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Reovirus-Induced Acute Myocarditis in Mice Correlates with Viral RNA Synthesis rather than Generation of Infectious Virus in Cardiac Myocytes

BARBARA SHERRY,* CATHERINE J. BATY, AND MARY ANN BLUM

Department of Microbiology, Pathology, and Parasitology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606

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The capacity for different reovirus reassortant viruses to induce acute myocarditis in mice correlates with cytopathogenic effect in primary cultures of murine cardiac myocytes. Multiple viral genes encoding proteins involved in viral RNA synthesis are determinants of this disease. We therefore evaluated the role of viral RNA synthesis in induction of acute myocarditis by infecting primary cultures of cardiac myocytes with a panel of myocarditic and nonmyocarditic viruses and quantitating RNA synthesis. RNA synthesis correlated with induction of myocarditis and with the S1 and M1 reovirus genes. Since one consequence of viral RNA synthesis is generation of infectious virus, we looked next at viral yield from cardiac myocyte cultures. Yield of infectious virus at an early time postinfection or as a final yield from primary infections did not correlate with myocarditis, but instead both correlated with the S1 gene. The S1 gene also determined the fraction of cells infected during primary infections in the culture, which varied dramatically between viruses. Viral yields per infected cell were similar for most myocarditic and nonmyocarditic reoviruses and did not correlate with induction of myocarditis or any reovirus gene. Together, the data provide two insights into reovirus-induced acute myocarditis in mice. First, while the S1 gene, which encodes the viral attachment protein σ1 (as well as a nonstructural protein, σ1s, of unknown function) does not determine the myocarditic potential of these viruses, it does determine the efficiency with which they infect cardiac myocytes. Second, while viral RNA synthesis is a determinant of acute myocarditis, this is not due to generation of infectious virus. This finding suggests that some other consequence of viral RNA synthesis, for example, induction of interferon, may determine reovirus-induced acute myocarditis.

Acute viral myocarditis (2) is an important disease in humans, with reports suggesting that 5 to 20% of the population have suffered some form of viral myocarditis (3, 25, 39). It is frequently fatal in infants (6, 16, 20), and the acute disease in children or adults may progress to chronic myocarditis or dilated cardiomyopathy later in life (1, 15, 21, 23, 32). Although enteroviruses (including coxsackieviruses) are the most frequently identified viruses associated with human acute myocarditis, (9, 15, 34, 35), many other virus families have been implicated as well (17, 19, 27, 38). Given the prevalence of these viruses, the limited ability to biopsy the heart, and the focal distribution of lesions in the heart, the frequency with which any of these other viruses induce myocarditis is largely unknown. Occasional anecdotes have associated reoviruses with cases of fatal acute myocarditis in infants, and reoviruses offer a tractable model for investigation of the molecular mechanisms involved in this disease.

Reovirus-induced acute viral myocarditis in mice is characterized by a mild inflammatory infiltrate with marked necrosis (11, 31, 33), in contrast to the massive cellular infiltrate characteristic of the enterovirus-induced disease (26). Indeed, evidence suggests that enterovirus-induced acute myocarditis is an immune system-mediated disease (8, 12), although there remains some controversy (7, 10, 13). In contrast, reovirus acute myocarditis in mice is not immune system mediated (30) and is instead most likely due to a direct cytopathogenic effect (CPE) of the virus on cardiac myocytes (4). Specifically, when a panel of nonmyocarditic and myocarditic viruses were used to infect primary cultures of murine cardiac myocytes in vitro, their CPE in these cells correlated with their potential to induce acute myocarditis. Interestingly, CPE in primary cultures of murine cardiac fibroblasts did not correlate with myocarditic potential, suggesting that viral interactions specific to cardiac myocytes are critical to disease.

Genetic analyses, using reassortant viruses containing combinations of double-stranded RNA gene segments from myocarditic and nonmyocarditic reoviruses, demonstrated that genes encoding viral core proteins determine reovirus-induced acute myocarditis (28, 29). In particular, the M1 gene, encoding a viral core protein μ2, was implicated in all genetic analyses. While the exact function of μ2 remains unknown, recent studies suggest a role in positive-strand RNA synthesis from reovirus cores in vitro (40), consistent with its likely association in the core with the viral polymerase λ3 (encoded by L1) and the guanylyltransferase λ2 (encoded by L2). Together, the results suggest the hypothesis that reovirus-induced acute myocarditis may be determined by viral replication in cardiac myocytes.

We report here the results of our investigations of RNA synthesis, efficiency of infection, and yield of infectious virus in primary cultures of cardiac myocytes. While RNA synthesis correlated with induction of acute myocarditis in mice, yield of infectious virus did not. Thus, some other consequence of viral RNA synthesis, such as cytokine response, is likely to be a determinant of the disease.

