Evolutionary relationships among orbiviruses

B. M. GORMAN*

Summary: The genus Orbivirus consists of 12 defined serological groups of arthropod-borne viruses. The taxonomic group was formed following the demonstration that the genome of bluetongue virus consisted of 10 segments of double-stranded RNA. Viruses with morphology and physico-chemical properties similar to those of bluetongue virus were included in the genus. As viruses representative of each serogroup are studied, it is apparent that the orbiviruses are a heterogeneous group and further reclassification of them will be necessary. The structural components and the distinct ecological features of some of the viruses suggest the basis for taxonomic revision.

Cross-reactivity in serological tests is often used as a measure of the evolutionary distance between proteins. There is no common generic antigen for orbiviruses but viruses in certain defined serological groups share antigens detectable in some serological tests. Viruses of the bluetongue, Eubenangee, epizootic haemorrhagic disease and Palyam groups probably form a sub-complex of the orbiviruses. The cross-reactive antigens may be indicative of a common evolutionary pathway for these viruses.

Genetic reassortment occurs between viruses of a defined serogroup but has yet to be demonstrated between viruses in different serogroups. It may be possible to infer evolutionary relationships among orbiviruses by defining genetically interacting groups of viruses and determining the importance of gene reassortment in generating diversity in natural populations of orbiviruses.

KEYWORDS: Antigen structure - Bluetongue virus - Genetic recombination - Genetics - Immune response - Nomenclature - Orbivirus - RNA viruses - Viral interference.

INTRODUCTION

In 1959 Sabin (26) proposed that a number of viruses classified within the ECHO virus group be reclassified as respiratory enteric orphan (reo) viruses. Although the definition was based on morphological and biological properties, the decision to establish the new group was vindicated when it was shown that their genomes consisted of double-stranded RNA (8). Until that time it had been assumed that double-stranded (ds) RNA occurred only as an intermediate in the replication of other viruses. The discovery of this "unique" stable form of genetic material and the demonstration that their genomes were packaged as discrete pieces led to considerable research into the structure and genetics of reoviruses. Despite their relative insignificance in medicine and that they form only a small part of the family of Reoviridae (reviewed in 17), the reoviruses have become one of the best studied groups of viruses.

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The family *Reoviridae* is divided into six genera of viruses containing 10 to 12 segments of dsRNA (Table I). The study of one of these viruses may provide clues to understanding the evolutionary relationships among them. The genome of wound tumour virus consists of 12 segments of dsRNA. The virus has been isolated only once in nature (from leafhoppers in 1941). It is maintained in the laboratory by serial passage from leafhopper to clover. Maintenance of the virus in the plant by vegetative propagation of infected plants results in defective virus populations; they are unable to replicate in insects or in insect cell culture. The detailed studies on wound tumour virus have been reviewed recently (22). Analysis of the genome segments of defective viruses revealed extensive deletions of certain segments (24). Nuss and Summers (23) showed that the variant dsRNA segments represented terminally conserved remnants of genome segments. This mechanism of deletion has been described for other viruses but one ex-vectorial isolate of wound tumour virus is interesting in that it has lost completely the gene coding for a major surface protein yet still retains its capacity to replicate in plants and in plant tissue culture.

Despite the Hennigian injunction that "there is no absolute coincidence between similarity of attributes and genealogy of objects" (6), the generation of a stable virus isolate with 11 segments replicating in plants without a major structural protein necessary for replication in insects suggests a possible pathway for the evolution of dsRNA viruses.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Genus</th>
<th>Segments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound tumour</td>
<td>Phyto</td>
<td>12</td>
</tr>
<tr>
<td>Colorado tick fever</td>
<td>&quot;Orbi&quot;</td>
<td>12</td>
</tr>
<tr>
<td>Infantile gastroenteritis</td>
<td>Rota</td>
<td>11</td>
</tr>
<tr>
<td>Bluetongue</td>
<td>Orbi</td>
<td>10</td>
</tr>
<tr>
<td>Reovirus</td>
<td>Orthoreo</td>
<td>10</td>
</tr>
<tr>
<td>Cytoplastic polyhedrosis</td>
<td>Cypo</td>
<td>10</td>
</tr>
<tr>
<td>Maize rough dwarf</td>
<td>Fiji</td>
<td>10</td>
</tr>
</tbody>
</table>

**ORBIVIRUSES**

Following the demonstration that the genome of bluetongue virus consisted of 10 segments of dsRNA (33), the proposition followed that all arthropod-borne viruses which were morphologically similar to bluetongue virus be classified as a separate genus of the *Reoviridae* (31, 2). The grouping was based on some of their physico-chemical properties as well as morphology. Borden *et al.* (2) suggested the name orbivirus for the group from the ring-like arrangement of capsomeres on the nucleocapsids of the viruses.

