

TECHSIGHTING
GENE THERAPYMailbox: www.sciencemag.org/cgi/dmail?53932a

Treating with HIV

Gene therapy is based on the idea that therapeutic agents or activities can be delivered in the form of nucleic acids. Central to the gene therapy schema is the delivery vector itself. Currently, there are two main vector types used commonly in clinical trials: modified DNA viruses and modified retroviruses. The latter are good delivery vehicles, because they are integrated into the target cell where they remain permanently. Unfortunately, because most retroviruses require a dividing host cell for integration, they cannot infect other important cell types, like neurons. On the other hand, DNA viruses, like adenovirus, produce a potent immune response that clears the virus-producing cells and usually prevents a second round of treatment.

Enter now the HIV virus. As a member of the lentivirus group, HIV has the curious ability to infect and to become integrated into nondividing cells. For example, HIV can be modified in the laboratory to widen its host cell range so that it can infect most dormant cells, such as neurons. So what then prevents HIV from becoming the perfect gene delivery vehicle? First, the gene therapeutic agent might produce live virus that could potentially recombine or interact with another virus to produce an active HIV strain. Second, the potential exists for one of the regulatory or accessory proteins of HIV to cause cell damage or disease. In fact, a recent report has shown that one HIV open reading frame, *nef*, can actually produce an AIDS-like disease if expressed in transgenic mice (1). All this could dampen the enthusiasm of those studying HIV-based vectors.

However, a group from the Salk Institute, led by Inder Verma, has pressed on and now reports in the journal *Virology* significant progress toward converting HIV into a safer delivery vehicle for gene therapy (2). They set out to produce an HIV vector that contained only the essential elements for gene transduction. They used a system in which they could package defective HIV genomes into a virion that had broad host range. Normally, HIV targets human CD4 cells through specific interactions with the virus' membrane-bound proteins. To broaden the host cell targets, they essentially inserted a surrogate targeting molecule (VSV-G) into the viral membrane. VSV-G can bind to many cells, in-

cluding neurons. Next, they modified the HIV genome to produce a minimal construct containing key viral *cis*-acting elements, and added the cytomegalovirus promoter, as well as the green fluorescence protein as a marker of infection. One of the key sequences in any retrovirus is the U3 region. During replication, U3 is duplicated such that it flanks the virus on both sides. Because U3 contains active promoter elements, it can drive synthesis of viral RNA and downstream cellular RNA, effects that are not desirable in a gene therapy tool. The researchers inactivated U3 with a deletion and showed that high-titer virus could still be produced with their helper system. The resulting viruses are called self-inactivating (SIN).

To test the ability of the HIV SIN viruses to infect neurons, the investigators injected preparations directly into rat brains. By staining, they could show high activity in neurons, specifically cholinergic neurons. They also injected the virus into rat retina and showed that a variety of retinal cell types could also be infected.

Challenges still lie ahead for HIV-based vectors. For one, the packaging cells used to obtain high-titer virus still contain HIV proteins, something that the authors have hinted can be eliminated in the future. It will be interesting to follow this story as scientists apply molecular techniques to turn a deadly pathogen into a tool for future therapies.

—ROBERT SIKORSKI AND RICHARD PETERS

References

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2. H. Miyoshi, U. Blomer, M. Takahashi, F. H. Gage, I. M. Verma, *Virology* **72**, 8150 (1998).

TECHSIGHTING
NET TIPSMailbox: www.sciencemag.org/cgi/dmail?53934Ten Tips to Building
Your Web Site

As scientists become more versed in the Internet, many want to set up their own World Wide Web site. Their goals vary greatly: some researchers plan to showcase the work done in their laboratory and use the site as a recruitment tool, others use it to post unpublished results, and some use their site to make protocols available electronically or to handle requests for laboratory materials.

Whatever your goal for setting up your own site, we have listed 10 tips you may want to follow as you build it.

1) Know why you are doing this. The Internet has been compared to the rush to the Wild West, where everyone wants to claim a small piece of "digital land." A principal investigator in a university or a company should, however, make sure that his or her site has a clear, cohesive message. Approach your Web site just as you would a manuscript. Know exactly what message you want to convey before lifting your digital pen and writing Web pages.

2) Stay organized. Managing your site will be much easier if you make sure your content is well organized. Separating art from text, using directory trees, and using a simple naming convention for files are all important ways you can use to keep the content organized and to facilitate navigation through your site.

3) Be aware of what is posted. While laboratory directors scrutinize all the details of the manuscripts their laboratory publishes, most of them delegate the publication of content on their Web site and have often not carefully reviewed what is being posted. Because materials published on the Web have the potential of being seen by your peers as well as other viewers, we advise you to be in charge of your lab's Web site content just as you would with a manuscript. In addition, realize that many print journals will not accept data that have already been published on a Web site. So think twice before posting original data on your site.

4) Stay away from code. Unless you spend most of your day using various Net programming languages, we advise you to stick to plain HTML when publishing your site. HTML is in its fourth version (HTML, HTML 2.0, HTML 3.2, and HTML 4.0), and new tags are being integrated with each new version. ZDNet provides an online reference to HTML 4.0 tags (www.zdnet.com/products/htmluser/href/). While the use of other languages such as Javascript, Java, or ActiveX might be attractive to some, realize that the various Web browsers behave differently with different languages. What may work with Internet Explorer 4.0 on a PC may not work if your viewers use Netscape 3.0 on a Macintosh, for instance.

5) Use graphics intelligently. The majority of scientists access the Net using the high-bandwidth network of their institutions, so the size of the files that are served probably does not matter so much. Nevertheless, an overzealous emphasis on graphics can negatively impact the user experience. After all, we are all in the information business, so always ask yourself what information you are trying to convey by adding an image to a Web page.