MATERIALS AND METHODS

Viruses and cells. Mouse L929 (L) cells were maintained as spinner cultures in minimal essential medium (S-MEM Joklik; Irvine Scientific, Santa Ana, Calif.)

* Corresponding author. Phone: (919) 515-4480. Fax: (919) 515-3044. Electronic mail address: bsherry@sn1.cvm.ncsu.edu.

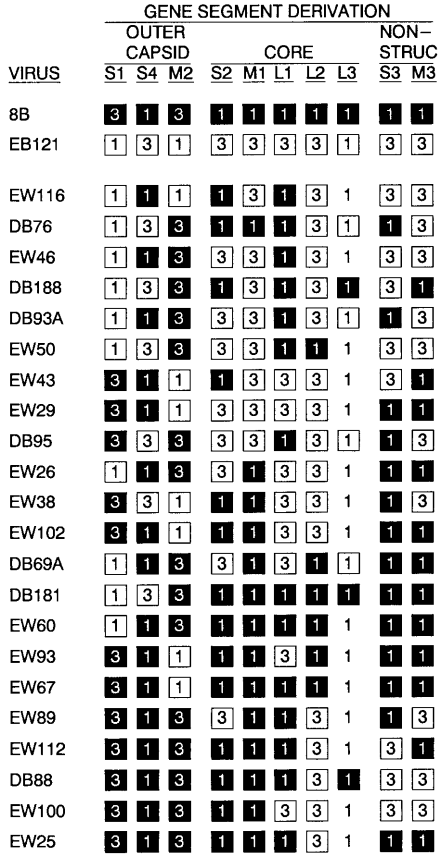

FIG. 1. Panel of 8B-derived reassortant viruses used for genetic analyses. Gene segment derivations are indicated in the following two ways: a black or white box indicates derivation from the myocarditic (8B) or nonmyocarditic (EB121) parent, respectively, while 1 or 3 indicates the T1L or T3D origin of the gene segment.

with 2.5% fetal bovine serum (HyClone, Logan, Utah), 2.5% viable serum protein (Biocell, Rancho Dominguez, Calif.), and 2 mM L-glutamine, in the absence of antibiotics.

All reovirus stocks (triply plaqued, passaged twice in mouse L cells) were characterized previously for their myocarditic phenotypes (31). Virus 8B is a reassortant virus derived from a mouse infected with strains serotype 1 Lang (T1L) and serotype 3 Dearing (T3D) (31). All other reassortant viruses (Fig. 1) were derived from mouse L cells infected with the indicated viruses (31). Viruses EB121 and E3 are reassortant viruses derived from T1L and T3D. All EW-series reassortant viruses were derived from 8B and EB121, while all DB-series reassortant viruses were derived from EW60 and E3.

Primary cardiac myocyte and fibroblast cultures were prepared as described previously (4). Briefly, Cr:NIH(S) term fetuses or 1-day-old neonates (National Cancer Institute) were sacrificed, and the apical two-thirds of their hearts were minced and trypsinized. The cell suspension was plated on 6-well clusters and incubated at 37°C in 5% CO_2 to allow fibroblasts to adhere, at which time the nonadherent cells were removed to fresh 96-well clusters (myocyte cultures, containing 5 to 20% fibroblasts as previously reported [4]). The 6-well clusters of fibroblasts were then trypsinized and replated on 96-well clusters (fibroblast cultures). Both cultures were incubated in Dulbecco modified Eagle medium (Gibco BRL) supplemented with 7% fetal bovine serum (HyClone) (completed DMEM) and with 0.06% thymidine in the myocyte cultures.

Infections and harvests. Two days after preparation, duplicate wells of primary cardiac myocyte cultures were trypsinized, and viable cells were counted (typically 2×10^5 to 4×10^5 per well) and infected at a multiplicity of infection (MOI) of 5 PFU per cell in completed DMEM with 0.06% thymidine. Cultures were incubated at 37°C in 5% CO_2. If indicated, the protease inhibitor *trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane (E64; Sigma catalog no. E-3132), solubilized in dimethyl sulfoxide (DMSO; Sigma catalog no. D-5879) and then diluted for a 10 mM stock in 7% DMSO, was added for a final concentration of 20 μM (and 0.014% DMSO). DMSO alone had no effect on viral replication.

Primary cardiac fibroblast cultures were infected as for cardiac myocytes except that there were typically 6×10^4 to 9×10^4 viable cells per well, and infected cells were incubated in completed DMEM without thymidine.

Plaque assays. Culture wells were frozen at −70°C, subjected to two additional freeze-thaw cycles, and then lysed in 0.5% Nonidet P-40. Virus titers were determined by plating serial dilutions on mouse L-cell monolayers, overlaying with agar, and staining with neutral red as previously described (29).

