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Enhanced replication of porcine reproductive and respiratory syndrome (PRRS) virus in a homogeneous subpopulation of MA-104 cell line

Brief Report

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Summary. Two different cell populations, high- (MARC-145) and low-permissive cell clones (L-1) to porcine reproductive and respiratory syndrome (PRRS) virus, were derived from MA-104 cell line (parent cell: P) by cell cloning. Maximum virus yields in MARC-145, P, and L-1 cell clones were $10^{8.5}$, $10^{3.5}$, and $10^{2.5}$ tissue culture infective dose 50 ($TCID_{50}$)/0.1 ml, respectively. The MARC-145 cell clone supported replication of all 11 different porcine reproductive and respiratory syndrome virus isolates that were tested. These results indicated that the MARC-145 cells will be useful for PRRS virus replication.

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Porcine reproductive and respiratory syndrome (PRRS) virus has recently been identified in North America and Europe as an important cause of reproductive failure in pregnant sows and respiratory disease in young pigs [2, 3, 5, 9, 11–13]. This virus infection causes a significant economic loss for the swine industry [7].

The PRRS virus is an enveloped RNA virus with a buoyant density of 1.18 to 1.23 g/ml on sucrose gradients [1]. Virions are estimated to be 48 to 80 nm in diameter [1]. Serologic studies indicated that antigenic variation may exist between American and European PRRS virus isolates [10]. Recent studies suggested that PRRS virus belongs to the *Arterivirus* group, which includes equine arteritis virus, lactate dehydrogenase-elevating virus, and simian hemorrhagic fever virus [4, 6, 8]. Virus replication has failed in many different

kinds of primary and established cell lines [11, 13]. Swine alveolar macrophages (SAM) are the only known primary cells that support virus replication [11, 13]. Advantages of SAMs include high sensitivity to PRRS virus infection and relatively high virus yields. However, there are several disadvantages of using SAMs for PRRS culture. These include the risk of contamination by unknown organisms present in the swine lung source, difficulties in obtaining lung macrophages with the consistent quality that is required for reliable results, the high purchase cost of animals, and technical difficulties in harvesting SAMs by pulmonary lavage. One established cell line, CL 2621, a proprietary cell line of the Boehringer Ingelheim Animal Health Inc., St. Joseph, MO, U.S.A., has been reported to support virus replication [3], but is of limited availability. The lack of an accessible established cell line permissive to PRRS virus has inhibited research and diagnostic efforts.

Initially, we found some evidence of PRRS virus replication in monkey kidney (MA-104) cells; however, virus titers were low and only focal cytopathic effects (CPE) were observed on infected monolayers. Virus infection on MA-104 cell monolayers was confirmed by an indirect fluorescent antibody test (Kim et al., pers. obs.). The present work was started under the assumption that MA-104 cells are heterogeneous with respect to PRRS virus permissiveness and that high- and low-permissive cell subpopulations could be separated from the MA-104 cells. This report describes the propagation and enhancement of virus replication in a selected homogenous permissive-cell population from the MA-104 cell line.

The PRRS virus isolates and MA-104 cell line used in this study were obtained from the swine virology laboratory (University of Minnesota, St. Paul, MN, U.S.A.). The cell line has been maintained in Eagle's minimum essential medium (MEM), supplemented with 5% fetal bovine serum (FBS), 0.15% sodium bicarbonate, and antibiotics. Reference-positive sera to PRRS virus were collected from weaned pigs which were infected experimentally with PRRS virus isolate MN-1 b [13]. Negative-reference sera were collected from the same pigs prior to inoculation.

Cloning of MA-104 cell subpopulations was performed by the limiting-dilution method. The trypsinized MA-104 cells, diluted at a mean concentration of 1 cell/well in MEM containing 30% FBS, were dispensed into 96-well tissue culture plates and incubated at 37 °C in an atmosphere of 5% CO_2. Following the identification of cell monolayers from the initial cloning, these subclones were subjected to 2 replications of further cloning.

In order to select for high- and low-permissive cell clones, PRRS virus MN-1 b was inoculated at multiplicity of infection of 0.01 onto each cell monolayer. The infected monolayers were fixed 3 days postinfection (p.i.) with pure, cold, absolute ethanol. An indirect fluorescent antibody (IFA) test was performed using reference-positive and negative sera, as previously described [14].

