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Togaviruses

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INTRODUCTION

At the time of writing this review, the family Togaviridae is composed of the genera alphavirus, flavivirus, rubivirus and pestivirus. Members of the family are characterized by spherical virions, 40 - 70 nm in diameter which possess a lipoprotein envelope with cellular lipids and virus-specified glycopeptides surrounding a spheric nucleocapsid of icosahedral symmetry. It contains a single molecule of single-stranded RNA (mol. weight about 4×10^6) with positive polarity which is infectious when extracted and assayed under appropriate conditions. Togaviruses multiply in the cytoplasm and mature by budding (Fenner, 1976). All species of the genus alphavirus and most flaviviruses multiply in arthropods as well as vertebrates and constitute the classical arthropod-borne viruses (formerly termed arbo-A and arbo-B viruses, respectively). Arboviruses are viruses which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by hematophagous arthropods (WHO Study Group, 1967). Essential to this ecologic definition is the term « biological » (as opposed to mechanical) transmission, by which is meant that an « extrinsic incubation period » (5 - 12 days) elapses between the moments when the arthropod vector becomes infected after a blood meal and when it can transmit the virus to a new vertebrate host. Biological transmission is not confined to the above mentioned togavirus genera : members of the Reoviridae and Rhabdoviridae families and all Bunyaviridae are also arthropod-borne.

On the other hand members of the rubivirus (rubellavirus) and pestivirus genus (hog cholera, bovine diarrhea viruses) as well as mouse lactic dehydrogenase virus and equine arteritis virus are nonarbo togaviruses (Horzinek, 1973). Simian hemorrhagic fever virus (Trousdale et al., 1975), the cell fusing agent of Aedes albopictus cells (Stollar and Thomas, 1975) and carrot mottle virus (Murant et al., 1969) most probably also belong to this family. The following review is intended to present average information mainly on the established togaviridae ; thus representative data may not have been obtained for all genera to the same extent, which is especially true for the pestiviruses. The literature references are kept to a minimum ; earlier data can be found in reviews by Horzinek (1973 a, b, 1975) and Pfefferkorn and Shapiro (1974).

VIRION STRUCTURE

Togaviruses are the smallest enveloped animal viruses ; alphaviruses and rubella virus (RUV) are about 60 nm in diameter, flavi- and pestiviruses appear smaller (about 45 nm). The size difference as determined by negative staining and thin section electron microscopy are reflected by the sedimentation coefficients which range between 240 and 300 S for the former and < 170 to 220 S for the latter group. Gradient analysis has shown that the buoyant density of infectious virions depends from the gradient substance used ; values vary between ⩽ 1.18 g/ml for sucrose and 1.24 for CsCl and other salts.

In the electron microscope togavirions appear as spheric particles consisting of an envelope and an isometric core. The envelope shows unit membrane characteristics and carries glycoprotein surface projections which in the case of alphaviruses are organized in a regular (icosahedral) surface lattice ; they are the substrate for a pH-dependend hemagglutinating activity. Exposure of virions to organic solvents or detergent inactivates their infectivity by disintegration of the envelope, leading to hemagglutinating membrane fragments (exception : pestiviruses) and to liberation of the nucleocapsid (30 - 40 nm across for alphaviruses and RUV, 20 - 30 nm for flavi- and pestiviruses). It shows a shape and substructure suggestive of icosahedral symmetry (Horzinek and Mussgay, 1969 ; Horzinek et al., 1971). In contrast to naked animal viruses, however, isolated togavirus capsids are non-rigid and susceptible to RNase which degrades the viral genome to small fragments. Since this treatment has no effect on intact virions it is the function of the envelope to protect the viral RNA from enzymatic attack.

The RNA of togaviruses is a single stranded, colinear molecule with an average molecular weight of 4.2×10^6, corresponding to about 12.000 nucleotides ; it has a sedimentation coefficient of 42 - 49 S in sucrose gradients. Studies with alphaviruses have indicated a significant degree of secondary structure of extracted RNA which is necessary for infectivity. Polyadenylic acid sequences located near the 3′ - terminus have been demonstrated, which, together with the absence of a virion-associated polymerase indicated that togaviral RNA is plus-stranded and might serve as the initial messenger molecule during infection. These assumptions were confirmed by the demonstration of parental virion RNA in polysomes of infected cells and by its translation into virus-specific polypeptides in cell free systems. Like other mRNAs from eukaryotic systems viral RNAs contain methylated « capted » structures at their 5′ - termini, which in alphaviruses are of the form mG (5′)pppAp.

