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The future of diagnostic virology

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Europe has an envied reputation for excellent epidemiologic monitoring of virus diseases, even when there were few antiviral drugs with which to treat them. More are now being developed and, because they are, and are likely to continue to be, highly specific for individual viruses (or, at best, groups of viruses), there will be a continuing need for rapid and equally specific diagnostic tests to identify acute infections with them. In this context, rapid really does mean exactly that. The answer has to be available in hours if an antiviral is to have any chance of being effective—even overnight will be too long in most cases, particularly where life-threatening disease is involved.

There have been, and are, useful and effective vaccines to prevent infections with some viruses, although others have proved intractable. The good vaccines included those against smallpox, polio and measles, and there is a reasonable hope that the latter two will join smallpox in oblivion early in the next Millenium. Respiratory viruses, like influenza and respiratory syncytial virus, offer more difficult challenges, yet to be solved fully [1,2], although the new antineuraminidase drugs for influenza show promise [3].

Underpinning the evidence of a need for an antiviral drug, and confirming its effectiveness in use, must be rapid and accurate diagnosis of virus infections. Many such diseases, particularly in the early stages, present as 'flu-like', with more characteristic features developing later, if at all. Hence, rapid diagnosis must be adequately specific and capable of distinguishing, if necessary, between closely related strains. Moreover, where vaccines are type-specific, and many are, only type-specific diagnosis can point to the need for a vaccine and monitor a vaccine's effectiveness. It, too, must be rapid to confirm an epidemic and to counteract common assumptions that all cases in an epidemic are due to the same virus—respiratory viruses frequently overlap in both symptoms and signs [4].

Two recent trends are threatening this availability of specific diagnosis, and are already having an effect in those countries in the world where there is a good diagnostic service. They are, first, the increasing and relentless pressure on the funds for health care, and, second, the increasing availability of commercially produced diagnostic kits. I wish to examine each in turn, though they are clearly linked, noting that the advent of the latter may be thought to be a help to the former.

PRESSURE ON HEALTHCARE FUNDS

Treatments for diseases of all kinds tend to become more expensive as new ones are developed and new diseases identified. Many conditions, inevitably fatal in the past, can now be cured, or alleviated. Cancers, leukemias and organ failures can be managed with cytotoxic drugs and/or transplants. New drugs to combat old conditions, drug-resistant bacteria and fungi, and organ rejection, are being evaluated and adopted into regular use. Few of them are cheap.

Not surprisingly, healthcare budgets everywhere are feeling great pressure, and both clinicians and administrators are being forced to decide priorities [5], including where diagnostic virology can fit into them. Many virus infections do not have specific drugs to cure them quickly, and most (in otherwise healthy individuals) are self-limiting anyway. Under these circumstances, there is a temptation to restrict diagnostic virology to what is simple and straightforward (e.g. the less expensive serology) and to reduce the number of specialist virology laboratories in each country to one or two centralized facilities. The main function of these central reference laboratories would be to serve the epidemiologic needs of the country. Routine diagnostic needs could then be met by multidisciplinary machine-based assays on serum samples.

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COMMERCIAL KITS

Before the development of monoclonal antibodies, commercially produced diagnostic kits or reagents were impractical—good polyclonal antisera were in very short supply, being produced, with a huge investment in time and effort, in small quantities by individual laboratories for their own use [6]. There was never a surplus, at any price, for any commercial enterprise to contemplate marketing kits based on them. Moreover, the realistic cost of such sera would have made any such kit unacceptably expensive for routine use.

Monoclonal antibodies completely changed the ground rules. Reliable antisera in almost unlimited quantities could now be produced and these could be used to standardize reagents for diagnostic use, as well as providing positive control sera. Kits followed rapidly, particularly for hepatitis B and HIV/AIDS, where there was, and is, a huge demand for screening blood and blood products for evidence of past infection before use [7]. The technology developed for these two viruses was soon adapted to others, and a variety of reagents, kits and systems for routine diagnosis have flooded onto the market—so much so that laboratories have found that, provided they used enough of the material or kits, they often did not have to buy the otherwise expensive machine on which to run them; it would be readily available on loan. Further, similar enzyme-linked assays became available for other branches of laboratory medicine, particularly biochemistry, and one machine might, in theory, fulfill everyone's needs. So far, this form of rationalization has been slow to catch on but, as money becomes ever tighter, the pressure towards it will rise. Such assays are based on measuring levels in serum, and, in virologic terms, this means measuring antibody levels. These rise far too slowly to be useful in rapid diagnosis, but many still believe that 'viral titers' provide the essence of virologic diagnosis. Virus titers may be helpful in some cases, but only in retrospect; they have no place in deciding antiviral treatment, except in continuing infections such as AIDS and hepatitis.

