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Gastroenteritis viruses: research update and perspectives

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Viruses are increasingly being recognized as the aetiological agents of acute gastroenteritis in addition to bacteriological and parasitological causes. Since the field was last reviewed comprehensively at an International Symposium in Sapporo, Japan, in 1995, progress has been rapid. This Symposium, bringing together 27 scientists from all over the world under the chairmanship of M.K. Estes (Baylor College of Medicine, Houston, TX, USA), was convened to review recent advances, identify gaps in knowledge and consider future work. The scene was set by an historical and up-to-date overview on gastroenteritis viruses delivered by R. Glass (Center for Disease Control, Atlanta, GA, USA).

Virus structure

Further developments of electron cryomicroscopy and X-ray crystallography techniques have now allowed detailed structural studies on many viruses causing gastroenteritis (rotaviruses, caliciviruses, astroviruses, adenoviruses). The work from several laboratories was comprehensively reviewed by V. Prasad (Baylor College of Medicine, Houston, TX, USA). A new aspect of research has opened since Prasad's group began to elucidate not only the contribution of different viral proteins to different parts of particle structure, but also to deduce details of replication functions from structural studies, for example, by investigating transcription-active subviral particles of rotavirus. These studies are beginning to provide a dynamic view of the virus replication process.

Rotavirus entry and replication

Rotaviruses initiate infection by interaction with a cellular receptor, and productive infections require proteolytic cleavage of the outer capsid spike protein. Understanding the molecules involved in the rotavirus entry process has been controversial and difficult. C. Arias (Instituto de Biotecnología, Cuernavaca, Mexico) reviewed

this area and provided data suggesting that breakthroughs are emerging. Key findings are that rotaviruses may bind to more than one cellular receptor and that the initial binding events are influenced by whether or not a particular rotavirus strain binds to sialic acid. It has recently been recognized that the human and most animal rotaviruses initiate infection in a sialic acid-independent fashion. Integrins and other newly identified cell surface molecules have recently been implicated in this important first step of infection, and there is hope that the individual roles of the multiple proteins can now be dissected using molecular biology and gene knockout or expression techniques.

Rotavirus RNA transcription and replication depend crucially on the 3' and 5' terminal sequences of the viral RNA and on the interaction of the RNA with several virus-coded, non-structural proteins in the infected cell. The sequences at the 3' terminus of mRNAs are also important for the initiation of translation. Progress in this area (by the groups of J. Cohen, M.K. Estes, J. Patton and F. Ramig) was reviewed by J. Patton [National Institutes of Health (NIH), Bethesda, MD, USA]. However, despite major efforts in several laboratories, no reverse genetics system has so far been devised for rotaviruses or for any other of the human gastroenteritis RNA viruses. It is not clear why this is so difficult to achieve, but for rotaviruses, very tight control of packaging RNA segments into nascent particles seems to be one of the main obstacles to understand and to overcome.

Pathology

Many genes of rotaviruses (*RNA3*, 4, 5, 7, 8 and 10) have been implicated in rotavirus pathogenicity. However, attention has recently been directed towards NSP4, the gene product of *RNA10*, which acts both as an intracellular receptor for double-layered particles (to process them further in the endoplasmic reticulum)

and also as a viral enterotoxin. M. Estes (Baylor College of Medicine, Houston, TX, USA), in whose laboratory this was discovered, gave a lively overview of the present state of knowledge. Based on previous data showing that both NSP4 and certain peptides derived thereof are able to produce diarrhoea in newborn mice, it was postulated that NSP4 is secreted (now confirmed as a truncated form), interacts with specific receptors (not yet identified) and then disturbs intracellular Ca^{2+} and Cl^{-} homeostasis, leading to an efflux of ions and water, through an intercellular signal-transduction cascade (some elements of which have now been identified). In an animal model, NSP4 has also been shown to have immunogenic potential (M.K. Estes, unpublished). Rotavirus infection may also be pathogenic by affecting the enteric autonomous nervous system.

Correlates of protection

It is becoming increasingly obvious that locally secreted copro-antibodies of the IgA subclass are the best correlate of protection against rotavirus infection. This work was reviewed by P. Offit (The Children's Hospital, Philadelphia, PA, USA). However, it remains to be determined which antibodies against which of the viral proteins are most important in protection. A cellular immune response against rotaviruses can be measured but seems to be less relevant for longer-term protection. The correlation between protection and the presence of antibody against caliciviruses is less clear.

Epidemiology

Rotaviruses are accepted as the main viral cause of infantile morbidity worldwide and also of mortality of children under five years of age in developing countries. Thus, the surveillance of rotavirus infections is long standing and has intensified over recent years. U. Desselberger (Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge,

UK) reviewed the data from many laboratories. So far, at least 14 different G and 20 different P types of group A rotaviruses have been distinguished, of which at least ten different G and 11 different P types are found in human isolates. The unifying observation in many countries is that rotaviruses of different types co-circulate at any one time. The G/P composition of virus isolates differs largely in various areas of the world. There is now good evidence that co-circulating viruses interact genetically by the formation of *in vivo* reassortants (M. Iturriza-Gómara *et al.*, unpublished). In addition, evidence is accumulating that some of the rotaviruses infecting man may originate from animals (domestic animals, pets) and, thus, co-surveillance of animal rotaviruses seems to be increasingly important (similar to the developments in influenza surveillance).