Immunocytochemistry. After infection and incubation, culture supernatants were aspirated and cells were trypsinized. DMEM completed with 20% fetal bovine serum was added, and half of each cell suspension was centrifuged onto glass slides according to manufacturer's protocol (Cytospin 3 Centrifuge; Shandon Lipshaw, Pittsburgh, Pa.). Slides were fixed in cold 2% paraformaldehyde (in 1× phosphate-buffered saline [PBS], final pH adjusted to 7.2 with HCl). Slides were rinsed in PBS and stored at 4°C in 70% ethanol until use for immunocytochemistry, performed according to manufacturer's protocol (Zymed streptavidin biotin peroxidase system; Zymed Laboratories, South San Francisco, Calif.), using a mixture of hyperimmune rabbit antisera prepared against T3D and T1L reoviruses.

RNA harvests and Northern (RNA) analyses. Medium was aspirated from infected cells, and 100 μl of TriReagent (Molecular Research Center, Cincinnati, Ohio) was added. Clusters were frozen, and RNA was harvested later according to the manufacturer's protocol. RNA from each well was resuspended in 2 μl of deionized, distilled H_2O and denatured by being mixed with 12.5 μl of loading buffer (final concentrations, 3.4 M formaldehyde, 42% deionized formamide, 1× morpholinepropanesulfonic acid [MOPS] buffer [20 mM MOPS, 50 mM sodium acetate, 10 mM EDTA, adjusted to pH 7.0 with NaOH], 0.1 mM EDTA, 4% glycerol, and 0.17 mg of bromophenol blue per ml), incubated at 65°C for 10 min, and immersed in ice. Half of each sample was electrophoresed on duplicate 0.25 M formaldehyde–1× MOPS–1% agarose gels in 0.25 M formaldehyde–1× MOPS buffer. After being washed in deionized, distilled H_2O for 25 min, gels were assembled in 10× SSPE (1.5 M NaCl, 100 mM NaH_2PO_4, 10 mM EDTA) for capillary transfer onto GeneScreen membranes (NEN Dupont) overnight and then subjected to UV cross-linking (Stratalinker; Stratagene, La Jolla, Calif.).

Membranes were prehybridized for 2 h at 42°C in 5× SSPE–50% formamide–1% sodium dodecyl sulfate (SDS)–5× Denhardt's solution (0.1% Ficoll, 0.1% polvinyl pyrrolidone, 0.1% bovine serum albumin)–100 μg of denatured salmon sperm DNA per ml. They were then hybridized overnight at 42°C in the above-described solution with 2.5×10^6 cpm of ^{32}P-labeled riboprobe (the positive strand was used for one membrane, and the negative strand was used for the membrane derived from the duplicate gel). They were then washed twice for 15 min each time at room temperature in 5× SSPE–0.5% SDS, twice for 15 min each time at 37°C in 1× SSPE–0.5% SDS, and then once for 5 min at 65°C in 0.1× SSPE–1% SDS. Membranes were scanned and quantified on a Packard Instant Imager (Packard Instrument Company, Meriden, Conn.), which revealed ^{32}P-labeled bands at the expected mobility for S4 single-stranded RNA (samples from mock-infected cultures always lacked this signal).

^{32}P-labeled riboprobes were prepared by using the Riboprobe II core system (Promega, Fisher Scientific, Pittsburgh, Pa.) according to manufacturer's instructions for T7 polymerase. DNA templates were *Bam*HI-linearized clones of the T1L S4 gene (derived from clones generously provided by Leslie Schiff) inserted in both orientations into the *Eco*RI site of pBlueScript II (Stratagene).

Statistical analyses. The nonparametric Kruskal-Wallis analysis, provided in Systat software (SPSS Federal Systems, Chicago, Ill.), was used. A P value less than or equal to 0.05 was considered significant.

RESULTS

Viral RNA synthesis correlates with induction of myocarditis. We infected duplicate wells of primary cardiac myocyte cultures and harvested total RNA for quantification by Northern analysis. We probed the S4 gene segment as a representative of general reovirus RNA synthesis, since the S4 gene is not a determinant of myocarditis and is transcribed efficiently at early times postinfection in mouse L929 cells. Duplicate membranes were probed with a riboprobe specific for the S4 gene positive or negative strand, and the signals were quantified on a scanning imager. RNA harvested at 4 h postinfection was not detectable (data not shown), indicating that all later signals were generated from newly synthesized RNA rather than input genomic RNA. RNA harvested at 8 h generated an undetectable signal for many viruses (data not shown), and thus RNA harvested at 10 h was used to calculate mean positive-strand and mean negative-strand synthesis (Fig. 2A). Since the viruses could (and did; see below) vary in their efficiencies of infection, and the fraction of cells infected would affect RNA synthesis measurements, we also calculated the ratio of positive- to negative-strand synthesis (Fig. 2B). This provided a measurement