More recently, nucleic acid amplification techniques (polymerase chain reaction (PCR), ligase chain reaction (LCR), nucleic acid sequence-based amplification (NASBA), etc.) have offered another new approach to diagnosis [8]. Their extreme sensitivity, combined with not having to recover live virus, has meant that, again in theory, very small quantities (down to a few genome copies) could be identified using primers made in unlimited quantities. Inevitably, diagnostic kits have appeared, and their use and value are being explored [9]. These kits are not cheap and need high-class laboratory techniques to be reliable, but they are being hailed as a possible way to avoid the need for cell culture. Whether they can be rapid and cheap enough for routine use remains to be seen but, since they require known primer sequences, they cannot work with novel viruses. Cell culture is one of the traditional diagnostic tools, but one that is expensive in skilled staff time, reagents and space and impossible to standardize.

Commercial kits also promise another bonus—that of standardization. If all laboratories use the same, or similar, equally and centrally validated kits, their results should be comparable [10]. The resultant data could then, in theory at least, give a more reliable continent-wide or worldwide picture of the epidemiology of individual viruses. Moreover, with the responsibility for developing and validating tests taken out of health service laboratories, it could be argued that the need for specialist virologists would then be reduced. They would only be needed in the few specialist laboratories; routine diagnosis could be supervised by microbiologists.

NEW VIRAL ACTIVITIES

Overall, this paints a gloomy picture for those who have chosen to specialize in virology. Do they have a future? Of course they do, because this concept of trivial infections being simply and easily diagnosed by serologic and nucleic acid amplification tests run on large machines using commercial kits is far too simplistic. Nonetheless, the reasons why this is so must be spelt out clearly, and quickly, before the virologists have become discouraged and disappeared.

The main reason is that viruses and their activities are continually changing. Such manifestations will include the appearance of new viruses, recognition of new activities by 'old' viruses or by new variants of 'old' viruses.

NEW VIRUSES

'New' viruses are still emerging, though most are not truly new—they have just not been encountered in humans before. Their new role in human disease comes from humans altering their environment and habits: holidaymakers go further afield than before, there is still a drift towards the cities, wars and deprivation are still common, diets change, and so on. Table 1 lists the viruses that have been recognized as new infections since 1970, and the list is not yet complete. When such novel infections surface, they may do so anywhere, at any time. How severe and widespread they will be cannot be predicted. It is essential that they are identified and confirmed as soon as possible, and their progress monitored.

Table 1 New or re-emergent viruses since 1970

Bornaviruses	
Bunyaviruses	
Hantaviruses: Hantaan, Sin Nombre, etc.	
Gastroenteropathy viruses	
Caliciviruses (including Norwalk and small round structured viruses), rotavirus, adenoviruses 40 and 41, astrovirus, small round viruses, fecal coronaviruses (?), picobirnaviruses (?)	
Hemorrhagic viruses	
Filoviruses: Ebola and Marburg (re-emergent)	
Arenaviruses: Guanarito, Junin, Sabia, etc.	
Hepatitis viruses	
Hepatitis C and GB variants, delta agent (hepatitis D), hepatitis E, more?	
Human herpesviruses	
HHV6, HHV7, HHV8	
Human retroviruses	
HIV-1, HIV-2, HTLV-1, HTLV-2	
Orthomyxoviruses	
Influenza A: H1N1 (re-emergent), H3N2 (drift strains), H5N1 and H9N2 (novel)	
Papovaviruses	
77 genotypes	

NEW ACTIVITIES BY 'OLD' VIRUSES

Two possible scenarios are possible under this heading. The first is importation of known viruses into new areas, and recent cases of yellow fever imported into Germany in 1999, and originally thought to be due to Ebola virus, provide a good example. Global warming may bring insects, and the viruses they transmit, to areas previously free from them [11].

Second, with increasing numbers of immunocompromised patients, either because their natural immunity is deficient or absent, or because they are on immunosuppressive drugs (for malignancies or following transplants), otherwise relatively harmless viruses can become seriously life-threatening. Cytomegalovirus (CMV) is normally a silent infection, but reactivation in transplant patients or those with AIDS can be very serious and is the most widely known example [12], although it is not the only one. When CMV recurs, high and apparently adequate doses of appropriate antiviral drugs often fail to eliminate CMV activity, and exactly why this happens is unclear [13]. Such patients are increasingly managed in district hospitals, but specialist virology has to be close at hand for this to be done properly. With more anti-CMV drugs becoming available to help to manage this problem, the patients must also be monitored for the appearance of resistant strains of the virus [14]. Just as importantly, progress in reducing virus activity must be assessed regularly. Failure to achieve undetectable levels can be due to developing resistance, but this may not be the only reason. Assumption is not enough; the real reason must be found if the problem is to be solved.

However, CMV is not the only virus to infect immunocompromised patients; others may be associated with very similar, and usually indistinguishable, clinical pictures [15]. A drug effective against CMV would be totally unsuitable for treating these other viruses, confirming the need for correct identification of both the true virus cause and its part in causing the clinical picture.

