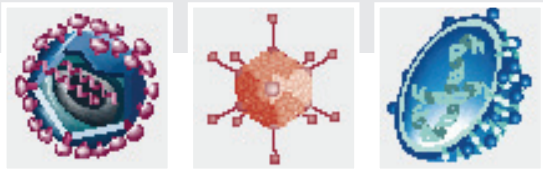


Bulletin Board

Death before infection: T cells pushed to suicide by failed HIV transcription

The major consequence of untreated HIV infection, AIDS, is caused by drastically lowered levels of CD4 T cells. The precise mechanism behind the destruction of this cell type remained mysterious, however, until a recent study led by Gilad Doitsh (Gladstone Institute of Virology and Immunology, CA, USA) uncovered the cause of the so-called 'bystander effect'.

Although one would expect that it is viral infection that leads to the HIV-related death of CD4 T cells, this did not previously seem to be the case. It is apparently-uninfected 'bystander' cells that die in this situation, giving the name to the bystander effect mentioned above. In order to study this strange phenomenon, Doitsh's group dissected the process of cellular HIV infection using a variety of anti-HIV drugs known to inhibit it at different stages. Human primary lymphoid tissue was used for this study in order to ensure a plentiful supply of CD4 T cells and a favorable environment for HIV replication.

The results of this study indicated that the cells that died were not in fact uninvolved bystanders after all; although drugs that prevented cell entry or the initiation of reverse transcription were effective at preventing CD4 T cell death, those that affect a later stage of the viral replication cycle failed to do so. These results implied that reverse transcription of the viral genome was the key to CD4 T cell death, and further investigation revealed that failed reverse transcription, presumably owing to a cellular defense mechanism, left a variety of incomplete transcripts and DNA intermediates in the cell that triggered apoptosis.

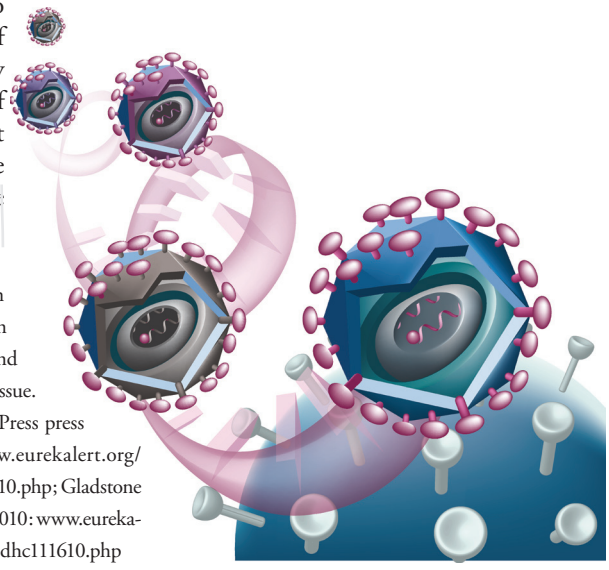
Although often a valid defense mechanism for the body as a whole, used to remove compromised cells from circulation, in this situation such self-destruction is the problem, not the solution. Even worse, in the process of dying, these cells also release cytokines that stimulate further immune reaction. As Dr Werner Greene (Gladstone institute for Virology and Immunology, CA, USA), senior author of the paper describing this study said, "That inflammation will attract more cells leading to more infection. It's a vicious cycle."

From the proportion of cells that die in this manner, it appears that approximately 5% of CD4 T cells are 'permissive' to HIV infection, allowing the virus to integrate itself and make them serve as an HIV source and reservoir. The remaining 95%, however, are nonpermissive, and meet the self-destructive end detailed above.

This news prompts a reassessment of the view of bystander cells. In the words of Dr Doitsh, "Our study reveals that the virus actually enters the CD4 T cells that are destined to die". Nevertheless, it is worth noting that this outcome is not an 'intentional' one on the part of the virus. As Dr Green puts it, "The cell death pathway is really not in the virus's best interest."

> "Our study reveals that the virus actually enters the CD4 T cells that are destined to die."

Although these findings do not change the effectiveness of current drug treatments, they may open up the possibility of developing new therapies that target the CD4 T-cell immune response, in order to avoid the 'vicious cycle' of death.



Sources: Doitsh G, Cavrois M, Lassen KG *et al.*: Abortive HIV infection mediates CD4 T-cell depletion and inflammation in human lymphoid tissue. *Cell* 143(5), 789–801 (2010); Cell Press press release. 24 November 2010: www.eurekalert.org/pub_releases/2010–2011/cp-aat112210.php; Gladstone Institute press release. 24 November 2010: www.eurekalert.org/pub_releases/2010–2011/gi-dhc111610.php

in the news...

