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EDITORIAL

Towards treatment of viral pathogenesis

Traditionally, antiviral drugs have been developed by targeting specific enzymes encoded by viruses. This not only explains the excellent specificity and selectivity of many of the drugs but also explains why we have no *potent* licensed broad spectrum antiviral drugs because the targeted enzymes are specific to virus families; (note that ribavirin, although broad spectrum, is not a *potent* antiviral drug and that cidofovir is not licensed for treatment of the several viruses against which it has activity). An exception is where viruses in two different families share a particular enzyme, such as reverse transcriptase in the case of HIV and HBV, so that drugs such as lamivudine have activity against both viruses.

Now the spectre of natural epidemics, or even bioterrorism, is demanding exploration of a different approach. A rapid response is required to any novel influenza virus which crosses into humans and threatens to cause a pandemic. A rapid response is also required for a whole host of rare infections where there is no financial argument for the development of compounds specific for each infectious agent. Would it therefore be possible, rather than targeting each infection itself, to target common components of the diseases triggered by different viruses? In this way, diseases would be seen as complications of infection and treatment would be directed at downstream pathogenetic effector mechanisms, which are shared by viruses from distinct families. Several possible examples present themselves.

First, the renin-angiotensin system, well known for its contribution to hypertension, is also involved in the diffuse alveolar damage known as acute respiratory distress syndrome (ARDS) which is a component of the respiratory disease caused by SARS coronavirus and influenza viruses. Angiotensin converting enzyme (ACE) cleaves angiotensin I to angiotensin II which can bind to alternative receptors in the lung which either cause (AT1 receptor) or decrease (AT2 receptor) protein transudation, lung oedema and tissue damage [1]. Angiotensin II can be cleaved to a benign form through the action of ACE2. Now, ACE2 is the receptor for SARS coronavirus [2–4] as well as the more benign, recently described, NL63 coronavirus [5]. A recent report in *Nature Medicine* shows that SARS coronavirus downregulates ACE2 [2] so probably exaggerating the ARDS response by decreasing inactivation of angiotensin II by ACE 2. Furthermore, recombinant ACE2 reduced lung injury in mice with diffuse alveolar damage [6] as did antagonists of AT1 receptor [7]. Although there remain some unresolved matters of detail [1], the therapeutic implications are clear. While reappearance of SARS appears unlikely, the imminent arrival of H5N1 influenza virus offers the potential to evaluate, in randomised controlled trials, the efficacy of already licensed anti-hypertensive AT1 receptor inhibitors and/or recombinant ACE 2, as adjuvant therapy to the neuraminidase inhibitors for the unfortunate humans who become infected.

Second, despite being caused by diverse members of the *Filoviridae, Arenaviridae, Flaviviridae and Bunyaviridae*, many, but not all, of the various haemorrhagic fevers share elements of pathogenesis [8,9]. The cardiovascular manifestations of hypovolaemia, hypotension, petechiae, mucosal haemorrhage and bleeding from venepuncture sites are not due to blood loss itself but to various combinations of disseminated intravascular coagulation (DIC), thrombocytopenia and adrenal insufficiency. The bleeding time, prothrombin time and activated partial thromboplastin time are frequently prolonged, with decreased synthesis of coagulation factors within the liver contributing to this cause of multiple organ dysfunction. These defects of clotting co-exist with defects of fibrinolysis, thus consuming the labile coagulation factors produced by the liver [10,11]. Over-expression of tissue factor in monocytes/macrophages is another component of pathogenesis [10]. The thrombocytopenia may result from interferon-induced arrest of megakaryocyte maturation [12]. Disruption of the

function of endothelial cells has also been suggested as a contributor to the haemorrhagic diathesis and Ebola virus glycoprotein can directly damage endothelial cells [13]. However, current evidence suggests that endothelial involvement occurs late in the disease process as a pre-terminal event [14,15]. The therapeutic implications are that drugs able to interfere with the coagulopathy induced by the haemorrhagic fever viruses might have broad clinical applicability. For example, DIC has, paradoxically, been treated in humans with the anticoagulant heparin which ultimately leads to cessation of the consumption of clotting factors. There is evidence from animal studies that interfering with clotting can improve the outcome of haemorrhagic fevers and some human anecdotes of the use of heparin [16]. Likewise, recombinant nematode anticoagulant protein C2, an 85aa protein which inhibits the tissue factor/factor VIIa complex, blocks clotting and improves survival when given to macaques infected with Ebola virus [17].

