et al.[15] have genetically and biochemically characterized a dual-specificity phosphatase (IphP) in the cyanobacterium *Nostoc commune*. As *N. commune* is free living, IphP probably evolved directly from prokaryotic ancestry. In addition, a potential target for IphP has been found in *N. commune*, a rare example of protein tyrosine phosphorylation in a prokaryote.

These results raise the possibility that tyrosine phosphorylation and its associated enzyme functions arose in evolution before the divergence of prokaryotes and eukaryotes. As eukaryotic organisms evolved and began to use protein tyrosine phosphorylation as a major mechanism to activate cellular responses in the immune system, microorganisms such as *Yersinia* seem to have acquired new genetic traits to subvert this process.

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State of the art: coronaviruses

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The 6th International Symposium on Corona- and Related Viruses discussed progress in the understanding of the molecular biology, immunology and pathogenesis of corona-, toro- and arterivirus infections. These large, enveloped animal viruses are responsible for a variety of common acute and chronic diseases in birds and mammals, including humans[1], and mainly cause infections of the respiratory, gastrointestinal and nervous systems[2]. In humans, coronaviruses cause 10–35% of common colds, have been implicated in some diarrheal diseases, and may be involved in multiple sclerosis, an inflammatory, autoimmune neurological disorder of multifactorial etiology[3]. In the veterinary field, corona- and related viruses cause economically very important losses in cattle, pigs and chickens[2].

Coronaviruses have the longest known RNA genome (27–31 kb), which is of positive polarity. Replication of the viral genome occurs by the production of a characteristic 3′-coterminal nested set of several subgenomic RNAs[4]. This replication strategy is also characteristic of the toroviruses and arteriviruses, although the latter have a smaller RNA genome[1].

Pathogenesis, immune responses and vaccines

Throughout the meeting, state-of-the-art speakers reviewed themes in the current research on corona- and related viruses. The opening lecture was an inspiring outside view of studies aiming to define host genes involved in susceptibility and resistance to various infections. The applications of this technology to coronaviruses have so far been undeservedly limited, although a good example has been the identification of a murine-hepatitis susceptibility gene of the fibrinogen family (Emil Skamene, Montreal General Hospital, Quebec, Canada).

The pathogenesis of coronavirus infections has been studied mainly with the murine coronavirus mouse hepatitis virus (MHV), a common mouse pathogen. Neurotropic strains of MHV cause demyelinating diseases of rodents that provide an animal model of human central nervous system (CNS) disorders, such as multiple sclerosis. The JHM strain of MHV has been used to identify determinants of tropism on both the virus and the target cells (Samuel Dales, University of Western Ontario, London, Ontario, Canada).

Several cellular receptors used by coronaviruses to enter target cells are now known. These include members of the carcinoembryonic antigen family for MHV, the aminopeptidase N for the 229E strain of human coronavirus and porcine transmissible gastroenteritis virus, and 9-O-acetylated neuraminic acid for bovine coronavirus. Binding domains on viral proteins are now starting to be identified, and it is

becoming clear that the presence of cellular receptors is not always sufficient to allow infection (Kathryn Holmes, Uniformed Services University of the Health Sciences, Bethesda, MD, USA).

The work on coronavirus attachment and penetration indicates that we have only just touched the surface of the molecular mechanisms involved in cell entry. The field is ripe for the discovery of the cofactors involved in virus penetration (virus binding to a receptor is not sufficient for entry into the cell). Also, the exact function of the hemagglutinin–esterase (HE) protein, which is expressed by some coronaviruses, but not by others, remains a subject of lively debate.

The structure and function of the numerous structural and nonstructural proteins of coronaviruses were ably summarized by Hubert Laude (Institut National de Recherches Agronomiques, Jouy-en-Josas, France). Although several studies on structural proteins (N, S, M and HE) have yielded much information, a great deal remains to be learned about the properties of the so-called nonstructural proteins that have been identified from the impressive nucleotide sequencing efforts accomplished for several coronavirus strains. One of these proteins, sM, was described recently to be present in small amounts on virions. Its function is unclear, but it is speculated that it acts as a channel protein (similar to the influenza M2 protein) and may be involved in virus assembly.