NEW VARIANTS OF 'OLD' VIRUSES

Influenza A is the best example of this problem. The RNA genomes of both influenza A and B are unstable, leading to antigenic 'drift', in which their surface antigens alter a little each year, possibly as a result of immune pressure, so that over several years mutants gradually outflank existing herd immunity [16]. In addition, influenza A can undergo major antigenic changes through acquiring new segments of RNA by genetic reassortment with animal strains [16]. These major 'shifts' occur at infrequent and unpredictable intervals, but are important because the new strains have changed their antigenic outer clothes to such an extent that no existing immunity in the community is effective and a worldwide pandemic may follow. Should this occur again (the last time was in 1968), there will be an urgent need for a new vaccine to protect the vulnerable. If the new strain is as pathogenic as Spanish flu was in 1918, the entire community will be at serious risk. The recent isolation of H5N1 [17] and H9N2 (J.S.M. Peiris, personal communication) strains of influenza A viruses in Hong Kong confirm that 'shift' changes occur and these dangers are not just theoretical. The formulation and production of any form of vaccine will depend on isolating and characterizing the new strain as quickly as possible.

New serotypes of other 'old' viruses are still being identified. These include adenoviruses, often in patients with AIDS [18]. Vigilance may reveal other examples.

Even without these new developments, we have not solved all the existing virologic problems. There remain annual epidemics of respiratory syncytial virus (RSV) affecting thousands of babies each year, many of them requiring hospital admission [19]. Routine diagnosis defines and reminds us of the size of the problem—it does not go away because the reports dry up. An effective vaccine has been sought for a long time but a satisfactory one has yet to be found [1].

CONCLUSIONS

All these viral activities should be monitored if measures to control them can be logically devised. Some are predictable, e.g. the annual RSV epidemic [20], though serologic diagnosis has been found to be far too unreliable [21], but the possibility of new viruses means that some catch-all techniques must be available. These are methods (cell culture and electron microscopy) which do not depend on pre-existing reagents, which will not be available for any novel viruses. Both methods, however, are under threat as being unacceptably expensive, but at present there are no practical alternatives. Neither method is suited to general microbiology laboratories because they require specialist skills which, to remain acceptably reliable, must be kept in regular daily use.

These factors encapsulate the virologic dilemma for health services—how many virus laboratories does a country need? For many European countries, a single centralized one might be thought enough, particularly in a geographically small country, but will not be adequate for two reasons. First, virus diagnosis works best over short (within city) distances, so that clinicians and senior laboratory staff can work in partnership, each knowing the other as individuals. Absence of contact leads to reliance on 'virus titers' alone. Secondly, the virologic staffing structure must be large enough to give the trainee virologists reasonable career prospects. These arguments would point towards a need for a virologist in every district general hospital.

As a virologist, I would personally welcome this conclusion, but this is (probably!) being unrealistic. There has to be a balance between too many and too few. There have to be as many laboratories as are necessary to ensure adequate and sufficiently broad monitoring, both epidemiologically and of individual patients, with properly trained and experienced staff in daily practice with the necessary catch-all techniques. At present, in the UK, too many senior vacancies are left unfilled—for example, during the last 10 years, four Consultant posts in Glasgow, two in Newcastle, one in Edinburgh, one in Leeds, one in Leicester. If this trend is not reversed, specialist virologists will become extinct through attrition, and because trainees will find their career prospects much greater in other specialities. The value of virology services directed by fully trained and in-practice specialists to transplant units, pediatricians, AIDS clinics, intensive therapy units (ITUs), epidemiologists, developers of vaccines and antiviral drugs (and those that evaluate them in trials) and commercial companies must be recognized, and their survival planned. Concerns about new viral activities developing in the community, and not being recognized until too late, were expressed as long ago as May 1989 at a meeting in Washington, DC on 'Emerging viruses: the evolution of viruses and viral diseases', sponsored by NIH in cooperation with The Rockefeller University. These well-justified anxieties were later published as a book [22], in which Donald Henderson, architect of the WHO Smallpox Eradication Campaign, proposed a network of nationally based surveillance laboratories [23]. This is, one would have thought, only common sense, but no network can survive without staff, trained and in regular practice, to do the basic tasks of looking for new viruses or changes in the pattern of 'virus-like' diseases. In other words, they must be in post, and kept in practice by being given routine tasks to do. Now, 10 years later, the situation has got gradually worse and there still seems to be far too little awareness of the danger of losing the necessary breadth of techniques necessary for adequate surveillance, staffed by fully trained and experienced card-carrying virologists deploying their traditional skills.

This review has focused on the laboratory techniques that a community needs to have available to monitor virus activities. They are, however, all but useless without suitably trained staff in adequate numbers to use them. A later review will discuss how they can be provided and maintained within hard-pressed health care budgets.

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