Antioxidant compound slows cell death

Through 20 years of research, Paul Wong and his team at NIH are paving the way for better treatments for debilitating degenerative and neurological diseases

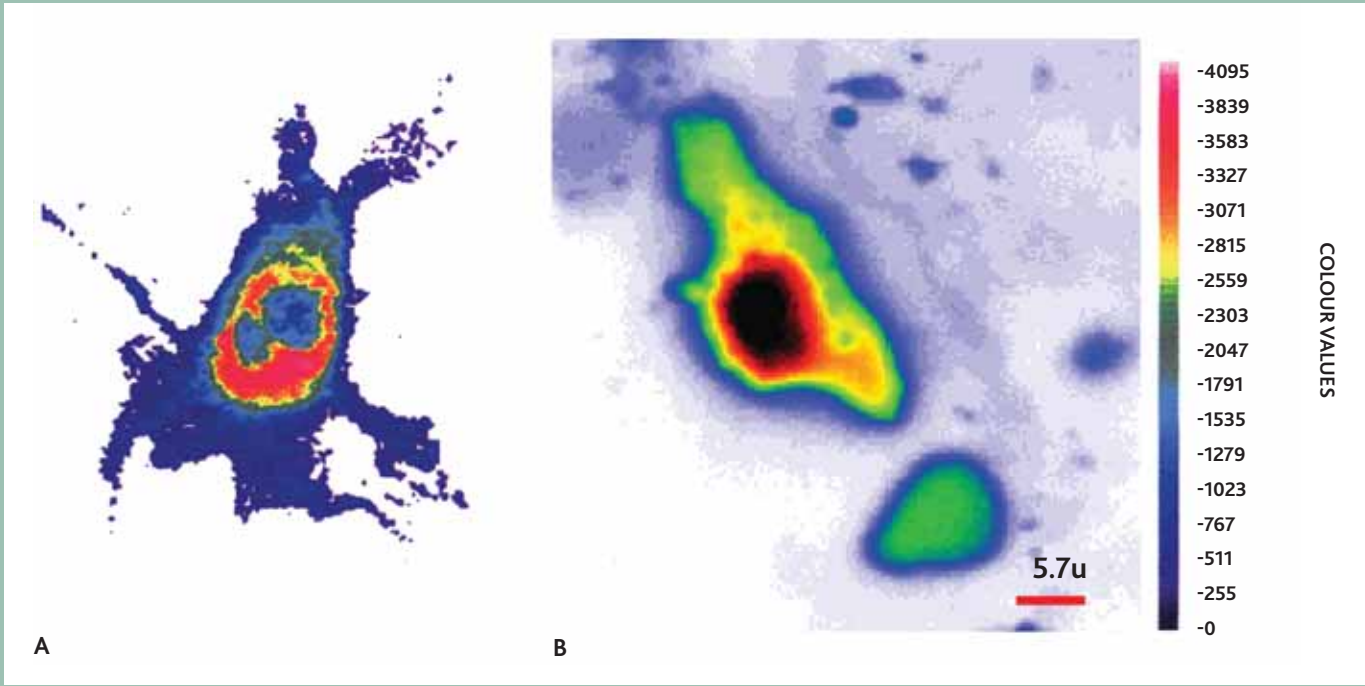
Could you give an insight into the overall aims and objectives of your research, and what prompted you to specialise in this area?

I isolated the murine retrovirus mutant ts1MuLV more than thirty years ago. Subsequently I and my co-workers discovered that infection of mice shortly after birth with ts1 induced a progressive disease leading to neurodegeneration and immunodeficiency, similar to HIV pathogenic symptoms. So we investigated the molecular mechanisms underlying the ts1-mediated cell death in the nervous and immune system and have since identified specific pathways that kill astrocytes and neurons in the CNS and T cells in the immune system in the ts1-infected mice. For both systems, the primary cause of cell death is accumulation of the mutant envelope protein leading to oxidative stress, endoplasmic reticulum (ER) stress, and mitochondria impairment. So the objective is to understand the molecular mechanisms by which retroviruses cause cellular damage and disease – particularly involving the immune and nervous systems - and to explore novel therapy to prevent cell death and disease caused by them.