After the third cell cloning of MA-104 cells, high- and low-permissive cell clones were established. Virus replication was evident by the CPE and intense

fluorescence color in the infected high-permissive cell monolayers on 3 days p.i., whereas, no CPE or fluorescence color was observed in low-permissive cell monolayers. These high- and low-permissive cell clones were designated as MARC-145 and L-1, respectively. The CPE and fluorescent staining of the infected MARC-145 and L-1 cell monolayers are illustrated in Figs. 1 and 2. One-step growth curves for released virus in the culture supernatant were determined for the MA-104 parent cell (P cell: uncloned MA-104 cell), MARC-145, and L-1 clones to compare virus yields among these three cell populations. Confluent cell monolayers were prepared in seven 24-well tissue culture plates. Each plate (containing 6 wells for each of three cell populations) was flooded with PRRS virus MN-1 b ($10^{4.5}$ $TCID_{50}$/0.1 ml) and absorbed for 1 h at 37 °C. Unabsorbed virus was removed, the monolayers were washed 3 times with MEM, and incubated after addition of 1.5 ml of MEM containing 3% FBS. Pooled culture supernatants from the wells for each cell population were collected from one plate per day for seven consecutive days. The supernatants were stored at − 70 °C until infectivity was determined using the MARC-145 clone. Maximum virus titers were significantly higher in the MARC-145 cell clone than those with P or L-1 cell clones (Fig. 3). Highest viral infectivity recorded in MARC-145, P, and L-1 cell clones were $10^{8.5}$, $10^{3.5}$, and $10^{2.5}$ $TCID_{50}$/0.1 ml, respectively.

Since the MARC-145 cell clone is optimal for the propagation of PRRS MN-1 b virus isolate, it was of further interest to examine whether MARC-145 is suitable for the propagation of other PRRS virus isolates. Virus replication of 11 additional PRRS virus isolates, including 2 viremic pig sera, was examined in the MARC-145 cell clone. Samples were diluted 20 times with MEM and then 100 µl of each dilution was inoculated onto the monolayers of the MARC-145 cell clone in a 24-well tissue culture plate. After 1 h incubation, 1.5 ml of MEM containing 3% FBS was added. The infected cell monolayers were frozen and thawed twice at 5 days p.i. The virus infectivity was determined in the MARC-145 cell clone.

In the experiment, all 11 virus isolates that were tested showed variable virus titers after first passage on the MARC-145 cell clone. Seven PRRS virus isolates showed infectivity titers that ranged between $10^{4.0}$ and $10^{6.0}$ $TCID_{50}$/0.1 ml, while four isolates had infectivity titers that ranged between $10^{6.0}$ and $10^{7.0}$ $TCID_{50}$/0.1 ml. The variation of virus infectivity between the isolates may have been due to different sensitivities of the virus isolates to MARC-145, or different amounts of virus in the inoculum.

In conclusion, homogeneous high- (MARC-145) and low-permissive cell clones (L-1) were derived from heterogeneous MA-104 P cells. The MARC-145, L-1, and P cells differed by virus yield, period of time before showing observable CPE, and intensity of CPE. The fact that MARC-145 cells supported replication of 11 PRRS virus isolates indicated the potential of these cells for diagnostic and research applications.

