in vitro selection to tightly and specifically bind proteins or other ligands (see figure 1).

In vitro selection is a process that produces nucleic acid binding species (aptamers) against a target (e.g. a protein from HIV-1; for review, see Ellington, 1994 and Famulok et al., 2000 (full references below)). This is accomplished by incubating a large, randomized pool of RNAs with the protein target. Most nucleic acids will not possess a tertiary structure capable of interfacing with the protein target and are removed. Those few that do bind are partitioned away from the non-binding species. The binding RNA species (aptamers) are then amplified and the process is carried out again under more stringent conditions, evolving a pool of “better affinity” RNAs. Ideally, once
The aptamers are expressed in the infected cell, they would bind to their corresponding HIV-1 protein and the protein would no longer be able to function.

The specific HIV-1 proteins that the Ellington lab focuses on are coded for by the Gag and Gag-Pol genes. Translation of the Gag gene produces the capsid proteins of the virus. Gag-pol translation produces HIV-1 protease, reverse transcriptase, and then RNase H and integrase (Atkinson 2003). Each of these proteins has a specific role in the replication cycle of the virus, but a few proteins are more crucial than others. The role of the nucleocapsid (nc) is to assemble the viral RNA into virons and the responsibility of reverse transcriptase (rt) is to reverse transcribe the viral RNA into DNA. The role of integrase (int) in the HIV-1 replication cycle is to guide the insertion of viral RNA into the cell genome (Hope 2000). Each of these proteins were separately used as targets to generate inhibitory aptamers (reviewed in Joshi 2003). The aptamers are the rt-aptamer, nc-aptamer, int-aptamer, (other anti-HIV-1 aptamers are available, but these are our aptamers of interest).
We have proposed the use of aptamers for intramer therapy and are now using aptamers to inhibit the effects of HIV-1. The aptamers generated through in vitro selection were amplified through polymerase chain reaction (PCR) and then ligated into a TOPO vector, sequenced, re-digested and then ligated into the final vectors (see figure 2). The purpose of the above process is to ensure that the desired aptamer sequence was accurately generated and then appropriately ligated into the expression vector.

The aptamers were ligated into two separate expression vectors: the Gilboa vector and a modified pcDNA3.1 vector. The expression vector is responsible for carrying the aptamer into the cell and then expressing the aptamer construct. The modified pcDNA3.1 vector is a 3.6kb long Invitrogen plasmid with the multiple cloning site removed and ribozymes added with internal restriction sites (Joshi 2002). The Gilboa vector is a 10.2kb long vector with a mouse leukemia virus backbone (MMLV) and a tRNA meti-pol III promoter (Lee 1994).
After the constructs are produced, they are co-transfected with a proviral plasmid into HeLa-CD4 cells. Transfection is a chemical process that allows for the transport of foreign DNA into a host cell. The transfection was carried out with FuGENE 6 transfection reagent (Roche Diagnostics Corporation).

The level of HIV-1 expression is then determined through an Enzyme Linked ImmunoSorbent Assay (ELISA) provided by Zeptometrix Corporation. This assay works by using an antigen/antibody complex to detect the level of HIV-1 infection in the tissue culture. Specifically, the antibody for the HIV-1 protein p24 is used to bind to the p24 antigen found the infected cellular media. Then the primary antibody/antigen pair is incubated with a secondary antibody that is conjugated to Biotin. Finally, Streptavidin-peroxidase is added to react with the biotin and produces a color. The color change is measured, and the level of HIV-1 infection is then determined by relating the change in light absorption to the concentration of the p24 found in the cellular supernatant. This assay was carried out over a 24, 48 and 72 hour time point in order to chart the effectiveness of the aptamer over time as compared with controls (infection in the absence of aptamer and no infection). This entire process has now been
carried out for the integrase, nucleocapsid and reverse transcriptase aptamers (figures 3 and 4).

In figure 3, all cells were transfected with HIV-1 and the Gilboa vector with the aptamer of interest as indicated on the graph. The level of inhibition and therefore effectiveness of the aptamer is determined via the comparison of the vector/aptamer constructs with infected cells alone.

Figure 4 represents the preliminary data for aptamers ligated into the pcDNA3.1 vector and their corresponding levels of inhibition. We are now in the process of testing the aptamers in combination (i.e., two aptamers in the same cell) and also comparing two vector systems. It is plausible that one vector system is better than the other at intracellular aptamer expression.
Further experimentation will lead to more conclusive results.

Intamer therapy is a therapeutic that could possible change the dynamics of medicine. Aptamers have moved from the experimental bench to animal model systems. In canines, an anti-thrombin aptamer was used for anticoagulation in heart surgery (DeAnda 1994). In rats, an anti-inflammatory aptamer was used to reduce lung inflammation (Bless 1997). More importantly, in humans, an aptamer that binds to vascular endothelial growth factor (VEGF) and prevents macular degredation has reached phase II/III testing (Eyetech Study Group 2003).

Intamer therapy is a nontoxic treatment for a fatal virus. It uses RNA designed aptamers to prevent further replication of HIV-1 and consequently prevent further harm to the human immune system. With the immune system safeguarded, existing medical procedures can work in coalition to halt the progress of HIV-1. In turn, an HIV-1 victim would be able to live a healthier and longer life.
References:


Eyetech Study Group


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