Rotavirus vaccine

A. Kapikian (NIH), whose group has been a major force in developing rotavirus vaccines, reviewed recent developments. A tetravalent (TV), rhesus rotavirus (RRV)-based, human-reassortant vaccine developed at the NIH has been found to be efficacious in phase III trials and was licensed by the Food and Drug Administration (FDA) in August 1998. Between September 1998 and July 1999, the vaccine was used in more than 1.5 million children in the USA. Under this implementation, cases of gut intussusception emerged that were found to be epidemiologically linked mainly with the first dose of the vaccine. At present, it is unclear what the detailed pathogenesis and the exact vaccine-attributable risks really are. However, the observation led to the decision of the Advisory Committee on Immunization Practices (ACIP) of the USA, in October 1999, to withdraw their recommendation for the RRV-TV vaccine to be used as a universal vaccine in infancy. The WHO, who had put great hope into the widespread use of this vaccine in developing countries, is at present reassessing the situation. Several other candidate rotavirus vaccines are on the horizon.

Human caliciviruses

Recent data on human caliciviruses were reviewed by I. Clarke (University of Southampton, UK) and M. Koopmans [National Institute of Public Health and the Environment (NIPHE), Bilthoven, The Netherlands]. These human viruses, which were previously known as small, round, structured viruses and classical human caliciviruses, are now divided into two genera called the 'Norwalk-like viruses' and 'Sapporo-like viruses'. These viruses are very frequent causes of sporadic infections as well as of outbreaks of gastroenteritis in adults worldwide. With the cloning of the Norwalk

virus and other calicivirus genomes and the development of new diagnostic assays, it has become apparent that there are many more calicivirus infections in humans than previously recognized and that many infections seem to be asymptomatic. Perhaps the biggest surprise has come from studies in Finland (T. Vesikan, *et al.*, unpublished) where the Norwalk-like viruses cause clinically significant disease in young children; these viruses were previously thought to only infect school-aged children and adults. It is now recognized that human caliciviruses of different genotypes co-circulate in the community and that recombination *in vivo* is possible, and this has been recorded. The significance of animals that are infected by various caliciviruses as a reservoir for human infections is still controversial. The availability of an infectious cDNA clone of the feline caliciviruses (K. Green, NIH, Bethesda, MD, USA) has allowed us to begin dissection of the replication strategy of these otherwise still under-researched viruses.

Human astroviruses

Human astroviruses replicate well in the gut and cause both sporadic infections and major outbreaks of gastroenteritis. They have now been studied structurally in some detail using cryo-EM and image reconstruction techniques, as reviewed by S. Matsui (Stanford University School of Medicine, USA). Since her laboratory has succeeded in producing an infectious cDNA clone of a human isolate, many questions of replication can now be investigated in a meaningful way. Epidemiologically, there is co-circulation of many genotypes (at least eight) as reviewed by S. Monroe (CDC).

Corona- and Toroviruses

Corona- and toroviruses are known as well-established causes of gastroenteritis in various animals, but their significance as a cause of gastroenteritis in humans is still not clear. Detailed structural studies on the interaction of the S protein of animal coronaviruses with cellular surfaces have been carried out and have allowed the identification of several cellular receptors, work reviewed by K. Holmes (University of Colorado, Denver, CO, USA).

Gastroenteritis viruses in the immunocompromised

In immunocompromised patients, all the above viruses tend to cause chronic diarrhoeas instead of acute, self-limiting infection and disease. In addition, human cytomegalovirus (HCMV) frequently causes chronic colitis in such patients, as reviewed by R. Pollok (St Bartholomew's Hospital and Royal London School of Medicine, London, UK). It was

interesting to learn that, with the advent of highly active antiretroviral therapy (HAART) against HIV infection, the clinical need to treat HCMV colitis with ganciclovir has decreased dramatically, presumably because of the improvement of immune defences under HAART treatment.

Therapy of viral gastroenteritis

M. Farthing (St Bartholomew's Hospital and Royal London School of Medicine) reviewed recent developments. Treatment of gastroenteritis consists mainly of oral rehydration therapy (ORT – an isotonic salt solution containing glucose), which had been introduced for the treatment of childhood diarrhoeas by WHO in developing countries more than 15 years ago. Based on new pathophysiological data, the use of hypotonic ORTs (which, by many accounts, are superior to standard ORT) has been proposed as an alternative. The application of newly developed anti-encephalase drugs (such as Racecadotril) seems to be clinically beneficial, while treatment with antimotility drugs is not generally advised.

Concluding remarks

There was agreement that considerable progress in knowledge has been made with regard to the molecular biology, pathogenesis and epidemiology of virus infections causing gastroenteritis in humans. However, there are still many questions remaining, particularly because of the lack of reverse genetic systems for most of the human gastroenteritis viruses. The setback with the RRV-TV rotavirus vaccine is hopefully temporary as new vaccine developments are underway.

The organization of the meeting was such that relatively short talks introducing particular topics were followed by extensive periods of discussions, and the consideration of negative and controversial data was encouraged. The reviews and discussions will be published in the Novartis Foundation Symposium Series as Volume 238.

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