Xenotransplanters Turn Xenovirologists

SIGHTINGS

Donor organs for transplantation are scarce, and this shortage has stimulated academic and commercial interest in techniques that employ animals as potential organ sources. One approach rapidly gaining ground is the use of pig organs for human transplantation. Though promising, pig xenotransplantation has a serious problem: hyperacute rejection (HAR) caused by preexisting human antibodies.

Pigs produce a specific sugar (α-gal) on the surface of their cells that is not found in human cells (or any other Old World primates). Consequently, all humans have existing antibodies that will target any α-gal–coated cells for destruction. Methods to either remove the HAR antibodies or decrease the expression of α-gal in the donor organ have reduced the severity of rejection and thus make xenografting a real possibility. Recent findings, however, show that there is a catch. Patience *et al.* (*1*) have discovered that pigs harbor endogenous retroviruses (PERV) that are capable of infecting human cells in vitro.

The normal germ line DNA of many vertebrates contains endogenous retroviruses (ERV) that act as molecular parasites, propagating via germline transmission and, if functional, through a productive infection as well. Some ERV can cause diseases such as leukemia, while others appear innocuous. Cross-species transmission of ERV from some animals such as cats (RD114) and baboons (BaEv) to humans has been shown to occur in the lab (*2*).

Given these data, it is a disturbing possibility that pig retroviruses might infect human cells following xenotransplantation. Patience *et al.* studied this by first confirming the existence of pig retroviruses in two commonly used pig cell lines, PK-15 and MPK. Electron microscopy showed the characteristic morphology of C-type retroviruses. Using supernatants from PK-15 and MPK cell cultures to infect human cell lines in vitro, they showed that PK-15 (but not MPK) -derived virus could infect the human kidney cell line 293. By cocultivating PK-15 with human cells, they then could infect a broader range of human hosts that included lines from lung (MRC-5), muscle (RD), and lymphoid (Raji, SupT1, and Molt4) origin. The message seems to be that productive pig-to-human viral infections can occur in the laboratory and that cell-cell contact increases their likelihood. Whether they occur in vivo is largely unknown.

The study went on to use degenerate PCR oligo primers directed at the conserved region of known retroviral proteases and reverse transcriptases to actually clone the PERV from both PK-15 and MPK cell lines. Both PERVs turn out to have more than 95% sequence similarity to each other and a strong identity to the gibbon ape leukemia virus (GALV). By blotting the cloned viral genomes to Southerns of normal pig tissue (from heart, kidney, and spleen), the investigators showed conclusively that the PERVs are indeed endogenous. The data were far from subtle: More than 50 complete or partial copies of PERV were shown to exist per swine genome.

Taken together, these results point out the surprises and challenges of working with an experimental system as complex as a whole animal. Just when promising strategies were developed to circumvent the rejection of xenografts, the specter of producing novel viral infections in the recipient has arrived. One solution could be to breed pig stocks that lack any known endogenous viruses. This will not be trivial given the abundance of integrants in the genome. Even more alarming is the possibility that unidentified pig DNA or RNA viruses exist in a human transmissible form. The question of whether endogenous viruses can escape the radar screen of present day detection techniques remains an open one.

References

1. C. Patience *et al.*, *Nature Med.* **3**, 282 (1997).
2. R. E. Beneviste *et al.*, *Nature* **248**, 17 (1974); R. M. McAllister *et al.*, *Nature New Biol.* **235**, 3 (1972); J. M. Coffin, in *RNA Tumor Viruses*, R. A. Weiss, H. E. Varmus, N. M. Teich, J. M. Coffin (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1985).

–Robert Sikorski and Richard Peters

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How to Use Usenet

NET TIPS

Usenet is a bulletin board system that was originally implemented in 1980 at Duke University and is now served by a network of computers throughout the world. At present, there are about 20,000 active Usenet newsgroup discussions that cover a broad range of topics. Usenet discussions are organized into seven major categories, including topics such as recreation and computers. Readers of *Science* will be most interested in newsgroups found under the "sci" category, such as sci.astro.research or sci.bio.microbiology. Within each group is a series of messages, numbered in order of receipt, with subject lines that should bear some relation to the content of the message. Users of Usenet can post new messages or respond to existing ones to create a continuing discussion.