Currently most workers who study orbiviruses recognise 12 distinct serological groups (Table II). As more information on the structure of representative orbiviruses is obtained it is apparent that they do not form a homogeneous group. An assessment of the evolutionary relationships among the orbiviruses is based on speculative extrapolation of the experimental data available. The taxonomic position of Colorado tick fever virus must be reassessed since Knudson (18) found that the genome consisted of 12 segments of dsRNA. The distinct ecology of the tick-borne Kemerovo viruses and differences in structure of viruses of the Kemerovo and Corriparta
TABLE II

Orbivirus serological groups

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Number of serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>African horse sickness</td>
<td>9</td>
</tr>
<tr>
<td>Bluetongue</td>
<td>23</td>
</tr>
<tr>
<td>Epizootic haemorrhagic disease</td>
<td>7</td>
</tr>
<tr>
<td>Eubenangee</td>
<td>3</td>
</tr>
<tr>
<td>Palyam</td>
<td>10</td>
</tr>
<tr>
<td>Equine encephalosis</td>
<td>5</td>
</tr>
<tr>
<td>Changuinola</td>
<td>12</td>
</tr>
<tr>
<td>Corriparta</td>
<td>4</td>
</tr>
<tr>
<td>Colorado tick fever (a)</td>
<td>2</td>
</tr>
<tr>
<td>Kemerovo</td>
<td>22</td>
</tr>
<tr>
<td>Warrego</td>
<td>2</td>
</tr>
<tr>
<td>Wallal</td>
<td>2</td>
</tr>
<tr>
<td>Umatilla (b)</td>
<td>2</td>
</tr>
</tbody>
</table>

(a) The genomes of Colorado tick fever viruses consist of 12 segments of dsRNA (18) and should be excluded from the genus Orbivirus.

(b) Knudson, personal communication.

serogroups from other orbiviruses suggests that evolutionary patterns may not be consistent among orbiviruses. Cross-reaction in certain serological tests between viruses of the bluetongue, Eubenangee and epizootic haemorrhagic disease (EHD) serogroups has led to the suggestion that the viruses be regarded as a taxonomic group. The data and arguments to support the proposition are reviewed in 11. Della-Porta (4) extended the proposition to include the Palyam serogroup of viruses and advocated recognition of sub-genera of orbiviruses.

Detailed descriptions of the structure and morphogenesis of orbiviruses can be found in recent review articles (11, 32) and will not be discussed here. There are, however, two aspects which must be considered in assessing evolutionary relationships among them. Purified orbiviruses contain seven structural proteins. After attachment of virus particles to susceptible cells, two surface proteins are removed and a transcriptase associated with the nucleocapsid copies the 10 genes into single-stranded RNA. These associate with ribosomes and are copied initially into 10 proteins. There may be secondary cleavage of some of these proteins to produce more than 10 virus-specified proteins in infected cells. In assembly of progeny virions one or more of these proteins attaches to each of the 10 single-stranded RNA molecules, converts them to dsRNA (a polymerase) and packages the 10 genes into a virus particle consisting of seven proteins. Two major virus-induced proteins which are not incorporated into virions probably have an important role in virus assembly.

In cells simultaneously infected with two related orbiviruses the genomes can be reassorted so that progeny viruses may derive genes from each of the parental viruses. The reassorted viruses may have a range of biological properties distinct from either of the parent viruses. It is important to define genetically interacting viruses and those that are excluded from such interactions. In this way potential gene pools of orbiviruses could be established and their significance in the evolution of the viruses could be assessed. Understanding the genetics of natural populations of organisms underpins our thinking about evolution but our knowledge of the genetic interactions between microorganisms observed in the laboratory has rarely been applied to the study of natural populations.
THE GENETIC BASIS OF SEROLOGICAL RELATIONSHIPS AMONG ORBIVIRUSES

In general the degree of antigenic sharing between proteins is assumed to be a measure of the evolutionary distance between them. The basis of the serological groupings of orbiviruses is that the proteins in the nucleocapsids of certain viruses are conserved. For example, viruses of the bluetongue serogroup give reactions of virtual identity in complement-fixation tests. One assumes that there are common ancestral patterns within the serogroup. Serotypes within each group are based on the reactions of specific neutralizing antibodies to the surface proteins. There are 23 distinct serotypes of bluetongue.