A minimum of three structural polypeptides has been identified for togaviruses, one of them associated with the nucleocapsid ; for alphaviruses and RUV this protein has a molecular weight of 30 000 - 35 000, for flaviviruses it ranges between 13 000 and 14 000. Hog cholera virus (HCV) also contains a non glycosylated 36 000 dalton polypeptide (Enzmann and Rehberg, 1977). Amino acid analyses have shown that the alphavirus capsid protein has a N-terminal lysine and is relatively hydrophilic, a property consistent with its extensive and rapid interaction with viral RNA.

Two glycosylated envelope proteins have been identified in RUV (50 000 and

63 000), HCV (55 000 and 46 000) and most alphaviruses (E1 and E2, 50 000 - 53 000) by SDS polyacrylamide gel electrophoresis ; using chromatographic techniques, three envelope proteins (52 000, 49 000 and 10 000) were isolated from Semliki Forest virus, an alphavirus. In flaviviruses only one envelope protein (50 000 - 60 000) is glycosylated whereas the other (8000 - 9000) seems to be buried in the lipid bilayer and may have a bridging function between the peripheral glycoprotein and the capsid. In alphaviruses both the E1 and E2 proteins occupy a superficial position for they can be stripped away by treatment with proteolytic enzymes and can be demonstrated by enzymatic iodination of undisrupted virions. They probably penetrate the lipid bilayer and interact directly with the capsid.

Togaviral lipid is confined to the envelope ; for alphaviruses it has been found that 25 - 31 % (by weight) is neutral lipid, predominantly cholesterol. Most of the remaining lipids are represented by phosphatidylethanolamine, phosphatidylserine, sphingomyeline and phosphatidylcholine, which is the principal phosphatide. Of the viral fatty acids oleic, palmitic and stearic acids predominate. In general, the composition of togaviral lipids reflects that of the host cell membranes as has been shown by a comparative analysis of Semliki Forest virus in BHK and Aedes albopictus cells, respectively (Pfefferkorn and Shapiro, 1974).

ANTIGENIC COMPOSITION

Assignment of a togavirus to a genus is made on the basis of immunological cross-reactions. Virion surface antigens responsible for adsorption to susceptible cells and erythrocytes can be identified and compared by neutralization (NT) and hemagglutination-inhibition (HI) tests ; using solvent-detergent extracted antigens in complement fixation, gel diffusion or radioimmuno assays, the total of antigen-antibody reactions is measured, including those with the capsid protein ; this has been described as carrying group-reactive determinants, possibly relating all alphaviruses (Dalrymple et al., 1972), similar to the common nucleoprotein (gs) antigen of the orthomyxoviruses.

Subgroups or complexes of more closely related viruses have been demonstrated by means of the less inclusive tests, namely by NT or by HI with the aid of sera from animals after a single injection of virus (Casals and Reeves, 1959 ; Karabatsos, 1975 ; de Madrid and Porterfield, 1974). RUV is unrelated to any other animal virus and has been classified as a togavirus exclusively for structural reasons. It is the only representative of the rubivirus genus. An antigenic relationship has been established between HCV and bovine diarrhea virus (BDV) ; border disease virus of sheep shares this relationship and is regarded as closely similar, if not identical with BDV (Harkness et al., 1977).

It has been possible to determine the antigenic function of the respective alphavirion surface polypeptides. The E1 glycoprotein of Sindbis virus carries the complete hemagglutinating activity while E2 appears important to infectivity since only antiserum directed against this molecular species effectively neutralized the virus. Glycoprotein E1 appears cross-reactive with antiserum to the closely related Western equine encephalitis virus and may therefore carry the determinant(s) responsible for defining the sero-

logical complexes ; E2 appeared virus specific (Dalrymple et al. 1976). By studying nucleotide sequence homologies between the nucleic acids of different alphaviruses it was demonstrated that the closely related viruses of Chikungunya and O'nyong nyong have only 13 % of the base sequences in common whereas < 1 % of RNA-RNA homologies occur between the other viruses of this genus. These rather limited homologies can be reconciled with the established serologic relationship by the assumption that the antigenic sites are composed of only a small number of amino acids and/or the degeneracy of the genetic code which allows a much larger homology to be present in the protein sequence than in corresponding RNA base sequence (Wengler et al., 1977).