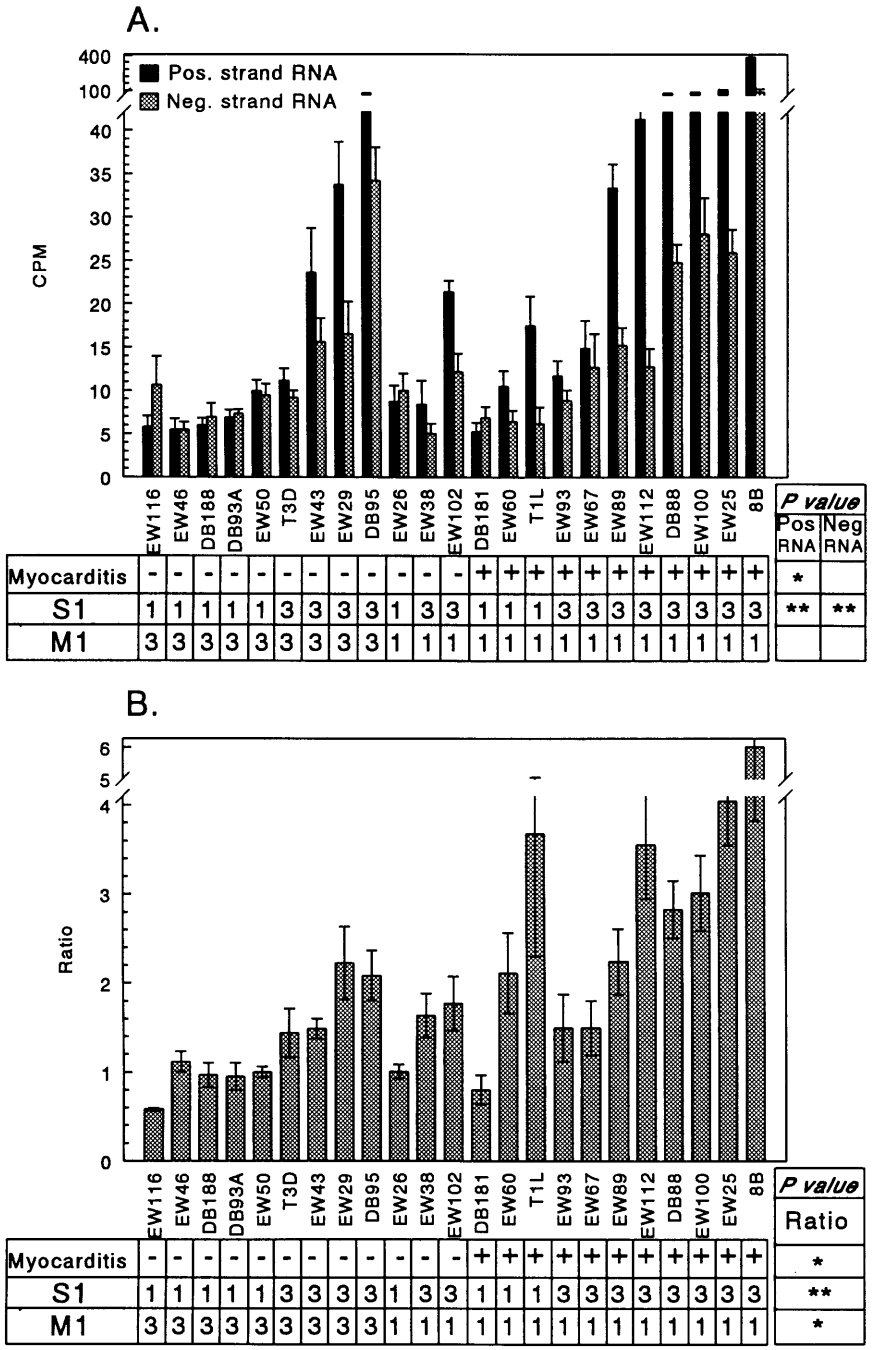

FIG. 2. Positive- and negative-strand RNA synthesis in primary cardiac myocyte cultures. In each of three experiments, duplicate wells were infected at an MOI of 5 PFU per cell with a panel of myocarditic and nonmyocarditic reassortant viruses, and RNA was harvested at 10 h postinfection for Northern analysis. Duplicate nylon membranes were probed with a riboprobe specific for the S4 gene positive strand (solid bars) or negative strand (stippled bars). *, $P < 0.05$; **, $P < 0.005$ (see text). (A) Positive- and negative-strand synthesis (mean ± standard error of the mean). (B) For each well, the positive-strand value was divided by the negative-strand value (mean ± standard error of the mean).

of RNA synthesis independent of infection efficiency. Statistical analyses were used to identify correlations between viral RNA synthesis and induction of myocarditis or reovirus genes. Notably, the induction of myocarditis correlated with both positive-strand synthesis and the ratio of positive- to negative-strand synthesis ($P = 0.036$ and 0.010, respectively). The S1 gene correlated with positive- and negative-strand RNA synthesis ($P < 0.000$ and 0.002, respectively). The S1 and M1 genes correlated with the ratio of positive- to negative-strand RNA synthesis ($P = 0.002$ and 0.023, respectively). We obtained similar results with primary cardiac fibroblast cultures (data not shown).