How easy is it to detect RNA viruses before they become endogenous?

Retroviruses can integrate into the host genome, so theoretically, this integrated viral DNA could become endogenous (if inherited in the germ line). However, the integrated viral DNA from exogenous virus can be distinguished from cellular endogenous viral DNA by specific sequences within the viral envelope gene. In fact, we do not know when or even whether retroviruses are originally evolved from endogenous DNA sequences of cells. Retrovirus RNA present prior to integration can readily be

detected using current technologies. We have asked this question for the ts1 system and know that viral RNAs can be directly isolated from cell extract or detected by in situ hybridization. In an indirect way, we can also measure reverse transcriptase activity and detect circularized



CAPTION GOES HERE

Retro Future

Through insight into the molecular process at their core, Professor Paul Wong is advancing our knowledge of retroviruses

WHILE THEIR EFFECTS can be very tangible, to really understand the process by which retroviruses interact with their host one has to look at the closest level.

Retroviruses are major health hazards because of their propensity for persistent infection of the nervous and immune systems. Consequently, they are often associated with degenerative neurological disorders and immune dysfunction with chronic oxidative stress and inflammatory responses.

This large family of enveloped RNA viruses is found in many species and is unique among viruses: they can induce reverse transcription from viral RNA to DNA in infected cells, and in this form integrate into the genome of the host cells. The integrated viral DNA is then passed on to offspring as part of the host genome. These permanent residents in the host are called endogenous viruses, which form a large part of the genetic makeup of humans.

A number of retroviruses, including the murine leukemia viruses (MuLV), the human HIV and HTLV-1, and the endogenous retroviruses, are associated with neurological disorders, immunodeficiency and cancer. A recently identified retrovirus called the xenotropic MuLV-related virus is linked to chronic fatigue syndrome in humans.

Studies in Professor Wong's laboratory on retrovirus-induced neuroimmunodegeneration in a mouse model have been focusing on a retrovirus called MuLV-ts1. This virus has a mutation in its envelope protein that renders it unable to fold properly upon synthesis.

The primary cause of cell death is oxidative stress from viral infection

As a result, it accumulates in infected cells, particularly in astrocytes and T cells, causing an increase in the amount of oxidants in the cell. Oxidative stress occurs in the cell when the production of oxidants exceeds the amount of cellular antioxidant. Oxidative damage to DNA, lipids and proteins in the cells leads to cell death – and Professor Wong's research shows that the primary cause of cell death is due to oxidative stress resulting from viral infection.

Mice infected with ts1 exhibit selective depletion of T cells and neurons, and the clinical characteristics of the disease resemble human AIDS. Like HIV, ts1 destroys T cells directly, but neurons indirectly, since these are generally not infected by retroviruses. The secondary loss of neurons is likely due to the primary loss of support and protection from infected astrocytes or to the local release of oxidants and toxins from these cells and from infected microglia.

The study reports that a novel antioxidant/anti-inflammatory drug called GVT (monosodium luminol) decreases intracellular oxidants and is effective in preventing the neuroimmunodegeneration that otherwise develops in ts1-infected mice, even though it does not significantly suppress viral replication.

Looking at oxidative stress as a primary mechanism for the T cell and neural cell death caused by cytopathic mammalian retroviruses, the work provides important insight into the pathology of oxidative stress in retrovirus-infected cells. Professor Wong hopes that mouse models for cytopathic retrovirus diseases will help us understand human retroviral diseases, enabling improved treatments. This has implications not only for retrovirus-induced neurodegeneration and immunodeficiency, but also other related chronic, debilitating diseases resulting from oxidative stress.

viral double-stranded DNA in the preintegration form. There is also extensive work by other researchers following the events in the life cycle of retroviruses.

Given that endogenous viruses comprise ~ 8% of the human genome, are they essentially 'sleeping' in healthy people?