New discovery of an ancient antiviral defense

Viruses have coexisted with animals for millions of years, and in that time there has been a great deal of mutual adaptation, an arms race that shows no signs of ending any time soon. Now, it appears that a research group led by Michael Diamond (Washington University School of Medicine, MO, USA) has found a long-discarded weapon from that arms race, in the form of a cellular defense mechanism that attempts to prevent the translation of viral genetic information.

The defense mechanism hinges on the methylation status of the 'caps' applied to cellular mRNA. The purpose of some of these caps is clear; they help to prevent the premature degradation of the mRNA. The purpose of another type of cap at the 2′ position, however, was less certain.

An answer to this question was suggested in the 1970s by Bernard Moss (National Institutes of Health, MD, USA), who observed that poxviruses added 2′ caps to all its mRNAs in an identical fashion to host cells. From this, it was theorized that these caps might be a method originally employed by animal cells to identify their own mRNA, since duplicated by viruses in order to escape detection. Certainly, viruses have been shown to go to great lengths to comply with this cap convention; the methods by which this marking is duplicated range from the synthesis of similar caps to the misappropriation of pre-existing marked RNA caps and their addition to viral sequences.

> "...the existence of such a system holds out the hope that therapeutic intervention might allow it to once again be effective against a broad range of viral pathogens."

In order to advance this concept beyond speculation, Diamond's group made use of a West Nile virus mutant in a mouse model. The mutant virus was able to add the mRNA cap required to prevent mRNA degradation, but lacked the ability to add the 2′ cap that was the focus of the investigation. Upon introduction to mouse hosts, the virus was not pathogenic, indicating that 2′ methytransferase activity was required for successful infection. Against a strain of mice deficient in type I interferon signaling, however, the virus was pathogenic, indicating that the necessity for the 2′ caps is mediated by this system. Following further investigation, it was found that viral 2′ mRNA caps did not prevent the triggering of interferon signaling, but instead provided resistance to antiviral proteins it can induce.

> "...we may be able to design inhibitors that prevent viruses from capping their RNA..."

Unfortunately, this mechanism is not currently relevant to modern viral pathogens. One of the reasons why it was not discovered earlier is because existing disease-causing animal viruses developed methods to evade it long ago. Nevertheless, the existence of such a system holds out the hope that therapeutic intervention might allow it to once again be effective against a broad range of viral pathogens. An initial suggestion along these lines came from Diamond: "We can look at the question of whether the human and viral enzymes that put the cap on are sufficiently different. If they are, we may be able to design inhibitors that prevent viruses from capping their RNA and make it much harder for them to replicate once the intrinsic immune system is activated."

Sources: Daffis S, Szretter KJ, Schriewer J: 2′-O methylation of the viral mRNA cap evades host restriction by IFIT family members. 468(7322), 452–456 (2010); Washington University in St Louis press release: Scientists identify antivirus system. Michael C Purdy: http://news.wustl.edu/news/Pages/21507.aspx

Priority Paper Alerts

Qi X, Lan S, Wang W *et al.*: Cap binding and immune evasion revealed by Lassa nucleoprotein structure. *Nature* DOI:10.1038/nature09605 (2010) (Epub ahead of print).

Lassa virus is a human pathogen responsible for thousands of deaths each year and for which there is currently no vaccine or fully effective therapy. This study reported on the first x-ray crystal structure determination for the Lassa virus nucleoprotein, which is known to have a role in viral RNA synthesis and in the suppression of immune response. The crystal structure of the protein was determined to a 1.80 Angstrom resolution, and was reported to possess N- and C-terminal domains not previously observed in viral nucleoproteins. The C-terminal domain possessed 3′–5′ exonuclease activity believed to be related to interferon signaling suppression, while the N-terminal domain took an appropriate shape, upon folding, for holding the mRNA cap structure essential for the transcription of viral genes. This high-resolution structural information is likely to be useful in developing therapies for this virus.

Frey G, Chen J, Rits-Volloch S, Freeman MM, Zolla-Pazner S, Chen B: Distinct conformational states of HIV-1 gp41 are recognized by neutralizing and non-neutralizing antibodies. *Nat. Struct. Mol. Biol.* 17(12), 1486–1491 (2010).

A major problem in raising an effective immune response to HIV is the generation of non-neutralizing antibodies. This study examined the different conformational states of the HIV envelope protein gp41, known to undergo several conformational changes during cell entry. Effective, infection-blocking, neutralizing antibodies were found to have a high affinity for gp41's fusion-intermediate state, while it was discovered that non-neutralizing antibodies have a high affinity for the stable gp41 conformation that exists after the fusion of HIV particles with the cell membrane. This latter capability would not be useful in binding to HIV particles prior to cell entry. This was theorized to be the basis of the ineffective humoral responses to HIV infection, and may also shed light on previous failures to develop an effective vaccine based on gp41.

