JOURNAL OF VIROLOGY, May 1989, p. 2385–2387
0022-538X/89/052385-03$02.00/0

Vol. 63, No. 5

Resistance to Foot-and-Mouth Disease Virus Mediated by *trans*-Acting Cellular Products

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Received 31 October 1988/Accepted 6 January 1989

Upon serial passage of BHK-21 cells persistently infected with foot-and-mouth disease virus (FMDV) C-S8c1, cells with increased resistance to the virus were selected (J. C. de la Torre, E. Martinez-Salas, J. Diez, and E. Domingo, J. Virol. 63:59–63, 1989). Two highly resistant cell clones, 74A11 and 74D12, were transformed to puromycin resistance (Pur^r) and were fused to BHK-21 cells transformed to neomycin resistance (Neo^r). The hybrid Neo^r Pur^r cells showed the specific resistance to FMDV C-S8c1 characteristic of clones 74A11 and 74D12. The results suggest that resistance to FMDV C-S8c1 is mediated by *trans*-acting cellular products. The possibility of engineering constitutive resistance to FMDV is discussed.

A remarkable variation in phenotypic properties of both the cells and the resident virus occurred upon serial passage of BHK-21 cells persistently infected with foot-and-mouth disease virus (FMDV) C-S8c1 (5, 7). In such cultures, termed C_1-BHK-Rc1, cell heterogeneity was rapidly generated (6), even though the cultures were established with cloned BHK-21c1 cells (5). Some clones isolated from late-passage C_1-BHK-Rc1 were highly resistant to FMDV C-S8c1, the virus used to establish persistence (6). However, the clones were productively infected by virus rescued from the carrier cultures at late passages, suggesting a coevolution of cells and resident virus (7). Cellular resistance was specific for FMDV (5, 7) and due to some intracellular block that resulted in a 100-fold decrease in the amount of FMDV RNA (7). Upon infection of monolayers of such clones with FMDV C-S8c1, the virus yields were 10^3- to 10^5-fold lower than those obtained with BHK-21 (6).

There is no evidence that either in uncloned populations of C_1-BHK-Rc1 cells at late passages or in clones derived from those cultures, defective FMDV particles or RNAs were present and interfered with FMDV C-S8C1 multiplication (6, 7). Thus, we considered two mechanisms of resistance to FMDV C-S8c1: (i) absence of a cellular component required for FMDV replication and (ii) production of factors that inhibit FMDV replication. To distinguish between these possibilities, we performed cell fusion experiments after introducing selectable markers into the cells. BHK-21 cells were transformed with plasmid pSV2neo (13), and G418-resistant transformants (BHK-21*neo*) were selected (13). Two FMDV C-S8c1-resistant clones, termed 74A11 and 74D12 (isolated from C_1-BHK-Rc1 cultures at passage 74 and belonging to groups 74R-1 and 74R-2, respectively [Table 1 in reference 6]), were transformed with plasmid pBSpac (4), and transformants (74A11*pur* and 74D12*pur*) were selected by their resistance to puromycin (4). Stable BHK-21*neo* transformants were fused to either 74A11*pur* or 74D12*pur* by the procedure of Robinson et al. (13). Hybrid cells were selected by their resistance to both puromycin and G418. Monolayers of hybrid cells were infected with FMDV C-S8c1, FMDV R59—the virus rescued from the carrier C_1-BHK-Rc1 culture (7)—or encephalomyocarditis virus. The results show that monolayers of the fusion products BHK-21*neo*–74A11*pur* and BHK-21*neo*–74D12*pur* were resistant to FMDV C-S8c1 but not to FMDV R59 or encephalomyocarditis virus (Fig. 1). The degree of resistance of the hybrid cells to FMDV C-S8c1 is similar to that shown by the parental 74A11 or 74D12 cells (6). FMDV R59 was able to partly overcome the intracellular block, as previously seen with uncloned C_1-BHK-Rc1p58 populations (7). BHK-21*pur*, 74A11*neo*, and 74D12*neo* were obtained and tested in parallel cell fusion experiments. Hybrid BHK-21*neo*–BHK-21*pur* was productively infected by all viruses tested (Fig. 1A and B). BHK-21*pur* fused to either 74A11*neo* or 74D12*neo* maintained the specific resistance to FMDV C-S8c1 (data not shown). The results suggest that clone 74A11 or 74D12 produces some *trans*-acting factors that interfere in a very specific fashion with FMDV C-S8c1 replication, resulting in a decrease of FMDV RNA in the cells (6, 7).

An intracellular restriction for viral development has been documented for other picornaviruses (16), such as strain GDVII of Theiler's murine encephalomyelitis virus in HeLa cells (15), for mengovirus in bovine cell line MDBK (3, 12) and other cells (2), and for encephalomyocarditis virus in monkey cells (9). In the latter system, analysis of monkey-mouse hybrid clones indicated that those possessing the greatest number of monkey chromosomes were the least permissive (9). It was suggested that a virus function could be inhibited by a gene product from the monkey cells (9). For C_1-BHK-Rc1, recent experiments have shown that the resistance extends to at least some subtypes of the European serotypes A, O, and C of FMDV (J. Diez, N. Parry, and A. Donaldson, unpublished results). Thus, FMDV R59 and other viruses rescued from late-passage carrier cultures have been selected as host range mutants by one or a combination of mutations not frequently fixed in natural populations of FMDV. We are now using such host range mutants to try to map on the FMDV genome the regions involved in overcoming the block in 74A11 or 74D12 cells.