We believe so - many recent studies now implicate the human endogenous retrovirus (HERV) envelope protein in certain human diseases. Since ~ 8% of the human genome is composed HERV of sequences, it is not surprising that some of these HERV genes encode proteins with cellular functions. When mutations occur in these HERV envelope genes, the altered proteins could cause a spectrum of phenotypes: accumulation of protein, endoplasmic reticulum stress, and oxidative stress. In the nervous system, HERV envelope proteins have been linked to multiple sclerosis and specific sequences to breast cancer. Studies on human subjects with HIV-1 suggest that integrated HIV 'sleeps' in large numbers of lymph node macrophages, dendritic cells and T cells, and CNS cells, during the long latent period. But, over time, localized or systemic events cause activation of these latent HIV sequence resulting in virus production.

How much is understood about retrovirus associations with the onset of neurological disorders, immunodeficiency, and cancer?

The association has been well established, but there is still much we do not understand about the mechanisms underlying the related disease pathogenesis. It is not clear whether HIV directly causes cancer, although HTLV-1 causes lymphoma. In our work on ts-1 infected mice, the pathological changes in the thymus are T cell death and thymic atrophy, and cell death in astrocytes and neurons is also apparent in the CNS. Neurons are generally not infected by retrovirus, so their loss is likely due to lack of support from nearby astrocytes. ts1 kills certain target cells as a consequence of protein accumulation, ER stress, oxidative stress, mitochondria stress and work from other laboratories has shown that other cytopathic retroviruses kill T cells and CNS cells via similar mechanisms. So much more work needs to be done to enhance our understanding of the disease mechanism mediated by these viruses.

What methods did you employ to study the role of retroviruses in human immunodeficiency virus diseases such as AIDS, and what were your findings with reference to oxidative stress in particular?

In collaboration with our colleagues Pramod Nehete and Christian Abee at the MD Anderson veterinary Center, in Bastrop TX, we have found that HIV-1 infections of cultured T lymphoblastoid cells cause a dramatic increase in intracellular ROS, associated with cell death. In HIV-infected cells treated with the antioxidant compound GVT (monosodium luminol), this does not occur and the infected cells survive, although they continue to produce virus. This mirrors ts1 infection, in which ROS elevation causes death in T-lineage cells, while GVT prevents ROS elevation and inhibits ts1-mediated T cell death. We therefore suspect that lowering systemic redox 'tone' in HIV-infected individuals, using selected antioxidants, may help delay or prevent disease development, as it does in GVT-treated mice infected with ts1.

MECHANISMS OF RETROVIRUS-INDUCED T AND NEURAL CELL DEATH

OBJECTIVES

Professor Wong's research aims to understand mechanisms for neurodegeneration and T cell loss in mice infected with the retrovirus ts1. As a result of more than twenty years of work with continuous funding from NIH, he and his team now know how neurons and T cells die in these mice, and have identified highly effective drugs for therapeutic treatment.

COLLABORATORS

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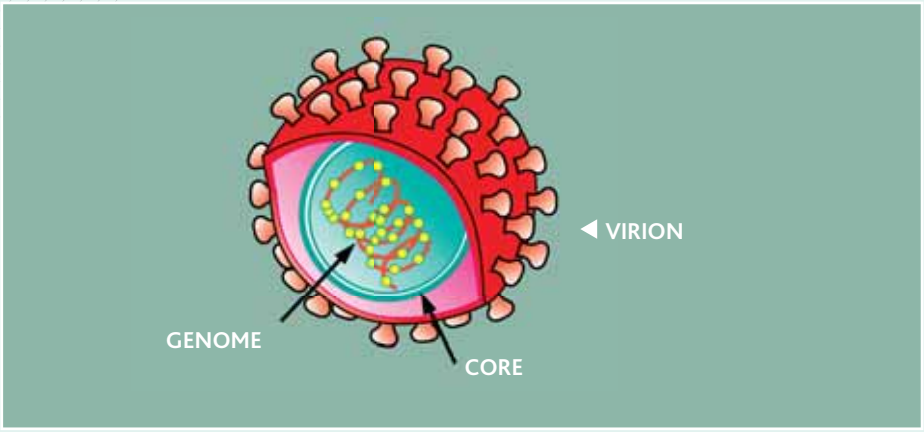
PAUL WONG is currently a professor at the MD Anderson Cancer Center, Science Park, in Smithville, Texas. His CV lists more than 100 peer-reviewed publications on mechanisms for oxidative stress-mediated cell death in two mouse models for human disease: one for ataxia telangiectasia, and the other for HIV-AIDS.



