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Cathepsin enzyme provides clue to SARS infection

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US researchers have found that inhibitors of the cathepsin L enzyme prevent severe acute respiratory syndrome (SARS) virus from entering target cells. 'This study demonstrates a new mechanism for how viral proteins are activated within host cells', said Paul Bates, Associate Professor in the Department of Microbiology in the University of Pennsylvania's School of Medicine (PA, USA). 'This paper changes the thinking of the field', said Bates about the findings published in the early August issue of *Proceedings of the National Academy of Sciences*. 'Everyone thought all of the activation steps were at the cell surface or due to the low pH environment in the vesicle. Our paper shows that it is not just low pH but the cathepsin proteases in the vesicles that clip the viral protein. This gives us a new target to address in the development of therapeutics against the SARS virus'.

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The role of cathepsins in SARS virus infection

A typical virus gains entry into a cell by binding to the receptors on the surface of the cell itself and it is taken up into a vesicle inside the cell. However, SARS virus needs one more step to infect the cell. Proteins within the membrane of SARS virus particles need to be cut by the cathepsin enzymes before they replicate within the host cell.

Cathepsins act in the low-pH environment inside the vesicle and this causes the viral membrane to fuse with the vesicle membrane, thus, viral proteins and nucleic acids can enter the cell (where viral replication occurs). The researchers found that several chemical inhibitors of cathepsin activity blocked the infection of human cell lines by the SARS virus. This research was conducted in a high-level-safety laboratory. These findings have led to a better understanding that the cutting of viral protein by cathepsins is necessary for viral infection and is probably not unique because both the SARS and Ebola viruses are now known to use a similar mechanism to invade their host cells.

Cytotoxic problems and extracellular protease involvement

'There is growing interest in cathepsins as mediators of viral entry', said Sue Delos (Professor of Research in Cell Biology from the University of Virginia, USA). 'In fact a role for cathepsins in reovirus and Ebola has been documented previously', she told *Drug Discovery Today*. Although Delos called the study 'thorough and convincing', she identified two problems with targeting cathepsins for inhibition. First, cathepsins are important for normal cellular function and second, extracellular proteases could also function in viral entry (extracellular protease-mediated entry, particularly at the sites of initial infection). 'Thus, cathepsin inhibition may be insufficient to prevent SARS infections', said Delos. 'Clearly, post-maturation proteolysis is required to activate each of these viruses. Targeting the proteolytic sites in the viral glycoproteins may provide more specific inhibition. Many labs are currently working to identify these sites'.

Polly Roy (Professor of Virology, at the London School of Hygiene & Tropical Medicine, UK) also had some reservations. She said the significance of the study for developing antivirals might be limited because cathepsin L was not SARS-CoV specific. 'Thus any inhibitor of this protease may disrupt other pathways associated with normal cellular processes and could be cytotoxic', said Roy. 'This work is of course very significant as cleavage of the SARS-CoV fusion protein does not occur during normal maturation of the protein. No doubt this will open up a new direction of research in the understanding of molecular events, not only in SARS-CoV virus entry mechanism but possibly for other enveloped viruses'.

Conclusions

SARS, caused by an emergent coronavirus, is a respiratory disease of unknown etiology that originated in mainland China in 2003. It is characterized by fever and coughing, difficulty breathing or hypoxia and it can be fatal. More than 8000 people were infected and 750 people were killed when the disease swept the world in 2003 forcing WHO to issue an unprecedented world-wide alert.

Currently there is no effective treatment although patients have been given steroids in an attempt to help their lungs cope with the infection. Peter Openshaw, a professor from the National Heart and Lung Institute at Imperial College London, UK, told *Drug Discovery Today* that 'a drug that works generically for CoVs would be a big step forward... But this is all *in vitro* and many drugs fall at the *in vivo* transition stage'.

Novel alternative for osteoarthritis

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A Phase III trial of 400 patients has demonstrated that extracting proteins from the patients' own blood can be an effective and safe treatment for osteoarthritis of the knee. Osteoarthritis is a common condition that leads to severe pain. In recent years, Cox II inhibitors have been used widely but emerging awareness of their severe side-effects led to the drug Vioxx (rofecoxib) being taken off the market worldwide in 2004. Over the past few years, scientists in Germany have been developing a method of extracting

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proteins from the blood of each individual to prevent further cartilage degeneration in joints. 'The therapy involves administration of knee injections of IL-1Ra protein, obtained from the patient's blood: as this is an autologous process, there are no allergic reactions and minimal side-effects', explains Antje Kassel, spokesperson for Orthogen (Dusseldorf, Germany), the biotech company responsible for orthokine.

'Orthokine acts on the cartilage directly, easing symptoms and changing the course of the disease…'

Interleukin-1 (IL-1) is produced in the body and contributes to the pathology of osteoarthritis and intervertebral disc degeneration and prolapse. Its antagonist, interleukin-1 receptor antagonist (IL-1Ra), acts against IL-1 as a protective mechanism against further damage but is present in the blood in only small amounts. Orthokine therapy was originally developed by orthopaedic specialist Peter Wehling (now CEO of Orthogen) and molecular biologist Julio Reinecke to boost the normally low levels of IL-1Ra. Blood is taken from the patient into a syringe containing small glass balls with a surface coating that simulates the presence of a wound. The blood cells in the syringe are stimulated to respond by producing IL-1Ra in large amounts. Within 24 hours, the syringe contains enough protein for treatment.

'The problem with conventional osteoarthritis therapies, such as painkillers, steroid and hyalauronic acid, is that they act on the symptoms but not on the underlying cause', comments Wehling. Orthokine acts on the cartilage directly, easing symptoms and changing the course of the disease. The value of orthokine is clear from the trial results. 'Compared to injections of hyalauronic acid and placebo, the therapeutic success was significantly greater in the orthokine treatment group', reports Axel Baltzer, Orthopaedic Surgeon from the Center for Molecular Orthopaedics, Konigsallee Clinic (Dusseldorf, Germany), who co-ran the trial. 'The highest success rate of 70% and also the highest satisfaction with therapy was seen with orthokine; at no time were there significant differences between hyalauronic acid and placebo', he adds.

Carsten Moser, Medical Manager of Orthogen Lab Services, says that orthokine treatment is used in Germany in nearly all joints and in conditions of the spine and has been available for about six years. 'We

conducted our trial on knee osteoarthritis as representative for all joints because it is the most frequently affected joint', he explains. Trialing is necessary to provide evidence of orthokine's effectiveness to enable it to become available more widely in Europe and Orthogen hope to apply for approval in the UK and other countries in the near future. 'We think this will now be straightforward; in addition to the trial results we have documented experience of nearly 30,000 intra-articular injections and 20,000 spine injections that show orthokine therapy to be extremely safe with an adverse event profile comparable to placebo injections', adds Moser.

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