Foot-and-mouth disease is a major animal health problem worldwide (1, 11). The extreme antigenic heterogeneity of FMDV (1, 8, 10a, 11) constitutes a drawback for the preparation of new synthetic vaccines. The results reported here suggest that cellular genes from susceptible cells, genes perhaps modified by mutation (6, 7), may be expressed to

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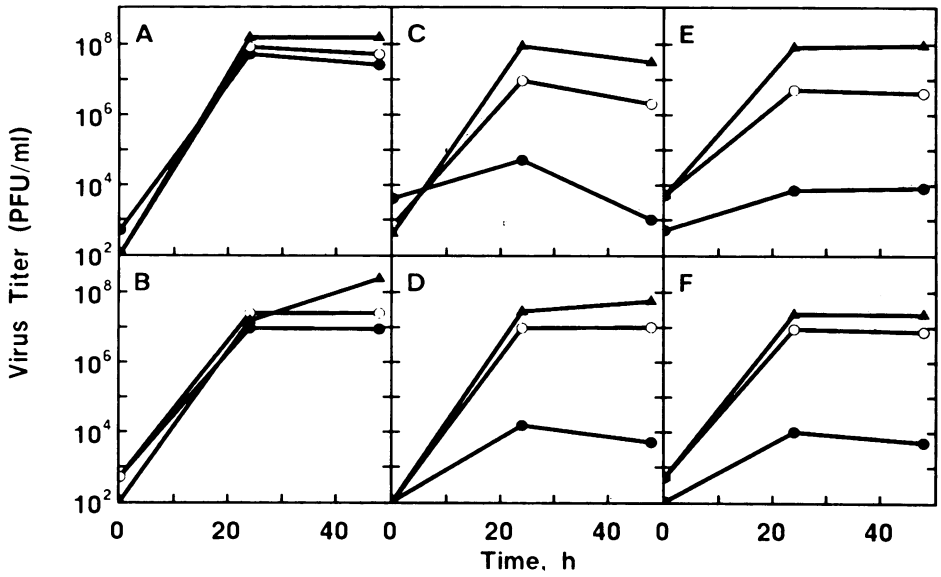

FIG. 1. Infection of hybrid cells with FMDV. About 2 × 10^6 cells were transformed with plasmid pSV2neo (13) or pBSpac (4) by the calcium phosphate precipitation technique (10, 17). After overnight incubation, the cells were diluted, plated, and maintained in the presence of G418 (1 mg/ml) for the pSV2neo cells or puromycin (10 μg/ml) for the pBSpac cells until transformed resistant colonies were apparent. Cells from single clones were obtained and grown in the presence of G418 (250 μg/ml) or puromycin (2.5 μg/ml). An identical procedure was followed for the transformation of 74A11 and 74D12. BHK-21*neo* cells (10^6) were fused to either 74A11*pur* or 74D12*pur* (10^6), as described elsewhere (12), and the hybrid cells were selected in the presence of G418 (1 mg/ml) and puromycin (10 μg/ml). Then the cells were grown in medium with G418 (250 μg/ml) and puromycin (2.5 μg/ml) to form monolayers of 0.5 × 10^6 to 1 × 10^6 cells for viral infections (5–7). Monolayers were washed and infected with FMDV C-S8c1 (●), FMDV R59 (○), or encephalomyocarditis virus (▲) at a multiplicity of 2 to 4 PFU per cell. At 0, 24, and 48 h, samples of the culture medium were titrated in duplicate. Results of two independent fusion experiments are given. (A and B) BHK-21*neo* fused to BHK-21*pur*; (C and D) BHK-21*neo* fused to 74A11*pur*; (E and F) BHK-21*neo* fused to 74D12*pur*. Virus yields as shown in panels A and B are similar to those obtained with ordinary BHK-21 cell monolayers (6).

inhibit FMDV multiplication. Their identification and subsequent engineering in transgenic animals could provide constitutive resistance to the virus and serve as a new approach to foot-and-mouth disease control. The feasibility of such a strategy will largely depend on the number and nature of the cellular genes responsible for the FMDV resistance phenotype and on the frequency of generation of viral mutants able to overcome the restriction.

We thank J. Ortín for valuable suggestions and M. Dávila and C. Martiñez for expert technical assistance.

This work was supported by Comisión Asesora para la Investigación Cientifica y Técnica, Fondo de Investigaciones Sanitarias, Consejo Superior de Investigaciones Cientificas, and Fundación R. Areces, Spain. S.L. and J.D. were supported by predoctoral fellowships from PFPI, and J.C.T. was supported by postdoctoral fellowships from Consejo Superior de Investigaciones Cientificas.

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