Because of the simplicity of the test system the hemagglutinating antigen has been studied most extensively (Clarke and Casals, 1958). For diagnostic purposes alkaline aqueous extracts, fluorocarbon, ether-acetone, sucrose-acetone or Tween 80-ether extracts of infected tissues (mostly mouse brain material for alpha- and flaviviruses) are used. Treatment with protamine sulphate results in precipitation of host material and improvement in the HA patterns. In contrast to most other viruses, the demonstration of HA by togaviruses is dependent from the ionic environment ; in general, alphaviruses show optimal activity at pH 5.8 - 6.2, flaviviruses at 6.2 - 6.4 and RUV requires Ca^{++} ions for HA. Dependence from the ionic environment indicates that a specific conformation of the envelope glycoproteins is essential ; it has been shown that the isoelectric point of Sindbis virus E1 protein is the same as the pH optimum for HA. — For pestiviruses, hemagglutinating activity has not been described so far.

Goose red cells are routinely used in many laboratories, but chicken (adult rooster and recently hatched) and pigeon cells (RUV) are also suitable. Optimal incubation temperature is 25 to 37°C for most togaviruses, some flaviviruses and RUV require + 4°C for HA. Spontaneous elution does not occur but may be induced by adjusting the reaction mixture to alkaline pH or, in the case of RUV, by removing divalent cations from the milieu. After elution there is no detectable alteration of the erythrocyte surface, since the cells are fully capable of adsorbing more virus of the same or other types. Neuraminic acid receptors do not play a role in togavirus HA.

REPLICATION

Adsorption of alphaviruses to susceptible cells occurs within a few minutes ; since it is dependent from the salt concentration (monovalent cations — whereas divalent cations inhibit attachment) and pH, but independent from the temperature, electrostatic forces are probably involved. The enhancing effect of DEAE-dextran, a polycation, further supports this assumption. Since alphaviruses are capable of growing in a variety of cells from phylogenetically unrelated species including mammalian, avian, reptilian, amphibian, piscine and arthropod, these viruses either have a broad range of receptors widely common in nature or they do not require specific receptors. In contrast, RUV and the pestiviruses have a very limited range of susceptible cells ; unadapted HCV e.g. attains appreciable titres only in cells of the porcine species. — Penetration of adsorbed virus is tem-

perature dependent (optimum at 37 °C) but independent from the ionic environment and follows first order kinetics. The virion is engulfed into a pinocytotic vacuole and subsequently uncoated.

Replication of viral RNA requires a new protein, a virus specific RNA-dependent RNA polymerase. For its synthesis, the input parental RNA serves as a messenger. The polymerase is bound to smooth cytoplasmic membranes, where it catalyses the synthesis of replicative intermediates. These consist of partially double-stranded RNA molecules containing one polynucleotide species serving as a template and a second species of complementary strandedness ; this latter consists of nascent chains of varying length which are partially base-paired to the template. The major species of alphaviral RNA synthesized in infected cells in not virion RNA (4.2×10^6) but a subgenomic RNA species of 26 S (1.6×10^6) which has the same polarity as virion RNA but contains only 1/3 of its nucleotide sequences. It serves as the mRNA for the structural proteins of the virion. Studies in Semliki Forest virus-infected cells and with cell free synthesizing systems have shown that the 5′ → 3′ gene order in 26S RNA is C, E3, E2 and E1 and that these proteins are synthesized as a polyprotein of about 130 000 daltons from a single initiation site. By a sequence of nascent and posttranslational proteolytic cleavages, this molecule is processed to give the structural virion polypeptides.

The remaining coding capacity corresponding to protein of about 300 000 daltons is used for nonstructural viral polypeptides. Since the 42S RNA is infectious, some, if not all of these are likely to be components of the viral polymerase. The nucleotide sequence of the 26S RNA is located inward from the 3′ end of the 42S RNA which implies that the genes coding for the nonstructural proteins must be situated in the 5′ terminal two thirds of the genome. Their synthesis is initiated at a single site near the 5′ end and terminated internally before the structural protein genes. Also the nonstructural polypeptides are synthesized as a giant precursor molecule which is subsequently processed by proteolytic cleavage.