Despite the fact that viruses of the bluetongue, Eubenangee, EHD and Palyam serogroups are regarded as distinct, the serological cross-reactions between viruses in those different serogroups lead to the conclusion that they may have common evolutionary origins and tendencies. The genetic basis of the serological cross-reactions has been studied using RNA-RNA reassociation techniques. The single-stranded RNA molecules synthesized in cells infected with orbiviruses are exact copies of the parental strand of each of the 10 genes. Isolated single-stranded RNA hybridizes with denatured dsRNA and the electrophoretic mobilities of the hybrids are identical to the corresponding native genome segments. The relationships between viruses can be determined by hybridizing the single-stranded RNA of one virus to the "complementary" strands of the genome of another. Mismatching of the nucleotide sequences leads to failure to form a hybrid duplex where the homology is low, or to incomplete duplex formation where homology is higher but the sequences have diverged significantly. The mismatched duplexes can be detected by comparing the electrophoretic mobilities of the hybrid molecules with the native genome segments. In cross-hybridization of RNA of a bluetongue virus and an EHD virus, Huismans et al. (16) found between 5% and 10% homology but were unable to detect stable duplexes by Polyacrylamide gel electrophoresis (PAGE). In immune precipitations of virus-induced proteins using antisera to each virus they located shared antigens on the major nucleocapsid proteins (VP3 and VP7) and on a non-structural protein (P6a).

Bluetongue virus type 20 and Tilligerry virus (a serotype of the Eubenangee group) cross-react to some extent in complement-fixation tests but their genomes cross-hybridize weakly and no duplexes could be detected after PAGE of the reaction mixtures (11). Roy et al. (25) used a cloned DNA copy of the gene coding for VP3 of a bluetongue virus to detect homologous sequences in the corresponding gene of all bluetongue virus serotypes tested. The probe failed to detect homologous sequences in the genome of an EHD virus. Clearly the homology between nucleotide sequences of the genomes of bluetongue, Eubenangee and EHD viruses must be low and an assessment of the phylogeny of the viruses depends on obtaining comparative nucleotide sequences for viruses in each serogroup.

GENETICALLY INTERACTING ORBIVIRUSES

Three bluetongue virus serotypes isolated in Australia are closely related by RNA-RNA reassociation assays. Eight duplex molecules can be detected after hybridizing single-stranded RNA isolated from cells infected with one virus with complementary sequences in the genomes of the other two viruses (11). Reassortant viruses can be isolated
from cells infected with the three viruses (13). Bluetongue viruses isolated in Australia
have diverged significantly in RNA sequence from viruses isolated in South Africa
and it is difficult to detect complementary sequences in the genomes by RNA-RNA
reassociation assays (12, 14). Despite the apparent sequence divergence a virus isolated
in Australia is serologically indistinguishable from bluetongue type 1 isolated in South
Africa (5). Reassortant viruses have been isolated from cells infected simultaneously
with a bluetongue type 1 virus isolated in South Africa and type 20 virus isolated
in Australia (13). The RNA-RNA reassociation assays are unreliable indicators of
the serological relationships between viruses and of the capacity of these viruses to
exchange genetic information.

No reassortant viruses could be isolated from cells infected simultaneously with
a bluetongue virus and an EHD virus or from cells simultaneously infected with a
bluetongue virus and a Eubenangee virus (10). However, insufficient experimental
data has been obtained to preclude the possibility of genetic interaction among viruses
in different serogroups. A simple extrapolation that genetic interactions occurred only
among viruses of a defined serogroup led to the proposition that the serogroup
represented a species of orbiviruses (10).

NATURAL POPULATIONS OF ORBIVIRUSES

From cross-hybridization of the RNA genomes of bluetongue viruses it is apparent
that geographic separation of viruses has led to significant sequence divergence be­
tween them. Huismans and Bremer (14) detected two duplex molecules after cross-
hybridizing RNA of a bluetongue virus isolated in South Africa and one from
Australia. The electrophoretic mobilities of the duplex molecules indicated substan­
tial sequence divergence in those two genome segments. By contrast, cross-
hybridization of the RNA genomes of bluetongue virus serotypes isolated in South
Africa yielded from six to eight duplex molecules (15). In similarly performed ex­
periments eight duplex molecules were formed on cross-hybridizing the genomes of
three serotypes isolated in Australia (11). Assuming common ancestry for bluetongue
viruses the observations are consistent with evolution in isolation of two distinct
populations of viruses and suggest the possibility that discrete gene pools of orbiviruses
evolve independently.