Nanotechnology offers new technique for rapid virus detection

The rapid and effective detection of specific viruses is a desirable goal for many reasons, ranging from the monitoring/control of viral epidemics such as the various infamous influenza outbreaks to the construction of early warning systems in case of bio-warfare.

Although there are already accurate diagnostic methods for virus detection, such as PCR- and ELISA-based techniques, their usefulness in the previously mentioned applications is hampered by the need for sample preparation and a lack of portability, both of which can significantly lengthen the interval between taking a sample and obtaining results.

A new development from a team led by Hatice Altug and John Connor (Boston University, MA, USA), an 'Optofluidic nanoplasmonic biosensor for direct detection of live viruses from biological media may prove to be a useful addition to the selection of viral diagnostics. Utilizing pathogen-specific antibodies bound to a metallic substrate containing 'plasmonic nanoholes', or openings 250–350 nm across, the system detects the binding of virus particles to the antibodies due to the unusual effects these nanoholes have on the amount and wavelength of light they transmit, and how these parameters change when the holes are occupied by virus particles.

The main advantages of this new system over current technology are its portability and its ability to analyze samples without preparation. Commenting on this, Altug explained that: "Unlike PCR and ELISA approaches, our method does not require enzymatic amplification of a signal or fluorescent tagging of a product, so samples can be read immediately following pathogen binding."

The antibody-based binding of target viruses means that the specificity of the test can be varied as desired. As Professor Connor said, "It will be relatively easy to develop a diagnostic device that simultaneously tests for several different viruses."

Sources: Yanik AA, Huang M, Kamohara O: An optofluidic nanoplasmonic biosensor for direct detection of live viruses from biological media. *Nano. Lett*. DOI: 10.1021/nl103025u (2010) (Epub ahead of print); Boston University College of Engineering press release: 'Novel biosensor could enable rapid, point-of-care virus detection'. www.bu.edu/phpbin/news-cms/news/?dept=666&id=57142

Phylogenetic analysis in the legal spotlight

The phylogenetic analysis of HIV has been applied to tracking the characteristics of individual infections for both therapeutic and epidemiological purposes, but such analysis was applied for somewhat different reasons in a recent court case.

> "Phylogenetic analysis allows us to reconstruct the history of the infection events."

Researchers led by David Hillis (University of Texas, TX, USA) and Michael Metzker (Baylor College of Medicine, TX, USA) were retained as expert witnesses over two criminal cases which concerned individuals aware of their HIV-infected status having sex with several partners without informing them of that status. The resulting infections from this at-best reckless, at-worst deliberate behavior were charged as 'aggravated assault with a deadly weapon'. The intention was to use phylogenetic analysis in order to establish whether or not the infections present in the victims were likely to have originated from the accused. In order to allow for impartial evidence gathering, the researchers conducting this study were kept blinded to the origin of each sample.

Gathering evidence for this sort of case is challenging, however. A problem with utilizing the results of phylogenetic analysis in epidemiological studies (and an enormous barrier to involving this sort of technique in legal cases) has been determining the direction of viral transfer – that is to say, who is the source of the infection and who is the recipient in any given interaction. A potential solution to this comes from the 'bottleneck effect'. As Metzker explains, "During transmission (of HIV), there is a genetic bottleneck in which only one or two viruses get transmitted to the recipient."

This limited transmission has a distinctive effect on the presence and distribution of HIV genotypes within the recipient which, as was recently demonstrated, can be distinguished from those present in the source of infection.

Now that the validity of this technique has been established (both scientifically and legally), it may have implications for future epidemiological studies. David Hillis explained that "Phylogenetic analysis allows us to reconstruct the history of the infection events. We can identify the source in a cluster of infections because some isolates of HIV from the source will be related to HIV isolates in each of the recipients."

Sources: Scaduto DI, Brown JM, Haaland WC, Zwickl DJ, Hillis DM, Metzker ML: Source identification in two criminal cases using phylogenetic analysis of HIV-1 DNA sequences. *Proc. Natl Acad. Sci. USA* DOI: 10.1073/pnas.1015673107 (2010) (Epub ahead of print); Baylor College of Medicine press release: Molecular evolution proves source of HIV infection in criminal cases. Glenna Picton www.bcm.edu/news/item.cfm?newsID=3042

About the Bulletin Board

The Bulletin Board highlights some of the most important events and research in the field of virology. If you have newsworthy information, please contact: Sean Cleghorn, Commissioning Editor, *Future Virology*; Future Medicine Ltd, Unitec House, 2 Albert Place, London N3 1QB, UK Tel.: +44 (0)20 8371 6090; Fax: +44 (0)20 8343 2313; s.cleghorn@futuremedicine.com