Early yield of infectious virus from cardiac myocytes does not correlate with induction of myocarditis. We next investigated consequences of this differential viral RNA synthesis that might determine myocarditis. We examined the yield of

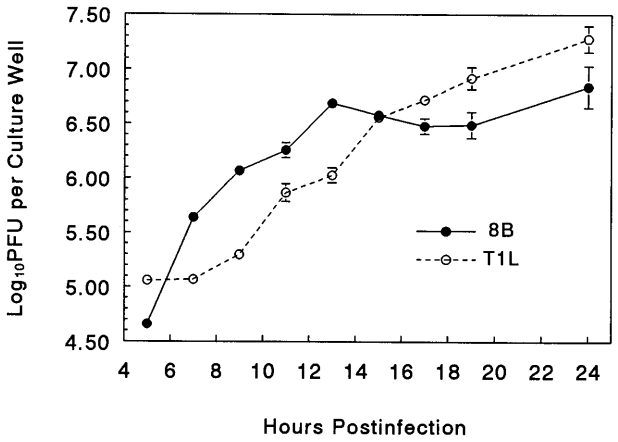

FIG. 3. Viral replication in primary cardiac myocyte cultures. In three separate experiments, primary cardiac myocyte cultures were infected at an MOI of 5 PFU per cell with the efficiently myocarditic virus 8B and the poorly myocarditic virus T1L. Cultures were lysed at indicated times postinfection, and PFU were quantitated by plaque assay (mean ± standard error of the mean). Each datum point is the mean of replicate wells from at least two experiments, except the 5- and 13-h datum points, which are the means of quadruplicate wells from single experiments.

infectious virus from primary cardiac myocyte cultures infected with 8B (efficiently myocarditic) and T1L (poorly myocarditic) (Fig. 3). At 9 h postinfection, 8B had achieved a titer 5- to 10-fold higher than that of T1L, and 8B subsequently reached its peak titer 4 to 6 h earlier than T1L. However, when virus replication had plateaued for both viruses, T1L had achieved a slightly higher peak titer than 8B. We obtained similar results in primary cardiac fibroblast cultures (data not shown).

To determine whether viral yield at 9 h postinfection correlated with induction of myocarditis, we infected primary cardiac myocyte cultures with a panel of 8B-derived myocarditic and nonmyocarditic reassortant viruses and quantified PFU at 9 h (Fig. 4). While the range of PFU did span greater than 2 log units, the titer correlated with the S1 gene ($P = 0.007$; all other genes, $P > 0.05$) rather than induction of myocarditis. We obtained similar results with primary cardiac fibroblast cultures (data not shown; correlation with S1 gene, $P = 0.003$).

Efficiency of infection does not correlate with induction of myocarditis. The S1 gene encodes the viral attachment protein σ1 (as well as a nonstructural protein, σ1s, of unknown function [reviewed in reference 24]), suggesting that the early viral yield could reflect efficiency of infection. To identify determinants of the efficiency with which reoviruses infect cardiac myocytes, we used immunocytochemistry to identify infected cells. However, at 1 day postinfection, only a fraction of the cells were infected (irrespective of virus, despite the MOI of 5 PFU per cell, and even when repeated at 50 PFU per cell [data not shown]). Given this inability to infect all cells during primary infections, and the different time courses of replication for T1L and 8B (Fig. 3), we needed to identify a technique for preventing secondary infections without affecting primary infections. The protease inhibitor E64 prevents productive reovirus infection, most likely by preventing the proteolytic cleavage in the acidified endosome that activates the reovirus transcriptase (8a). We determined that when E64 was added to primary cardiac myocyte cultures 8 h postinfection, it inhibited secondary infections without affecting primary infections (data not shown).

Thus, to identify determinants of the efficiency with which reoviruses infect cardiac myocytes during primary infections, we infected cultures with a panel of viruses, added E64 8 h postinfection, and harvested cultures for immunocytochemistry at 30 h postinfection (Fig. 5). While the efficiency of infection varied dramatically between viruses, it correlated with the S1 gene ($P = 0.004$; all other genes, $P > 0.05$) rather than myocarditis ($P > 0.05$).