The concept of “gene pools” of orbiviruses is so attractively simple that its adop­
tion may lead to an erroneous view of the evolution of orbiviruses. The term is relative­
ly new (see 1) but implies a population of organisms able to exchange genetic
information. The gene pool becomes a resource for evolutionary change. Despite the
observation that closely related viruses will reassort genes in simultaneous infection
of cells in culture, there is little firm evidence that the phenomenon is important in
nature. Sugiyama et al. (29) compared oligonucleotide maps of genome segments of
bluetongue viruses of types 10 and 11 isolated in the USA and concluded that one
virus isolate was a natural reassortant between prototypes 10 and 11. The
oligonucleotide maps suggested that nine segments of the virus were derived from
the type 10 virus and one (segment 3) from the type 11 virus. Subsequent studies using
a cDNA copy of segment 3 of a bluetongue virus as a hybridization probe revealed
that the gene is highly conserved in bluetongue viruses (25). Comparison of complete
nucleotide sequences of segments 3 of a type 10 virus and a type 17 virus showed
95.5% homology (7). The interpretation of oligonucleotide maps to suggest reassor-
tants in segment 3 should be viewed with caution. Collisson and Roy (3) compared the oligonucleotide maps of vaccine strains of type 10 used in the USA and concluded that one strain was a natural reassortant and that the oligonucleotide map of segment 10 was more like the corresponding segment of a prototype 11 virus. The authors concluded that “it appears very likely that reassortment between different BTV serotypes probably occurs continuously in nature although whether it takes place in the vectors or in the animal host (or both) is not known”. In a previous study Sugiyama et al. (30) analysed composite fingerprints of segments 1-3, 4-6 and 7-10 of various isolates of bluetongue virus type 11. Each strain was related to, but distinguishable from, a prototype strain. In general the results indicated significant sequence divergence between isolates of one serotype over 12 years, but one strain isolated in Colorado in 1970 differed from the prototype strain in only 2 of 101 large oligonucleotides. The changes were in segment 10. The interpretation of oligonucleotide maps of genome segments as providing evidence for naturally occurring reassortant bluetongue viruses is not convincing.

It is easy to assume from laboratory-based experiments that the genetic interchange observed between organisms actually occurs in nature. In fact there has been little study of natural populations of microorganisms. In one of the first studies of the population genetics of Escherichia coli the conclusion was made that natural populations were essentially panmictic (20) but subsequent studies have shown that, although genetic recombination (parasexuality) occurs in E. coli, the populations in nature are essentially clonal (27) and that genetic recombination is a very rare event in natural populations. According to Levin (19) “the most compelling evidence that E. coli is composed of a modest number of widespread and nearly monomorphic lineages is the recovery of essentially identical ... clones... in the small sample examined”. Similar studies of the genetic structure of populations of Legionella pneumophila and of Haemophilus influenzae have shown that the populations are basically clonal (28, 21). From the limited data available it is premature to infer that gene reassortment is a major determinant in the genetic structure of populations of bluetongue viruses. Caution seems justified when one considers the results of cross-hybridization of the genomes of a bluetongue virus isolated in Cyprus in 1971 and the original strain of type 4 isolated by Theiler in South Africa in 1900. Huismans and Howell (15) detected nine hybrid molecules after hybridizing single-stranded RNA of the Cyprus isolate with the genome of Theiler's bluetongue virus isolate. Slight migrational differences in PAGE of the hybrid and native segments indicated that mutational change had occurred but the genetic composition of the two viruses was remarkably conserved despite their geographic and temporal isolation. It is difficult to believe that such conservation could occur in freely panmictic populations of bluetongue viruses.

It is important that natural populations of orbiviruses be defined. The biological properties and medical significance of a virus isolate may depend on the role of gene reassortment in generating diversity within the group to which the isolate belongs. Despite the demonstration in the laboratory of genetic reassortment among viruses of a serogroup, the phenomenon might not occur or might be a rare event in nature. Isolates of bluetongue may represent independent clones with numerous clones evolving separately. The association of each clone with disease in animals could be established. If, however, the isolate represents only a transient genetic constellation drawn from a “bluetongue virus gene pool” then any biological property becomes a potential trait of all bluetongue virus isolates. Is the isolate a “stable” genetic entity with its own evolutionary tendencies or a transient assembly of genes? In the latter case
the determination of virulence of a particular isolate would be meaningless and the use of live attenuated viruses in polyvalent vaccines would be counter-productive.

