Foot-and-Mouth Disease Virus O₁Lombardy is Biochemically Related to O₂ Isolates

OTTHEINZ KREBS,¹ HANS-GERHARD BERGER,² WIESLAW NIEDBALSKI,³ AND OTFRIED MARQUARDT⁴
¹Federal Research Centre for Virus Diseases of Animals, Tübingen, FRG
²Friedrich-Miescher-Laboratory of the Max-Planck-Society, Tübingen, FRG
³Zakład Badania Przyszczycy, Zdunna Wola, Poland

Received August 13, 1990
Accepted November 13, 1990

Requests for reprints should be addressed to Dr. Otfried Marquardt, Federal Research Centre for Virus Diseases of Animals, P.O. Box 1149, D-7400 Tübingen, FRG.

Key words: FMDV subtype O₂, VP1, nucleotide and amino acid sequence, RNase mismatch cleavage method

Abstract

The capsid protein VP1-encoding RNA regions of the foot-and-mouth disease virus isolates O₁Lombardy/1946 and O₂Brescia/1947 were sequenced and found to be closely related to each other and to O₂Normandy/1949, despite some sequence differences. The O₁Lombardy sequence was expected to be more closely related to those of the subtype O₁ isolates of 1965 and later (e.g., O₁Kaufbeuren/1966), but this was not the case. The serological subtyping of both the Lombardy and the Kaufbeuren isolate as O₁ strains was possibly due to identical VP1 C-terminal sequences, since all the subtype O₂ isolates differ here from the O₁ isolates at residue 209. Considerable dissimilarity of other O₁Lombardy and O₂Brescia genome parts to those of O₁Kaufbeuren was qualitatively shown by analyzing the sizes of RNase-treated hybrids formed with virus RNA and defined subgenomic fragments of O₁Kaufbeuren-specific antisense cRNA. These hybrids were fragmented into oligonucleotides, but others containing O₁Kaufbeuren virus RNA were protected.

Introduction

Foot-and-mouth disease is caused by a picornavirus (FMDV). The virion is an icosahedron consisting of 60 copies each of four viral polypeptides (VP1-4), which encapsidate the single-stranded RNA genome. FMDV has been classified into
seven antigenically distinct types (serotypes A, C, O, SAT 1, SAT 2, SAT 3, and Asia 1; reviewed in 1), which are further distinguished by neutralizing reference sera into 64 subtypes (2).

Antigenic variation of FMDV is mainly due to sequence changes in those parts of VP1 that form the major antigenic sites. These are residues 135–160 and the C-terminal residues (reviewed in 3) that protrude from the viral surface and interact with each other while belonging to different molecules (4). Together they contribute to a nonlinear neutralization epitope (5).

Even different isolates of the same subtype may vary in the VP1 sequence, especially between positions 135 and 160 (compare for instance: 6–12). FMDV O1Lombardy, a 1946 isolate, differs also in its VP1 sequence from other O1 isolates (present report), but it is unusual since it is biochemically closer related to its contemporary isolates subtyped O2 than to the O1 strains isolated during a more recent epizootic. The inconsistency of serological (13) and biochemical data concerning the relation of FMDV strain Lombardy to other type O strains should be realized, as it is still studied as a laboratory strain (14).

A FMDV subtype, which has never caused a field outbreak since 1975, was analyzed because its strains Lombardy, Brescia, and Normandy were isolated, at times, where vaccination against FMD was not carried out (reviewed in 1). Knowledge on antigenic variations under such conditions may be valuable in the future, because vaccination will be stopped after 1991 in the EC. Animals will therefore no longer be protected against accidental FMDV infection.

Materials and Methods

Virus strains, cells, and infection. Baby hamster kidney-21 (BHK-21) cells (15) were infected with the following FMDV strains: O2Brescia, O1Kaufbeuren, and O1Lombardy wt (26 times passaged); and its mutants that either produce thermo-stable capsids (41 times passaged), induce the production of interferon (43 times passaged), or are attenuated to grow at room temperature (411 times passaged). Cell culture and virus infection procedures have already been described (16,17).

Virus RNA

When a beginning cytopathic effect became visible, the exact time depending upon the virus strain, virus-infected cells were lysed and total RNA was extracted by the acid guanidinium thiocyanate/chloroform/phenol method (18). The RNA contained enough virus RNA for sequencing reactions (19). Oligodeoxynucleotide primers were synthesized with an automated DNA synthesizer (Biosearch 8700; New Brunswick, Heusenstamm, FRG). Sequencing gels were run at standard conditions in LKB units (Pharmacia/LKB, Freiburg, FRG).