In the cytoplasm of the alphavirus-infected cell the viral capsid is assembled around the RNA and then attaches to the inner surface of the host cell plasma membrane after that membrane had been modified by the insertion of the virus specified glycoproteins. The membrane at this stage contains the definite E1 protein and a precursor to the second glycoprotein. The envelopment of the nucleocapsid takes place as it is progressively wrapped into the modified membrane while moving from the cytoplasm to a position physically outside the cell. During the terminal stages of this « budding » process the precursor polypeptide is cleaved to form the E2 glycoprotein.

Intracytoplasmic vacuoles are the first evidence of subcellular alteration associated with virus replication. Alpha- and flaviviruses differ with respect to their morphogenesis, the former enveloping their capsids by budding preferentially from the marginal membrane whereas the latter emerge from internal vacuolar membranes ; for RUV the general picture is like that of alphaviruses, pestiviruses rather resemble flaviviruses in their morphogenesis. Only in alphavirus-infected cells assembled capsids may be detected around cytoplasmic vacuoles.

Different togaviruses vary in the intensity of their cytopathic action which may

depend from the cell species on one hand and from environmental conditions (medium, pH, temperature, virus dose) on the other hand. In vertebrate cells, growth of alphaviruses is rapid. A few hours after infection, first newly formed virus is released, the virus production continues at a constant linear rate for up to 10 or 12 hrs, approaching 1000 infectious units per cell. Flaviviruses show a latent period of about 12 hrs and a further 10-20 hrs are required to achieve maximal titres of extracellular virus (Mussgay et al., 1975 ; Pfefferkorn and Shapiro, 1974). RUV and BDV may multiply without apparent cytopathic alterations ; the cell species and virus strain determine, whether changes in cell appearance will occur. HCV is notoriously non cytopathogenic, with the exception of one virus strain (Gillespie et al., 1961).

PROBABLE TOGAVIRUSES

Although their taxonomic status has not officially been approved, lactic dehydrogenase virus of mice (LDV), equine arteritis virus (EAV) and simian hemorrhagic fever virus would appear further Togaviridae family candidates. LDV (for review see Rowson and Mahy, 1975) in size and morphology resembles an alphavirus without projections (Horzinek et al., 1975). It possesses an infectious RNA (Notkins, 1964) and three structural polypeptides of molecular weights 13-15 000, 17-18 000 and 24-44 000 (Michaelides and Schlesinger, 1973 ; Brinton-Darnell and Plagemann, 1975). For EAV similar results have been obtained (v.d. Zeijst et al., 1975 ; Zeegers et al., 1976). Since structural similarities sofar were always shown to be accompanied by serologic cross reactions, we have recently compared LDV and EAV by neutralization, immunofluorescence and radioimmuno precipitation tests (Zeegers and Horzinek, in press). No antigenic relationship could be shown to exist between both viruses. Upon a comparative re-examination of the molecular weights it was found that the LDV capsid polypeptide appeared distinctly smaller than the corresponding EAV protein ; this is at variance with published data (10 000, as compared with 13 000 — Michaelides and Schlesinger, 1973, Brinton-Darnell and Plagemann, 1975) and would place LDV and EAV in taxonomically different groups.

Not unexpectedly, because of their arthropod transmission, togaviruses have also been identified in insect cells ; the cell fusing agent found in Aëdes albopictus cultures is an invertebrate Togaviridae family member candidate (Igarashi et al., 1976). From umbelliferous plants, a lipid containing RNA virus was isolated (Carrot mottle virus) which in many respects resembles the animal togaviruses (Murant et al., 1969). If this taxonomic position can be confirmed, togaviruses would turn out to be very versatile parasites, occurring in vertebrates, invertebrates and plants ; other virus families with a similar host spectrum are the Reo- and Rhabdoviridae, which also possess arthropod-borne members. Since the insecta and acarina represent phylogenetically old classes of animals, they might have played a crucial role in the evolution and cosmopolitan distribution of the Reo-, Rhabdo- and Togaviridae.

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