It is equally important to establish that the bluetongue, epizootic haemorrhagic disease, Eubenangee and Palyam groups of viruses represent independently evolving groups or distinct species. That they may have evolved from a single ancestral species becomes a question not of academic interest but of significance in disease control. Understanding the evolutionary origins of viruses will suggest ways of controlling them.

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RELATIONS ÉVOLUTIVES ENTRE LES ORBIVIRUS. — B.M. Gorman.

Résumé: Le genre Orbivirus comprend 12 groupes sérologiques définis de virus transmis par les arthropodes. Ce groupe taxonomique a été créé après la mise en évidence du fait que le génome du virus de la fièvre catarrhale ovine était composé de 10 segments d'ARN double-brin. Les virus possédant une morphologie et des propriétés physico-chimiques semblables à celles de ce virus ont été regroupés dans le genre. Au fur et à mesure de l'étude des virus représentatifs de chaque sérogroupe, il paraît évident que les orbivirus forment un groupe hétérogène et qu'une classification nouvelle de ces virus va devenir nécessaire. Les composants structuraux et les caractéristiques écologiques distinctes de certains d'entre eux pourraient servir de base à la révision de la taxonomie.

Dans les épreuves sérologiques, la réactivité croisée est souvent utilisée pour mesurer la distance évolutive entre protéines. Il n'existe aucun antigène générique commun à tous les orbivirus, mais les virus appartenant à des groupes sérologiques bien définis partagent des antigènes décelables dans certaines épreuves sérologiques. Les virus des groupes «fièvre catarrhale ovine», Eubenangee, «maladie hémorragique enzootique» et Palyam forment probablement un sous-ensemble au sein des orbivirus. Les antigènes à réaction croisée pourraient indiquer que ces virus ont suivi une voie d'évolution commune.

Un réarrangement génétique peut se produire entre virus d'un sérogroupe défini mais reste à démontrer entre virus de sérogroupes différents. L'évolution des relations entre les orbivirus pourrait être déduite de la définition des groupes de virus ayant entre eux des interactions génétiques et de la détermination de l'importance du réarrangement génomique, source de la diversité des populations naturelles d'orbivirus.

RELACIONES EVOLUTIVAS ENTRE LOS ORBIVIRUS. — B.M. Gorman.

Resumen: El género Orbivirus incluye 12 grupos serológicos definidos de virus transmitidos por los artrópodos. Se creó este grupo taxonómico cuando se descubrió que el genoma del virus de la lengua azul estaba compuesto de diez segmentos de ARN de doble hebra. Se agruparon en el género los virus que tienen morfología y propiedades fisicoquímicas parecidas a las de este virus. Conforme se va adelantando en el estudio de los virus representativos de cada serogrupo, resulta obvio que los orbivirus forman un grupo heterogéneo, por lo que se requiere una clasificación nueva de estos virus. Los componentes estructurales y las características ecológicas distintas de algunos de los mismos podrían servir de base para revisar la taxonomía.

En las pruebas serológicas, a menudo se usa la reactividad cruzada para medir la distancia evolutiva entre proteínas. No existe ningún antígeno genérico común a todos los orbivirus, pero los virus que pertenecen a grupos serológicos perfectamente definidos comparten antígenos detectables en algunas pruebas serológicas. Los virus de los grupos "lengua azul", Eubenangee, "enfermedad hemorrágica enzoótica" y Palyam probablemente forman un subconjunto dentro de los orbivirus. Los antígenos de reacción cruzada podrían indicar que estos virus siguieron una vía evolutiva común.

Se puede producir una reordenación genética entre virus de un serogrupo definido, aunque está por demostrar entre virus de serogrupos distintos. La evolución de las relaciones entre los orbivirus podría deducirse de la definición de los grupos de virus que tienen entre sí interacciones genéticas y de la determinación de la importancia de la reordenación genómica, fuente de la diversidad de las poblaciones naturales de orbivirus.

PALABRAS CLAVE: Estructura del antígeno - Genética - Interferencia vírica - Nomenclatura - Orbivirus - Recombinación genética - Respuesta inmunitaria - Virus de ARN - Virus de la lengua azul.

REFERENCES