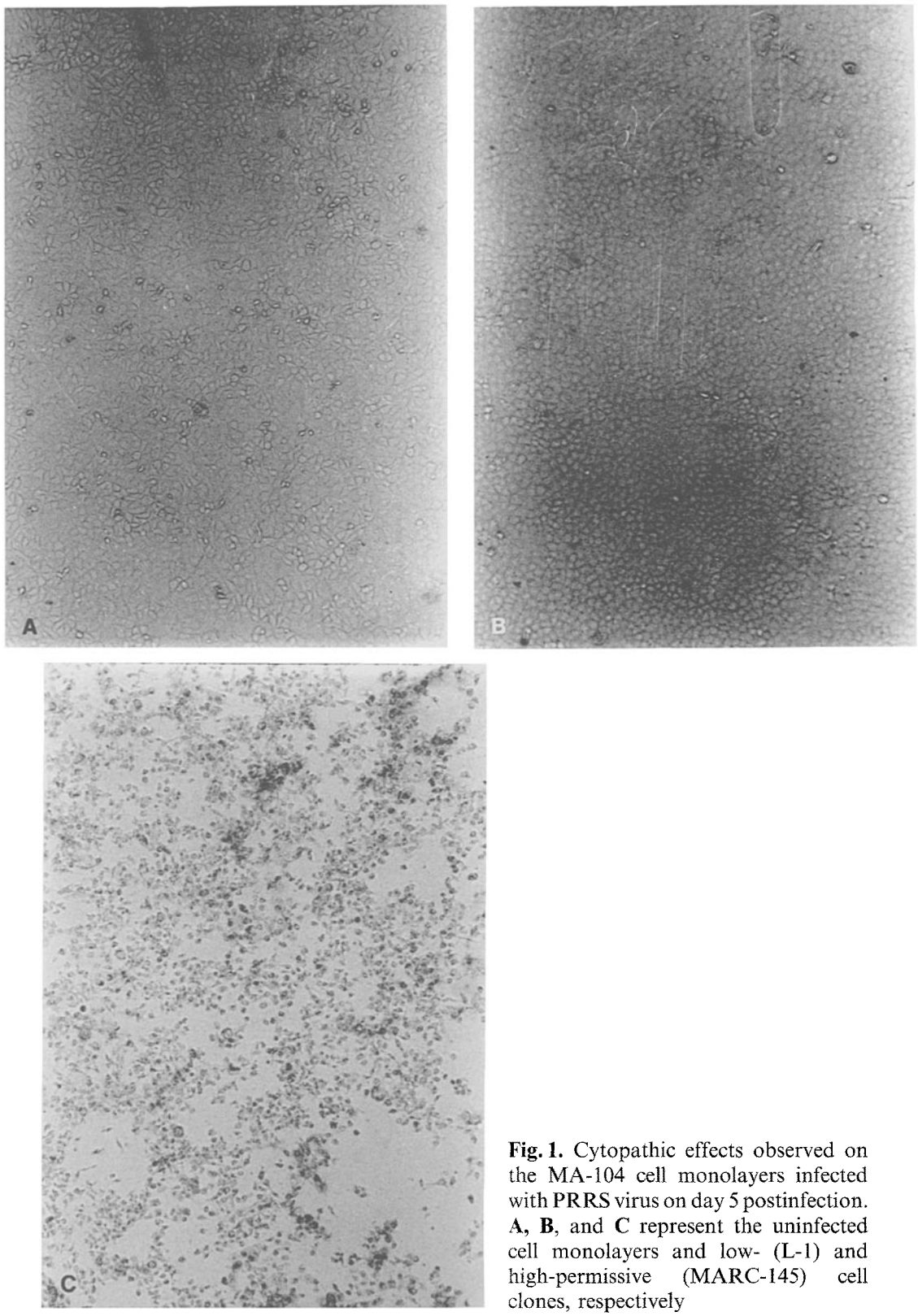

Fig. 1. Cytopathic effects observed on the MA-104 cell monolayers infected with PRRS virus on day 5 postinfection. **A, B**, and **C** represent the uninfected cell monolayers and low- (L-1) and high-permissive (MARC-145) cell clones, respectively