MHV is a model system for studying the immune components involved in protection from coronavirus infection. Although antibody protects from acute infection, viral clearance from the CNS requires $CD8^+$ and possibly $CD4^+$ T cells, and may be mediated by several locally released cytokines (Michael Buchmeier, Scripps Research Institute, La Jolla, CA, USA). Although release of cytokines in the CNS may contribute to MHV-induced immunopathological disease, several studies have now established the critical importance of T cells in protection against virus infection of the brain. Nevertheless, the protective role of neutralizing antibody against acute disease remains a significant factor and the role of the idiotypic network has still not been examined fully. Studies on the function of mucosal immunity, although essential to understand the first line of defense against corona- and related viruses, have been largely neglected, but work is currently under way on porcine coronaviruses. There is also some evidence that coronaviruses induce alteration of B and T cell functions, which may, in some cases, lead to immunopathology or immunosuppression. Coronaviruses, including human strains, are now known to infect cells of the immune system. Furthermore, coronavirus neurotropism may also involve infection of brain endothelial cells, a finding that opens up an exciting avenue of investigation.

Because this group of viruses is of major economical importance, the development of vaccines is a priority. Successful immunization has been achieved for feline coronaviruses, and there has also been progress in using multiple antigen peptide constructs and adenovirus recombinants for immunization. Various other biotechnological approaches are possible. One provocative possibility is to genetically alter the host animal to make it resistant to infection through transgenic expression of antiviral antibodies (Luis Enjuanes, Centro Nacional de Biotecnologia, Madrid, Spain).

Transcription and replication

The complete genomic sequences of four coronavirus species are now known; avian infectious bronchitis virus, MHV and human coronavirus 229E sequences have already been reported, but the sequencing of porcine transmissible gastroenteritis virus was reported at this meeting (Jean-François Eleouet *et al.*, Institut National de Recherches Agronomiques, Jouy-en-Josas, France).

There is enormous research activity on the characterization of the complex of proteins encoded by the 5′ end of the coronavirus genome, which is probably involved in forming the RNA-dependent RNA polymerase complex. There has been progress recently on the characterization of the mechanisms of polyprotein processing, including the identification of the cleavage sites and the proteinase activities involved. The mechanism of RNA transcription and replication and its regulation, in particular, the mechanism of discontinuous transcription of subgenomic mRNAs, are still not completely understood at the molecular level. A leader-primed transcription mechanism was proposed more than a decade ago and remains highly plausible, although the presence of subgenome-size negative-strand RNAs has forced a debate as to the precise mechanisms of transcription and replication, and their regulation. Putative cellular factors that may be involved in such RNA synthesis need to be studied (Michael Lai, University of Southern California, Los Angeles, CA, USA). Various innovative models for replication were proposed, including the possible involvement of 5′ and 3′ noncoding sequences. This would not be unexpected considering the data on other positive-strand RNA viruses.

Studies of coronavirus protein synthesis, protein–protein interactions and intracellular transport have progressed rapidly. Examples include elegant studies on the expression of proteins, either alone or in combination, that lead to the formation of virus-like particles containing only the M and sM proteins (Harry Vennema and Peter Rottier *et al.*, Utrecht University, The Netherlands). There has been rapid progress in understanding the molecular genetics of the economically important Lelystad virus (porcine reproductive and respiratory syndrome virus; the prototype arterivirus of swine).

The common and distinctive features of corona-, toro- and arteriviruses emphasize the wisdom of organizing a meeting where discussions on all these viruses could take place (Willy Spaan, University of Leiden, The Netherlands). Increasingly, corona- and related viruses are attracting attention, both as models for studies of pathogenesis and RNA replication, and as important pathogens in the veterinary and medical fields.

Acknowledgements
Discussions held at the meeting will be published as a proceedings volume[5], which should be consulted for detailed information. The next international symposium will be held in Spain in 1997, and will be organized by Dr Luis Enjuanes.