Discussions in the scientific groups often turn to methods and materials, so Usenet can be a very rich source of up-to-date information on scientific technology.

Although some Usenet discussions are moderated (reviewed before posting), most are not. Not surprisingly, the quality of moderated newsgroups tends to be higher than those that are fully open. In order to access Usenet you will need special software, a newsgroup reader that interprets NNTP (network news transfer protocol)—the networking language of Usenet. This is analogous to using a browser to interpret HTTP (hypertext transfer protocol)—the networking language used to surf the World Wide Web. Fortunately, a newsgroup reader can be found bundled with the latest release of the most popular Web browsers.

If you do not currently use Netscape Navigator 2.0 (or above) or Internet Explorer 3.0 (or above), you should start by downloading the most recent version of whichever one you prefer. Alternatively, you can buy or download separate newsgroup reader software that you can access through the links featured on our Web site (see address below). You will then need access to a news server, through a lab or office computer, or through your Internet Service Provider (ISP). It is the news server that actually connects, or subscribes, to newsgroups. You will then need to configure your newsgroup reader so that it knows the address of the NNTP server you will be dialing into. You should find out this address from your systems administrator or from your ISP.

Once you know this address, go to the "Options" pull-down menu in the Netscape browser. Select "Mail and News Preferences," select the "Servers" tab, and, in the "News (NNTP) Server" dialog box, type in the address of the news server. (These directions apply to Netscape 3.0 for Macintosh, Windows 95, Windows NT, and UNIX.) For Internet Explorer (version 3.0 for Windows 95 and NT), select the "Go" pull-down menu and choose "Read News." An "Internet News Configuration Wizard" will be launched; just follow the directions to configure the newsgroup reader. If the wizard does not pop up under the "News" pull-down menu, select "Options," click on the "Server" tab, then click on the "Add" button and enter the address of the news server there. [For Internet Explorer 3.0 on the Macintosh, you will have to run a separate application (Internet Mail and News 3.0). Select the "Preferences" menu, then "News," and enter the NNTP server address.] Because you will be accessing newsgroups that the server has been programmed to read, you can only receive messages from those newsgroups that your ISP or system administrator supports or subscribes to.

Once your system has been configured, you can find an extensive listing of newsgroups by querying your news server. A list of those groups dealing with various topics in science is also available at the following address: tile.net/news/sci17.html. Another way to obtain a list of all available newsgroups is to send an e-mail message to mail-server@rtfm.mit.edu. Make sure to put the following text in the body of the message: "send usenet-by-group."

It is often useful to be able to search for specific messages across many groups. There are two search engines dedicated entirely to collecting and archiving all of the messages posted in Usenet. These engines are accessed and searched with a regular browser, not a newsgroup reader. In fact, this may be the first place to start, since you don't need any additional software. Deja News (www.dejanews.com) is the most complete database and offers a very user-friendly interface. Reference.COM (www.reference.com) is another site entirely dedicated to newsgroups. You can even store frequently run queries and schedule automatic queries on a regular basis. Companies, for instance, could regularly review every Usenet post pertaining to their organization or their products. Finally, several Web search engines such as AltaVista, Excite, HotBot, and InfoSeek will let you query newsgroups archives in addition to searching Web pages.

It is a good idea to try out the system by posting a test message to "news:alt.test" before participating in an active newsgroup. This will avoid cluttering up the active newsgroups with junk postings. (The correct form of the URL is "news:alt.test"; do not type "http"). When you access a new newsgroup, it is prudent to monitor the messages for a while prior to posting your first message. Then you will be sure that this newsgroup is indeed the right one for your message.

For more about newsgroups, the latest browsers, and news readers, stop by www.MedsiteNavigator.com/techsight/tips.html

–Richard Peters and Robert Sikorski

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