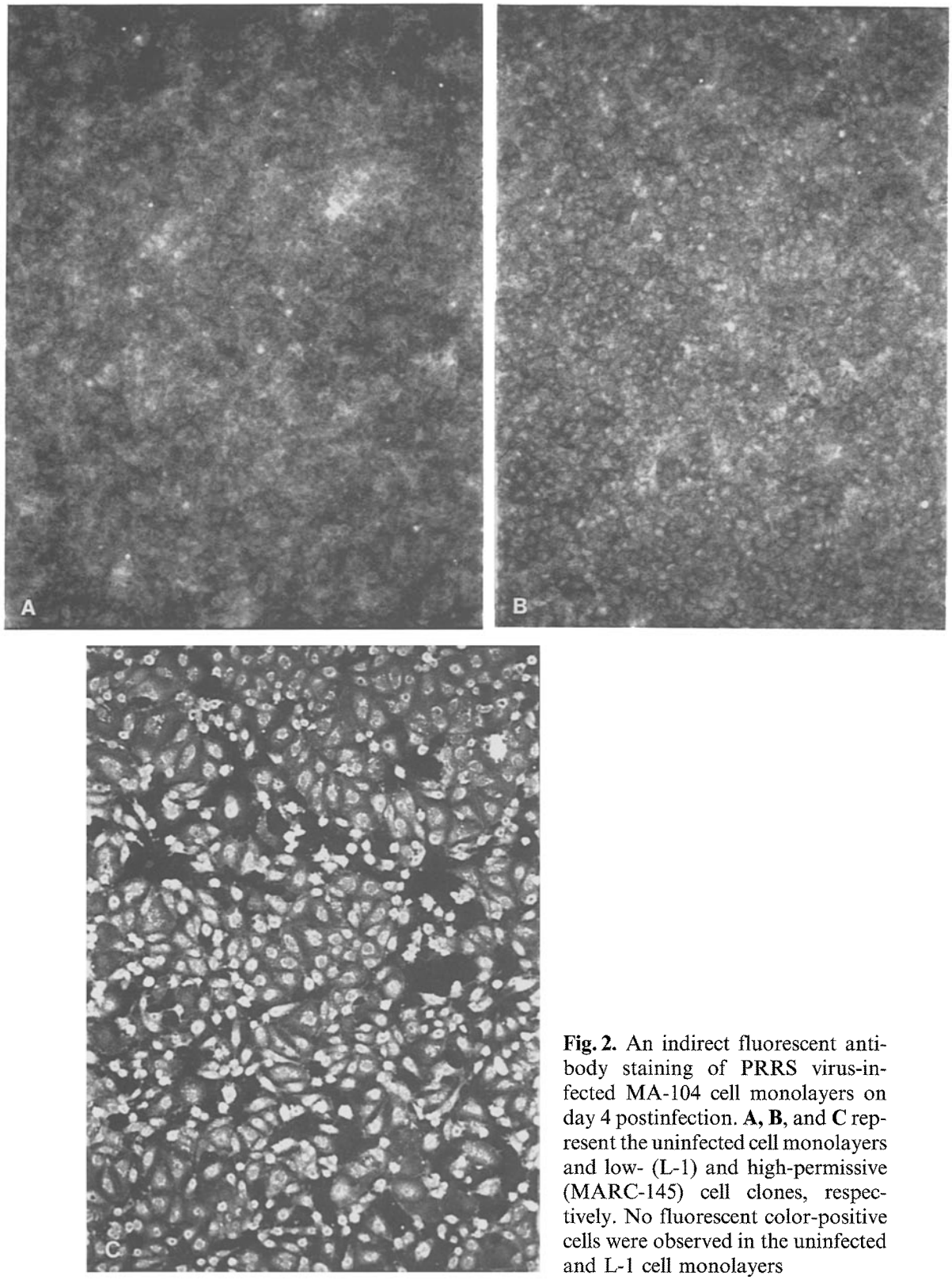

Fig. 2. An indirect fluorescent antibody staining of PRRS virus-infected MA-104 cell monolayers on day 4 postinfection. **A, B,** and **C** represent the uninfected cell monolayers and low- (L-1) and high-permissive (MARC-145) cell clones, respectively. No fluorescent color-positive cells were observed in the uninfected and L-1 cell monolayers

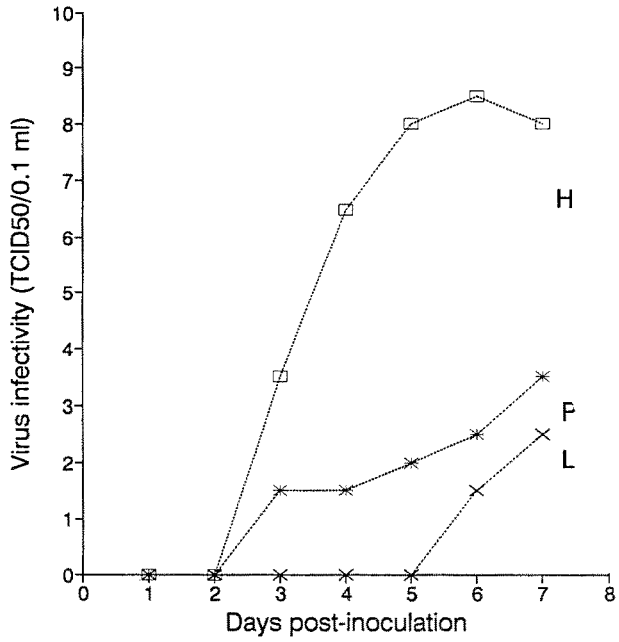

Fig. 3. The one-step growth curves of PRRS virus in three different MA-104 cell subpopulations. *H, L,* and *P* represent the high- (MARC-145) and low-permissive (L-1) cell clones and parent cells, respectively

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