Virus yield per infected cardiac myocyte varies little between viruses and does not correlate with induction of myocarditis. T1L generated more infectious virus (Fig. 3) but infected fewer

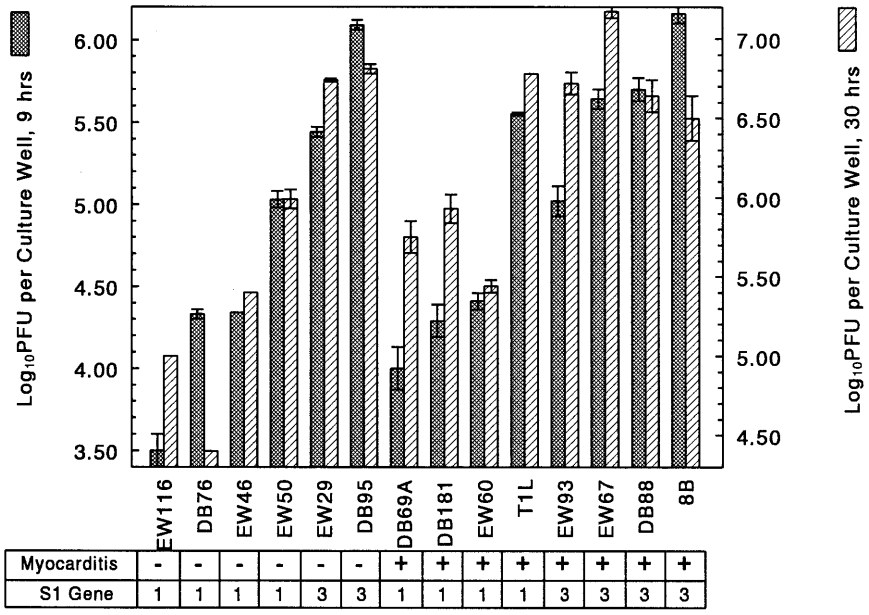

FIG. 4. PFU yield at 9 and 30 h postinfection of primary cardiac myocyte cultures. Duplicate wells of primary cardiac myocyte cultures were infected at an MOI of 5 PFU per cell with a panel of myocarditic and nonmyocarditic reassortant viruses. Cultures were lysed at 9 h postinfection, or the protease inhibitor E64 was added at 8 h and cultures were lysed at 30 h postinfection. PFU were quantitated by plaque assay (mean ± standard error of the mean).

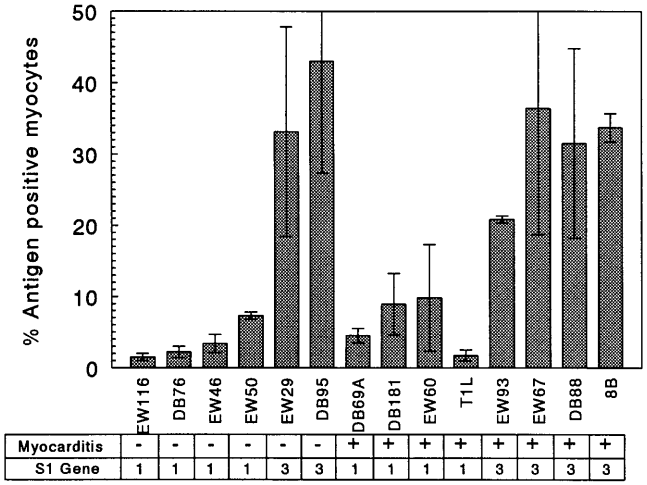

FIG. 5. Efficiency of infection of primary cardiac myocyte cultures. Duplicate wells of primary cardiac myocyte cultures were infected at an MOI of 5 PFU per cell with a panel of myocarditic and nonmyocarditic reassortant viruses. The protease inhibitor E64 was added at 8 h, and cultures were harvested at 30 h postinfection for immunocytochemistry.

cells (Fig. 5) than 8B during primary infections, suggesting that high yield per infected cell might correlate with poor myocarditic potential. Therefore, we infected cardiac myocyte cultures with a panel of viruses, added the protease inhibitor E64 at 8 h postinfection to inhibit secondary infections, and harvested duplicate cultures for immunocytochemistry and quantification of PFU. The yield per infected culture well varied considerably (Fig. 4) and correlated with the S1 gene ($P = 0.004$) just as the 9-h yield did, most likely reflecting the S1 gene function in determining infection efficiency (Fig. 5). The results were combined to determine the PFU per infected cell for each virus (Fig. 6). With the exception of T1L, most of the viruses (including 8B) generated similar yields of infectious virus. Thus, yield did not correlate with induction of myocarditis or any