Will your work be a catalyst for progress in understanding and treating chronic fatigue syndrome?

For a long time, the exact cause of chronic fatigue syndrome (CFS) in humans has not been known and in a recent Science article, it was linked to a retrovirus called xenotropic murine leukemia virus-related virus (XMRV). However, we do not know how this virus is transmitted to humans, though it has been suggested this could be through sexual or intimate contact, or blood-borne. The pathogenic potential of XMRV in humans is also unclear, namely whether or not all infected humans develop CFS. However, it is clear that multiple-system disorders involving neurological maladies and immune dysfunction, associated with CFS, are also caused by many retroviruses, including ts1 MuLV, which are related to XMRV. So CFS in humans may also be caused by variants of retroviruses and our study may shed light on the pathogenic mechanisms for CFS. Since we have successfully treated the disease caused by ts1 MuLV, similar treatment may reduce oxidant load and have potential implication for therapy for CFS.

How striking were the results of injecting Galavit (GVT) in reducing cell death in ts1-infected mice?

Compared to uninfected, untreated mice, infected mice treated with GVT maintain normal thymic and body weights for many weeks, and the oxidative stress markers and pathology caused by ts1 in the brain do not occur. Furthermore, infected mice come down with immunodeficiency and paralysis. We have tested a number of anti-oxidants and found that GVT is the most effective drug for the symptom of ts1-mediated disease. We are exploring the possibility that potent GVT-supported antioxidant defenses allow a "truce" for CNS and T cells in ts1-infected mice, but this ends when thiol reducing agents in the tissues is depleted. Interestingly, HIV-AIDS patients have markedly depleted serum and tissues levels of cysteine, suggesting that this may accompany the development of AIDS in HIV-infected individuals. Accordingly, we are testing agents which are cysteine donors, with GVT for thiol redox protection. This drug combination may extend the latency of disease onset in ts1-infected mice.

How far off do you think a practical application for your research is?

So far our work has identified specific pathways that kill astrocytes and neurons in the CNS and T cells in the immune system in ts1-infected mice. These include oxidative stress, ER stress, and mitochondria dysfunctions which are known to be associated with HIV-AIDS, Parkinson's disease, Alzheimer's disease, multiple sclerosis, CFS, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), and ataxia-telangiectasia.

Within 5 years, we anticipate we will have extended our studies from GVT treatment and thiol supplement to treatment of some of these diseases in humans. GVT is already in clinical use in Russia for treatment of neurodegeneration and other inflammatory conditions, involving oxidative stress. After NIH reviews this data, the findings, along with our planned GVT work with SHIV-infected rhesus macaques, should shorten the time between this primate work and human trials for GVT.

What is your vision for your research in the next decade?

We hope through steady progress elucidating the retrovirus mediated pathogenic mechanisms, and development of drug-based and novel therapies, we will obtain palliative or curative treatment for many human neurodegenerative and immunodeficient conditions, to the extent that their pathology involves oxidative stress. Since retroviruses, like HIV and ts1, cause treatable oxidative stress in all infected organs, we hope GVT treatment can scavenge toxic free radicals to fully protect infected cells - as well as their uninfected neighbours - from the virus-induced oxidative stress. However, since this antioxidant treatment does not eliminate the virus, prolonged antioxidant treatment along with antiviral therapy may be necessary. Our best hope is either to eliminate all cells having latent HIV, or to find out how to get rid of latent virus in infected cells.