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EDITORIAL

Orphan viruses, orphan diseases: still the raw material for virus discovery

This journal's title specifies reviews of *medical* virology, and it is important not to lose sight of this, its primary purpose. The inter-relationship between virological research, clinical disease and public health should be at the heart of the journal's contents.

The viruses of humans are diverse, and the RNA viruses especially are unstable life forms on a continuing journey towards symbiosis with their host species. During this evolution, they may trade virulence for transmissibility, but they probably never establish complete harmony with their host. So, whenever a 'new' virus is isolated and is suspected of being a pathogen, it is important to ask what its range of virulence is, even if such questions may be difficult to answer comprehensively.

It is now 60 years since monolayer cell culture was first applied to the isolation and propagation of viruses. It soon uncovered a superfluity of isolates in human faecal extracts and throat swabs, many of them of uncertain pathogenicity. There was no doubt about the virulence of the three poliovirus types, nor of some other 'enteroviruses', such as the Coxsackie viruses that were pathogenic for mice and also associated with acute human illness; but there were other serologically distinct enteroviruses that seemed to be non-pathogenic both for humans and experimental animals. Only when the moment came that their occurrence coincided with local outbreaks of rash or other illness did their pathogenicity become apparent, and they were more closely investigated.

At first, therefore, many enteroviruses were referred to as ECHO, that is, enterocytopathic orphan viruses, the term 'orphan' being applied because they lacked a parent disease. This term was, according to Gilbert Dalldorf, conceived by a group of pioneers of virus cell culture '*in a moment of conviviality*' (Dalldorf, a New York pathologist, was the investigator who had previously shown that Coxsackie viruses caused paralysis in newborn mice but not in humans).

Many so-called orphan viruses were subsequently linked to diseases. However, by the mid-1960s, the use of cell monolayers had become so routine in diagnostic laboratories that it seemed unlikely that more new associations between virus and human disease would be made unless by adopting more advanced cell culture techniques or developing other means of virus discovery. Maintaining monolayer cell cultures for longer periods did, then, allow cytomegaloviruses, polyomaviruses and hepatitis A viruses to be grown and linked to disease. Also, the culture of Epstein–Barr virus in lymphocytes suspended in liquid media led to it being shown to be the cause of infectious mononucleosis. Later, a similar culture technique revealed human immunodeficiency virus (HIV) as the cause of AIDS.

Meanwhile, viral structures and complete virus particles were being visualised by electron microscopy, and these were associated with 'serum' hepatitis, with different forms of gastroenteritis and, in the case of erythrovirus B19, with 'fifth' disease. Molecular techniques have since led to the discovery of the viral causes of two additional forms of infectious hepatitis, C and E, and more recently have suggested that a herpes virus might be the cause of the unusual rash illness, pityriasis rosea [1].

So, just as, earlier, orphan viruses were linked to specific diseases, various 'orphan' diseases have since been linked to their causal viruses. Indeed, Nobel prizes have been awarded for the work that led to the discovery of the viruses of hepatitis B and AIDS. Even now, when a disease with characteristics suggestive of a viral infection is scrutinised by virologists who have the necessary skills and techniques, an unrecognised causal virus may be found. It will probably not be a virus that grows in ordinary cell culture though there have been recent exceptions to this. The viruses of the trans-species emergent coronavirus infections, SARS and Middle East Coronavirus (MECoV), although not discovered by that route, have both proved to grow readily in cell monolayers [2,3]. It is a reminder that the viruses of feral animals have not been studied with anything like the same intensity as those of humans and of domestic and farm species.

What are the features of diseases of uncertain aetiology that suggest a viral cause or a virus trigger that sets them off, and how should investigation

proceed? The virologist will be most interested in the clinical features that characterise the onset of the illness, for instance the herald patch of pityriasis rosea. While the most prominent clinical features of disease are likely to be mediated by host immunopathic responses, the peak of virus replication has probably been reached earlier. Does the setting or does a particular host behaviour suggest from where a causal virus might have been contracted? Did an incoming virus leave clinical footprints, perhaps a transitory fever, loss of appetite, aches and malaise, a sore throat or a fleeting rash? Such 'prodromes' are well known sometimes to precede serious illnesses with long incubation periods—the prodromes of the viral hepatitides are obvious examples. Virus-induced disease in the immunocompromised, in others with an unusual host susceptibility and in the fetus may follow a trivial initial illness. So, was there illness in close contacts before or soon after, perhaps among children in the same family, and are they also being investigated? Could a virus have acted as the trigger for a disease process which was self-sustaining but for which the viral stimulus might no longer be detectable by routine means?

A different investigative approach is to look for rare but serious clinical manifestations attributable to a familiar virus, either one retrospectively detected by serological means or perhaps one shown by whole genome sequencing to be a variant with an unusual tropism. Such attempts to associate a seemingly benign virus with an important disease of unknown aetiology are often prompted by an excess of wishful thinking, but some diseases are of such gravity that speculative investigation is justified. Take, for example, type 1 insulin-dependent diabetes. The early work on the susceptibilities of mice to Coxsackie viruses showed that type B4 caused rapid onset of diabetes in mice, and this has led to unsuccessful attempts stretching over several decades to associate that virus with human diabetes [4]. More recently, it has been suggested that erythrovirus B19 is associated with autoimmune disease, specifically rheumatoid arthritis and Graves' disease. However, an investigation comparing Graves' with multinodular thyroid disease has found that, unexpectedly, B19 seropositivity is much more frequent in the latter than the former [5]. Yet, the possibility of viral triggers of autoimmune disease, not the least type 1 diabetes, remains open to investigation; for diabetes, the search could well be broadened to include other picornaviruses and, for instance, hepatitis E virus (HEV). HEV is certainly hepatotropic and might also be pancreatotropic. In late pregnancy, an immunosuppressed and proto-diabetic state, HEV is at its most virulent [6,7]. Incidentally, PCR is a detector tool that has greatly widened the opportunities for virus discovery in 'fishing' expeditions of this sort.

But it is not only current molecular developments, powerful though they are, that will drive future clinico-virological studies nor should interest just be concentrated on emergent, usually rare, infections, such as the novel coronaviruses. The systematic study of prodromic and syndromic illnesses will also advance virus discovery and may now be empowered by means not previously available, such as internet search engines and the social media. When, for instance, the public resorts to the Web for information about illness, how much and in what circumstances does that traffic fluctuate, and what does this imply about the changing epidemiology of an infectious disease? What observations are then being shared, e.g. on *Facebook*, about symptoms and signs? The ability to investigate common diseases in these new ways may be constrained by confidentiality issues, but it could reveal much about the range of host susceptibilities to viruses [8]. These data could then be further informed by the fast expanding knowledge about human genetic micro-diversity and consequently about individuals' susceptibility to serious infectious disease. Both these strands are likely to contribute to medical virology in the future.

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