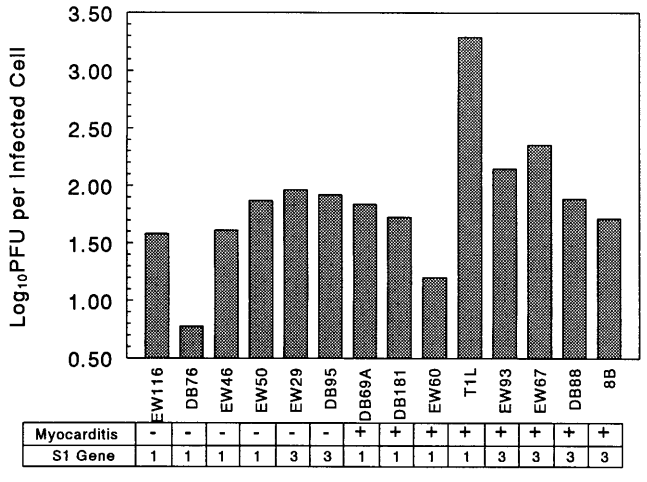

FIG. 6. PFU yield per infected cell at 30 h postinfection of primary cardiac myocyte cultures. Two sets of duplicate wells of cardiac myocyte cultures were infected at an MOI of 5 PFU per cell with a panel of myocarditic and nonmyocarditic reassortant reoviruses. The protease inhibitor E64 was added at 8 h, and the duplicate cultures were harvested at 30 h for immunocytochemistry or quantification of PFU by plaque assay. The viral yield (PFU) was divided by the number of infected (antigen-positive) cells to calculate PFU per infected cell.

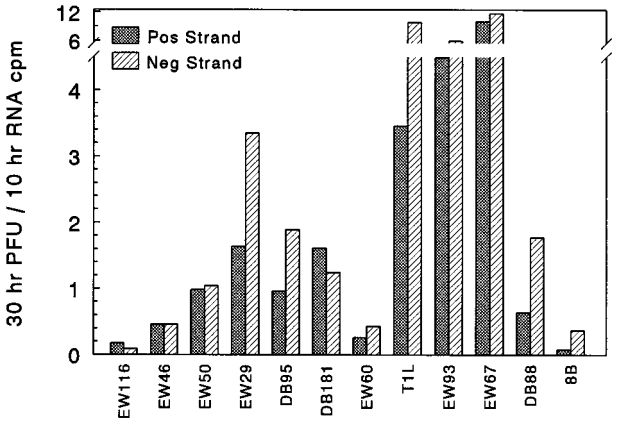

FIG. 7. Relationship of RNA synthesis to PFU yield. For each of the viruses indicated, the 30-h PFU yield from primary infections (Fig. 4) was divided by the hybridization signals for positive- and negative-strand RNA synthesis at 10 h postinfection (Fig. 2A). Ratios were then divided by 100,000 for simplification.

reovirus gene ($P > 0.05$), suggesting that some other consequence of viral RNA synthesis determines myocarditis.

Viral RNA synthesis does not determine yield of infectious virus. Viral RNA synthesis at 10 h postinfection as well as viral yields at 9 and 30 h postinfection all correlated with the S1 gene. Yet viral RNA synthesis correlated with induction of myocarditis, whereas viral yield did not. To evaluate the role of viral RNA synthesis in viral yield, viral yields at 30 h postinfection were divided by hybridization signals for positive- and negative-strand RNA synthesis (Fig. 7). The ratios varied across a 2-log-unit range, with similar results for ratios calculated from 9-h viral yields (data not shown), suggesting that RNA synthesis is not a major determinant of viral yield from cardiac myocytes.

DISCUSSION

To determine the role of viral RNA synthesis in reovirus-induced acute myocarditis in mice, we infected primary cardiac myocyte cultures with a panel of myocarditic and nonmyocarditic reassortant viruses and quantitated positive-strand and negative-strand RNA synthesis. In addition, we calculated the ratio of positive- to negative-strand synthesis as a measure of RNA synthesis independent of efficiency of infection. Induction of myocarditis correlated with both positive-strand synthesis and the ratio of positive- to negative-strand synthesis.

Reovirus RNA synthesis is fully conservative; the double-stranded RNA genome directs synthesis of the positive strand, and the nascent positive strand directs synthesis of the negative strand to regenerate double-stranded RNA, which in turn serves as template for late positive-strand synthesis as well as progeny genome for packaging (reviewed in reference 24). Interestingly, there was much greater variation between viruses for positive-strand synthesis than for negative-strand synthesis (Fig. 2A), resulting in significant variation in the ratio of positive- to negative-strand RNA synthesis (Fig. 2B). Since early positive-strand synthesis serves as template for negative-strand synthesis, one explanation for this variation is varying late positive-strand synthesis. Since positive-strand synthesis and the ratio of positive- to negative-strand synthesis both correlated with induction of myocarditis, but negative-strand synthesis did not, myocarditis may be correlated with efficiency of progression to late-strand RNA synthesis.