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Horizons: a selection from recent publications

From wire wool to cables

Burkholderia (formerly *Pseudomonas*) *cepacia* is a ubiquitous organism that frequently colonizes the lungs of patients with cystic fibrosis, and is associated with a poor prognosis of disease. Colonization of the lungs may be aided by novel giant fibres termed cable (Cbl) pili. Sajjan *et al.* previously identified a 22 kDa mucin-binding pilus-associated adhesin, and have now purified the 17 kDa major pilin subunit, and isolated and sequenced its chromosomal gene, *cblA*.

Sajjan, U.S. *et al.* (1995) Cable (Cbl) type II pili of cystic fibrosis-associated *Burkholderia* (*Pseudomonas*) *cepacia*: nucleotide sequence of the *cblA* major subunit pilin gene and novel morphology of the assembled appendage fibers *J. Bacteriol.* 177, 1030–1038

This group has also shown that different strains of *B. cepacia* express one or more of at least five morphologically distinct classes of pili: cable (Cbl), filamentous (Fil), spine (Spn), spike (Spk) and mesh (Msh). The types of pilus expressed seem to correlate with the source of the *B. cepacia* isolate. The Msh fibres are very fine and curly, and form a steel-wool-like mesh that is constitutively coexpressed with Cbl, Fil and Spk pili, suggesting that cooperative pilus interactions are involved in the colonization of different environments. Adhesion by *B. cepacia* is clearly a complex business!

Goldstein, R. *et al.* (1995) Structurally variant classes of pilus appendage fibers coexpressed from *Burkholderia* (*Pseudomonas*) *cepacia* *J. Bacteriol.* 177, 1039–1052

Talking rough

Neisseria gonorrhoeae lipopolysaccharide (LPS) undergoes phenotypic variation, which alters both the invasiveness and the resistance to host defences of the bacteria. Schwan and colleagues have shown that the *N. gonorrhoeae* gene *lsi-1*, known to be involved in synthesis of inner-core LPS, is the homologue of the *Salmonella typhimurium* gene *rfaF*, encoding heptosyltransferase II, which catalyses addition of the second heptose to the inner core in LPS biosynthesis. The *lsi-1* gene can complement *rfaF* mutants and has a high degree of sequence similarity with *rfaF*. Interestingly, *N. gonorrhoeae lsi-1* mutants have a reduced ability to bind to the human epithelial Chang cell line, and cannot invade these cells. One explanation of this effect is that LPS mediates tight binding to or entry into cells; potentially, LPS mutants could be used to probe the LPS structures involved in such interactions. An alternative explanation for the phenotype of LPS mutants may be the indirect effect of altering the architecture of the outer membrane, possibly by changing the conformation of the opacity protein, which is known to be necessary for invasion.

Schwan, E.T. *et al.* (1995) Gonococcal *rfaF* mutants express Rd_2 chemotype LPS and do not enter epithelial host cells *Mol. Microbiol.* 15, 267–275

LCMV model for immune suppression

Virus-induced generalized immune suppression in viral diseases including AIDS and measles is clearly of immense clinical importance, but the mechanism is still unclear. Borrow and colleagues have studied this phenomenon using as a model system the generalized immune suppression induced by persistent infection of adult mice with certain strains of lymphocytic choriomeningitis virus (LCMV). The mechanism of immune suppression in this case is particularly puzzling as LCMV only poorly infects lymphocytes. Borrow *et al.* have shown that infection with an immunosuppressive LCMV strain results in the loss of periarterial interdigitating dendritic cells from the spleen, while a parental nonimmunosuppressive strain differing in only two amino acids has little effect on these cells. The immunosuppressive, but not the parental, strain has a tropism for periarterial interdigitating dendritic cells and, while the virus itself is noncytolytic, host cytotoxic T cells destroy these critical accessory cells. Generalized immune suppression results because dendritic cells are essential for presenting antigen to naive T cells to initiate a primary response. Is this mechanism important in other immunosuppressive viral diseases, such as AIDS? Evidence is accumulating that HIV does indeed infect and impair the function of dendritic cells from an early stage in infection, and this may be a crucial factor in the development of AIDS.

Borrow, P., Evans, C.F. and Oldstone, M.B.A. (1995) Virus-induced immunosuppression: immune system-mediated destruction of virus-infected dendritic cells results in generalized immune suppression *J. Virol.* 69, 1059–1070