New insights into factors that affect HIV-1 transmission

The path towards successful therapy or an effective vaccine against infection by the human immunodeficiency virus (HIV) is a difficult one. But, says Phalguni Gupta, Professor of Infectious Diseases and Microbiology at the University of Pittsburgh School of Public Health (Pittsburgh, PA, USA), 'The more that we learn about the events of early infection and the destiny of HIV in the body, the more likely it is that drugs will improve and a vaccine will become a reality.'

Modelling the female genital tract

Gupta has just completed two studies that provide new information on heterosexual transmission of HIV-1. In the first study[1], Gupta tackled the lack of a suitable model of the female genital tract that could be used to study HIV infectivity. Until now, modelling has relied on monolayers of cells[2], but these do not accurately reflect the *in vivo* situation. Gupta's organ culture model is derived from squamous cervical tissue[1]. It is an improvement on previous models, as it provides a natural tissue architecture, including epithelial cells, submucosa and immune cells such as T cells, macrophages and Langerhans cells.

Having developed the organ culture model so that it remained stable and biologically functional for seven days, Gupta and his colleagues used the system to elucidate the virological and host factors responsible for the transmission of HIV-1 into the female genital tract. The cervical tissues were exposed to infectious doses of cell-associated or cell-free HIV-1 for 24 h, after which the virus inoculum was removed. Viral transmission in the organ culture model was then followed during the course of the next 5–6 days. Cell-free virus showed the highest level of transmission on day 1, while cell-associated HIV-1 showed the highest level of transmission on day 6. Controls and tests that assessed the transmission of blue dextran through the tissue indicated that the transmission observed occurred across the mucosa and was not a mechanical process caused by leaks in the system.

Tissues from the model were frozen on the last day of culture (day 6) and were then analyzed to determine which cell types became infected during viral transmission across the mucosa. Viral RNA was detected mostly in memory $CD4^+$ T cells located immediately beneath the epithelial layer. To a lesser extent, viral RNA was also detected in scattered $CD14^+$ macrophages. No infection was detected in Langerhans dendritic cells.

'The experiments we have conducted so far indicate that this model system mimics *in vivo* transmission of HIV-1 very well', concludes Gupta, who now plans additional studies to investigate the different kinetics of transmission between cell-free and cell-associated virus.

Viral compartments and HIV-1 shedding in men

The main findings of Gupta's second study were presented at the end of January this year, at the *Seventh Conference on Retroviruses and Opportunistic Infections* (30 January–2 February 2000, San Francisco, CA, USA) and will be published in the summer[3]. This prospective longitudinal study of 18 HIV-1 infected men from the Pittsburgh portion of the Multicenter AIDS Cohort study revealed that HIV-1 shedding in semen followed three different patterns: 28% of the men did not shed the virus at all; 28% shed continuously and the remaining 44% showed patterns of intermittent shedding. None of the patients, however, showed any change in blood plasma viral load during the 10 weeks that weekly samples of semen and blood were taken.

Phylogenetic analysis of the envelope sequences of HIV-1 RNA in semen and blood revealed distinct virus populations in intermittent shedders, while similar virus populations were detected in the semen and blood of continuous shedders. The most probable explanation for this observation is that men who shed the virus intermittently have a separate compartment of HIV infection in the prostate and this is usually a different HIV strain from the virus found mainly in the blood.

This new knowledge of compartmentalization between semen and blood indicates that any vaccine prepared from blood-borne HIV might not be effective against sexual transmission of HIV in all men. In addition, potent retroviral therapy could have a different effect on HIV in semen than it does on the virus in blood. Gupta's team has now begun testing patients in whom retroviral therapy failed, to see if their seminal virus is different to that present in their blood. They are also speculating that the source of the strain of HIV found in the semen could be the prostate and are performing follow-up genetic analysis of

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HIV from the prostate, testis, semen and blood to more accurately pinpoint where this second compartment of infection lies. 'The impact on the direction of research on AIDS vaccines and treatments could be significant', concludes Gupta.

REFERENCES

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Mouse model of spinal muscular atrophy

Researchers from Ohio State University (OSU; Columbus, OH, USA) have for the first time replicated the condition, spinal muscular atrophy (SMA), in a mouse model and found that large quantities of the protein Survival Motor Neuron 2 (SMN2) corrects the SMA phenotype[1]. Their findings support the conclusion that SMN2 could act to prevent the damage caused by SMA, which has led to the search to isolate a compound that will activate production of SMN2 to treat SMA.

Spinal muscular atrophy (SMA) is the most common inherited cause of childhood mortality, with an incidence of 1 in 10,000 live births[2]. It is an autosomal recessive disorder, with one in every 40 people carrying the gene that causes SMA (Ref. 3), and it is characterized by the destruction of the α-motor neurons in the spinal cord that control voluntary muscle movement.

The genetic basis of SMA

SMA is caused by mutations in the telomeric survival motor neuron gene *(SMN1)*, but patients retain at least one copy of a highly homologous gene, centrometric SMN (*SMN2*; Fig. 1)[4]. Both genes are found on chromosome 5 where they are located ≈500 kb from each other. 'Sequence analysis of the SMN genes show there is only one functional nucleotide difference between the two genes. This lies in exon 7 and affects splicing,' explains Arthur Burghes, Associate Professor in the Departments of Neurology, Medical Biochemistry and Molecular Genetics, OSU, and the lead investigator of the project. The result is that for the *SMN1* gene, approximately 90% of the transcript is full length containing exon 7, but for the *SMN2* gene, only 10% of the transcript contains exon 7.

The significance of exon 7 is that it contains crucially important information for the SMN protein. One function that is affected by the deletion of this exon is the ability of the SMN protein to oligomerize. This single difference causes the quantity of protein produced to differ between the two loci. The result is that the *SMN1* gene produces high levels of SMN protein, while the *SMN2* gene does not produce sufficient SMN protein (Fig. 2). Increasing the number of *SMN2* genes present, however, results in the production of more SMN protein[1].

Humans carry different numbers of the *SMN2* gene as unequal crossing over at meiosis results in some people carrying two copies of the *SMN2* gene and others having none, while most have one copy. 'If you are carrying an extra copy of the gene, you will double the quantity of SMN2 protein produced,' says Arthur Burghes. This results in a reduction in the severity of the SMA symptoms.

Species other than humans have only one SMN gene that is the equivalent of the human *SMN1* gene. In mice, a homozygous knockout of the SMN gene results in early embryonic lethality following massive cell death. Coupled with the fact that SMA patients lacking the *SMN2* gene have never been reported, this suggests that SMN plays an essential role during embryonic development.

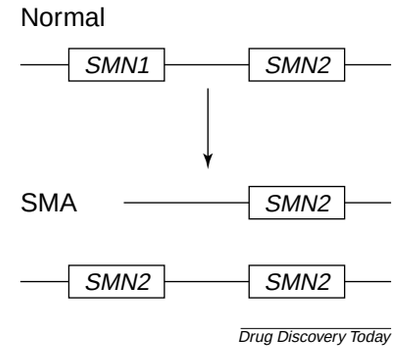

Figure 1. *Diagram of mutations giving rise to spinal muscular atrophy (SMA). The loss of the SMN1 gene can occur by deletion or conversion of SMN1 to SMN2.*

Mouse model of SMA

In collaboration with Michael Sendtner's group from the University of Würzburg (Würzburg, Germany), Burghes and his team have created a mouse model by introducing the entire human *SMN2* gene onto a null SMN background. This mimics the situation in human SMA patients, where *SMN1* is

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