6) Tool up. There are now a number of software tools that can help you publish

Web pages. For instance, the most popular word processors, WordPerfect and Word, will let you save your files as HTML documents. Examples of more specialized HTML editors are BBEdit for the Mac and FrontPage 98 for the PC.

7) "Water" your site. Unless you plan to invest at least a few hours each month in maintaining your Web site, you might as well not start at all. Links decay and content becomes obsolete, so you owe it to anyone who takes the trouble to visit your site to maintain it and to keep it up to date.

8) Back up. If you follow the previous tip, you realize that soon you will have invested days of work in the site and there is nothing worse than losing all that effort by clicking the wrong computer key! So, before you make any changes to a file, make a copy of the old version. Finally, back up the entire site at least once a month.

The last two tips do not apply to those of you who plan to use someone else's server to publish your site. In that case, you are probably using a department's shared server or even an Internet Service Provider. The only thing you need to do in that instance is to transfer your files to that computer. If, however, you want to run your own server, there are two more tips to keep in mind.

9) Use the right hardware. While almost any computer can be hooked to the Net as an Internet server, you should make sure that your machine has sufficient CPU power and RAM to handle simultaneous service requests. Fortunately, this is something you should not have to worry about until you start receiving several thousand simultaneous requests. Nevertheless, if your site becomes quite popular, you should be aware of your hardware's limitations. You can read more about Internet and intranet servers at www.computers.com/reviews/editorial/0,53,0-13-979-3,00.html.

10) Use the right software. Setting up the hardware is just half the battle. Next, you need to set up Internet server software. For the Unix operating system, Apache Web Server and Netscape's Enterprise Server are very popular choices. For the Windows operating system, Apache and Microsoft's Internet Information Server tend to be the favorites among webmasters. For the MacOS, Microsoft's Personal Web Server or WebStar are all good choices. Finally, there are important security issues when you install a Web server. For instance, hackers will exploit security holes in your Web server to break into your system, thereby using your computer to impersonate you. So, before setting up your own server, make sure you understand security issues (1).

Additional resources are available on our Web site at <http://mednav.com>.

—RICHARD PETERS AND ROBERT SIKORSKI

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TECHSIGHTING LIGAND BINDING

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Molecular Barbells

Some proteins have multiple ligand-binding sites. For instance, ligand-gated ion channels, transport proteins, and many allosteric enzymes have two or more binding sites. To design new drugs that can bind to these proteins, one would ideally want to know the targets' structure. Another approach is to use combinatorial chemistry, by screening a large number of random combinations of chemical groups as potential ligands. Yet another alternative just published in the journal *Nature* consists of taking an existing ligand and creating a dimer by linking the two units with a polymer molecule (1). The authors reasoned that once one ligand unit has bound to a site on the target, the probability of binding of the other ligand unit will increase, thus increasing the overall affinity of the dimer.

To test their hypothesis, the authors synthesized cyclic guanosine monophosphate (cGMP) dimers joined through a polyethylene glycol (PEG) link. The resultant "barbell" molecules can have different sizes when the length of the PEG link is varied. The researchers measured the binding affinity of polymer-linked ligand cGMP dimers of various lengths by incubating them with cyclic nucleotide-gated channels in excised patches from rat olfactory chemoreceptors or bovine rod photoreceptors. They noted that there was an optimal length for the PEG link between the two cGMP molecules: for the olfactory channels, a 2-kD PEG gave the greatest increase in apparent affinity, with a decrease in $K_{1/2}$ by a factor of nearly 1000. Dimers with a shorter or longer PEG link did not fare as well. The authors hypothesize that the 2-kD PEG link closely matches the distance between two binding sites on the channel; a shorter link cannot span the distance between two binding sites, whereas a longer link introduces too many degrees of freedom. Interestingly, kinetic experiments indicate that the dissociation rate of these barbell molecules is slower than for cGMP by a factor of several thousand. Indeed, electrophysiological studies indicate that the

channels remained open for minutes after exposure to the dimer compared with a few milliseconds with cGMP alone.

Additional experiments revealed that the optimal length of the PEG link varied depending on the channel that was used for the binding experiments. For instance, the size of the optimal polymer link for activating cyclic nucleotide-gated channels of rod photoreceptors is 1.2 kD, whereas for activating olfactory channels, it is 2 kD. It is worth pointing out that the olfactory channel has been reported previously to have a larger pore; so these results are consistent with the hypothesis that the barbell molecules span the channel. The length of the PEG link can only be used to estimate rather than to measure accurately the exact distance between the binding sites, because the PEG link probably has much flexibility.

Finally, the authors tested the effect of these cGMP dimers on protein kinase G. The optimal PEG link turned out to be 282 Daltons in size and gave an apparent affinity that was 30 times that for monomeric cGMP. Because the length of the PEG link in this instance is very short, however, the authors hypothesized that the cGMP dimers were binding across two homologous subunits rather than binding on the high- and low-affinity cGMP-binding sites of a single protein kinase regulatory domain.

Clearly, a method that helps in the discovery of ligands that can bind tightly to proteins with multiple substrate sites is an important breakthrough for the pharmacologist and medicinal chemist. Indeed, such proteins (from hemoglobin to ion channels) often have very important physiological functions. The high potency and specificity of the dimers generated by the approach makes the method all the more interesting. The door is now open to try the method with other ligands and with other polymers besides PEG. For instance, more lipid-soluble polymers might be developed as highly potent and specific lead compounds that could be delivered intracellularly. Coupling ligands to branched polymers might lead to the development of compounds optimally tuned to receptors with any number of binding sites for a given ligand, or even for several different ligands.

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