Third, hepatitis B (HBV) and hepatitis C (HCV) frequently establish chronic infections which lead, over decades, to cirrhosis and liver failure with or without hepatocellular carcinoma. A common component of the pathogenesis of cirrhosis is fibrosis, which leads to distortion and permanent remodelling of liver architecture. This explains the haemodynamic effects of cirrhosis which allow toxins, absorbed from the gut, to reach the brain causing hepatic encephalopathy. Fibrosis can be defined as a wound maintaining chronic signals for tissue repair [18]. TGF-β is the master switch for profibrotic genes as shown by studies in radiation induced fibrosis [18], while transgenic mice with TGF-β under the control of the albumin promoter showed hepatocyte specific expression of TGF-β and produced extensive hepatic fibrosis [19]. When carbon tetrachloride was used as a liver toxin in normal mice and in TNF receptor knockouts, the acute infection was similar, but chronic inflammation, TGF-β levels, and fibrosis were significantly decreased in the knockout mice, implicating TNF-α as also being profibrotic [20]. In humans, transplant or HIV patients show accelerated fibrosis so that immune control of viral replication must decrease liver inflammation. However, immunity may act as a dual-edged sword; studies of intrahepatic T-cells reveal that virus-specific cells provide protection while non-specific cells contribute to inflammation [21]. In addition, when genes from the HCV genome were linked to a reporter gene under the control of the TGF-β promoter, the core protein produced activation with evidence that the mitogen activated kinase pathway was involved in directly stimulating fibrogenesis [22].

Irrespective of how TGF-β is stimulated, the fibrosis is similar [23]. Stellate cells are profibrotic when activated and transdifferentiate to become myofibroblasts [23]. Matrix metalloproteinases produced by fibroblasts and macrophages digest the fibrotic extracellular matrix but are inhibited by tissue inhibitor of metalloproteinase (TIMP). Decreasing inflammation reduces TIMP, thus allowing activated stellate cells to be removed through apoptosis [24]. Regression of fibrosis can occur even in cirrhotic livers, but the ultimate clinical response is limited by the degree of matrix cross linking produced by tissue transglutaminase blocking the function of the metalloproteinases [25]. This argues in favour of intervening therapeutically as soon as fibrosis is identified. Conventionally, fibrosis is detected by histopathological examination of liver biopsies, but quantification of mRNA for profibrotic genes is also being evaluated [23]. Less invasively, serial biochemical assessments of blood are showing improved ability to identify liver fibrosis. For example, an international multicentre evaluation of a panel of 9 biomarkers, which measure various elements of matrix synthesis or matrix degradation, had a 90% sensitivity and 92% negative predictive value for identifying fibrosis on liver biopsies from over 1000 patients [26].

What are the implications for treatment? Fibrosis regresses when hepatitis C responds to interferon/ribavirin treatment [27]. In addition, novel drugs could be developed to interfere specifically with the development of fibrosis. The multiple points at which profibrotic signals could potentially be blocked by candidate compounds are reviewed elsewhere [23,28] and a controlled trial is planned for at least one compound, Cu–Zn superoxide dismutase [29]. Initially, patients who are non-responders to interferon will be recruited, but the potential of studying combination interferon/anti-fibrotic therapy is obvious. Thus, inhibition of fibrosis within the liver has the potential to delay the progression to cirrhosis triggered by HBV and HCV, despite their

classification as members of the distinct *Hepadnaviridae* and *Flaviviridae* families.

PD Griffiths

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