The S1 gene correlated with positive- and negative-strand

RNA synthesis, and the S1 and M1 genes correlated with the ratio of positive- to negative-strand RNA synthesis. The S1 gene correlation most likely reflects its function determining efficiency of infection (see below). The M1 gene correlation is consistent with its correlation with positive-strand RNA synthesis from viral cores in vitro (40), but its specific role remains to be defined.

The efficiency of infection and the total yield of infectious virus from primary infections did not correlate with induction of myocarditis. Instead, they correlated with the S1 gene, most likely reflecting its function encoding the viral attachment protein σ1 (reviewed in reference 24). In earlier investigations (22), we had examined viral yield from cardiac myocyte cultures infected with T1L/T3D reassortant viruses at 4 days postinfection and had found that yield (representing both primary and secondary infections) did not correlate with the S1 gene but instead correlated with the M1 gene ($P < 0.000$), the L1 gene (encoding the viral polymerase λ3; $P < 0.000$), and the L3 gene (encoding a viral core protein, λ1, of unknown function; $P = 0.001$). Thus, while the S1 gene dominates productive infection during primary infections, it becomes less significant when virus is given time to spread through the culture, and then the role of viral core proteins as determinants of yield becomes apparent. Using the myocarditic potentials of the T1L/T3D reassortant viruses (28), we have determined that yield from primary and secondary infections correlates with induction of myocarditis (Kruskal-Wallis analysis, $P = 0.013$). Thus, viral yield from primary infections does not correlate with myocarditis but viral yield from primary and secondary infections does, suggesting that efficiency of virus spread is a key determinant.

RNA synthesis at 10 h postinfection was not a strong determinant of viral yield (Fig. 7), suggesting that other rate-limiting parameters of viral replication not relevant to myocarditis determine assembly of infectious virus. Indeed, viral yields per infected cell were similar for most myocarditic and nonmyocarditic reoviruses (Fig. 6) and did not correlate with myocarditis. Interestingly, this is in contrast to results from similar experiments conducted with cardiovirulent and acardiovirulent coxsackievirus strains in cardiac myocyte cultures (36). While a cardiovirulent strain did synthesize more RNA, there was a concomitant increase in yield of infectious virus. Thus, while RNA synthesis is an important determinant of myocarditic potential for both reoviruses and coxsackieviruses, the underlying mechanisms are likely to be different for the two virus types.

Viral RNA synthesis (Fig. 2) and CPE in cardiac myocytes (4) are both determinants of myocarditis, suggesting that viral RNA synthesis may induce CPE in cardiac myocytes, resulting in acute myocarditis. Indeed, the Kruskal-Wallis analysis (treating CPE as all or none, with groups defined as greater than or less than 80% viable cells) identifies positive-strand synthesis, negative-strand synthesis, and the ratio between the two as determinants of CPE ($P = 0.036$, 0.036, and 0.046, respectively, using data from Fig. 2 and reference 4). However, while the S1 gene is a strong determinant of RNA synthesis, it is not a determinant of CPE ($P > 0.1$), nor does it determine myocarditis in 8B-derived viruses. Importantly, we measured RNA synthesis in primary infections but measured CPE induced by both primary and secondary infections. Therefore, as proposed above, the S1 gene may be a critical determinant of primary infections but becomes less significant relative to other determinants of secondary infections, which in turn determine myocarditis. The correlation between RNA synthesis in primary infections and CPE in primary and secondary infections is consistent with a mechanism whereby RNA synthesis in primary infections determines the efficiency of spread to secondary infections. This does not eliminate the possibility that RNA synthesis in primary infections directly determines CPE in those cells as well.

Together, the data suggest that reovirus RNA synthesis in primary infections of cardiac myocytes is a determinant of reovirus myocarditic potential, primarily by affecting virus spread from cell to cell and concomitant CPE, irrespective of viral yield from the primary infections. One obvious mechanism would be induction of interferon (IFN) during primary infections, which would subsequently affect efficiency of secondary infections. Since the results were generated in primary cardiac myocyte cultures, the IFN would most likely be IFN-β. In general, viruses that were more myocarditic synthesized greater quantities of positive-strand RNA, and since viral RNA is a potent inducer of IFN in infected cells (reviewed in reference 37), one might have predicted the opposite. Yet greater (or faster) RNA synthesis would most likely result in a concomitant increase in translation of reovirus proteins, including the σ3 double-stranded RNA-binding protein, which inhibits double-stranded RNA-activated cell proteins (5, 14, 18). Thus, while myocarditic viruses synthesize more positive-strand RNA, they may also induce less IFN or may decrease the double-stranded RNA activation of IFN-induced proteins that directly mediate the antiviral state (such as the protein kinase PKR or 2′5′-oligoadenylate synthetase [reviewed in reference 37]). While reoviruses have been demonstrated to induce IFN in various cell types (reviewed in reference 24), the role of IFN in reovirus disease has not been established and will be addressed in future studies.

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