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Letter to the Editor

CYTOMEGALOVIRUS INFECTION OF HUMAN ARTERIAL SMOOTH MUSCLE CELL CULTURES CAN DELAY TERMINAL PASSAGE

Dear Editor:

Replication of cytomegalovirus (CMV) (strain AD-169) in human, arterial smooth muscle cell (SMC) cultures causes persistent infection (1) characterized by temporary, limited reconstitution of cultures. Ultimately, restorative capacity is depleted, and cultures, consisting of surviving non-dividing, terminal cells, slowly deteriorate as a result of "senescent" degeneration and sporadic CMV cytopathic effects (2). Neither transformed nor continuous persistently infected, human SMC cultures have been established after infection with CMV.

These experiments were done to determine the influence of infection with different strains of CMV at different multiplicities of infection (MOI) on the repeated subcultivation of SMC cultures to termination, i.e., to the first distinct decline in growth and beyond. The Towne strain (3) was obtained from the American Type Culture Collection (Rockville, MD). The Mj strain (4) was obtained from Dr. F. Rapp (Pennsylvania State U., Hershey, PA). Mycoplasma was isolated on two separate occasions from fibroblast (IMR-90) (5) (American Type Culture Collection) cultures infected with the original CMV (Mj) suspension and maintained in medium with antibiotics (100 U/ml penicillin G and 100 mcg/ml streptomycin). This combination and concentration of antibiotics was the same as used by Rapp et al. (4). The mycoplasma was identified by Dr. G. McGarrity (Coriell Inst. for Med. Res., Camden, NJ) as M. orale, which increases polyploidy, and chromosome breakage and rearrangements in diploid fibroblast cultures (6). The mycoplasma used here as a positive control in standard agar tests was identified as M. arginini. No mycoplasma was isolated from any uninfected fibroblast or SMC cultures during numerous attempts before and after isolation of M. orale. One ml of spent medium containing CMV (Mj) and M. orale was added to 6.8 ml of sterile distilled water containing kanamycin (1470 mcg/ml) and tylocine (220 mcg/ml) and incubated 16 h at 37° C. Then, streptomycin (550 mcg/ml) and erythromycin (280 mcg/ml) in 18 ml of sterile distilled water were added to the above suspension, which was incubated additionally 24 h at 37° C. After the above treatment, mycoplasma could no longer be isolated from fibroblast cultures infected with CMV (Mj) and maintained in medium without antibiotics. Suspensions of CMV (Mj) rid of mycoplasma were used for all experiments and repeatedly tested to confirm the absence of mycoplasma.

Human SMCs were isolated from the arteries of umbilical cords as described previously (2). The identity of cells derived from umbilical arteries was established by the indirect immunofluorescence technique with a monoclonal, anti-SMC antibody (7) (Enzo Biochem., Inc., New York, NY) and by topographic patterns of growth. SMCs were seeded at low densities in 25-cm^2 plastic flasks (Corning Science Products, Corning, NY) and infected with 0.01 to 0.03 or 1.0 to 3.0 50% tissue culture infective doses ($TCID_{50}$) per cell of either of the two strains of CMV. Thereafter, at about 2 to 3-wk intervals, cells were removed with trypsin-versene, pooled from four flasks generally, suspended in Hanks' balanced salt solution without phenol red, and counted in the presence of trypan blue with a hemacytometer to determine the number of viable cells. The number of viable cells per flask was regarded as the total pooled number divided by the number of flasks. Growth medium, Dulbecco's modified Eagle's medium containing 20% heated (56° C, 30 min) bovine, neo-natal, colostrum-free calf serum (Sigma Chemical Co., St. Louis, MO) and antibiotics (100 U/ml penicillin G and 100 mcg/ml streptomycin), was replaced at 5 to 7-d intervals.

Figs. 1 and 2 show progression of SMCs derived from donor #12 to terminal passage. Fig. 1 records a delay of three passages in reaching the terminal passage by SMCs infected with a MOI of 0.01 of CMV (Mj) compared with uninfected SMCs. The same comparison in Fig. 2 shows uninfected and infected cells arriving at termination similarly, but arrival of SMCs infected with the low MOI of CMV (Mj) appeared more irregular given the rise in cell number in Passage 10. Fig. 3 also shows a delay in reaching the terminal passage by SMCs derived from donor #40 and infected with a MOI of 0.03 of the two CMV strains. Retrospectively, culture termination was initiated in Passage 5 in uninfected cultures and in Passage 6 in cultures infected with the low MOI of CMV (Mj). SMCs infected with a MOI of 0.03 of CMV (Towne) also continued to proliferate in Passage 5 before terminating abruptly in Passage 6. CMV (Mj) reportedly transforms human embryonic lung cells (8), but transformation was not apparent in SMC cultures infected with CMV (Mj) at a MOI of 0.01 and surviving 155 d (Fig. 1) and 225 d (Fig. 2).

Based on a study of the atherosclerotic lesions of four women heterozygous for glucose-6-phosphate dehydrogenase, Benditt and Benditt (9) suggested transformation of SMCs by viruses or chemical mutagens to explain the monoclonal nature of atherosclerotic plaques. In a more detailed and extensive similar study, Thomas et al. (10) rejected monoclonalism as an exclusive origin of monotypism in plaques, and presented observations supporting non-mutational causes of monotypism. Eskin et al. (11) concluded that their comparison of the proliferative potential of SMCs derived from atherosclerotic plaques and from uninvolved sites did not support the monoclonal or transformation hypothesis.

The phenomenon described here may occur in vivo. Conceivably, a CMV-induced delay in the normal progression of dividing SMCs to a non-dividing, terminally differentiated state in vivo during vessel wall repair may cause a gradually occluding accumulation of SMCs during a lifetime of cycles of periodic repair at focal sites of recrudescence of latent (12) infection. Delay in possibly regular or chaotic termination could be influenced in part by the strain of CMV, by MOI presumably controlled by immune status and local available concentration of neutralizing antibody, and by cytokine interactions.

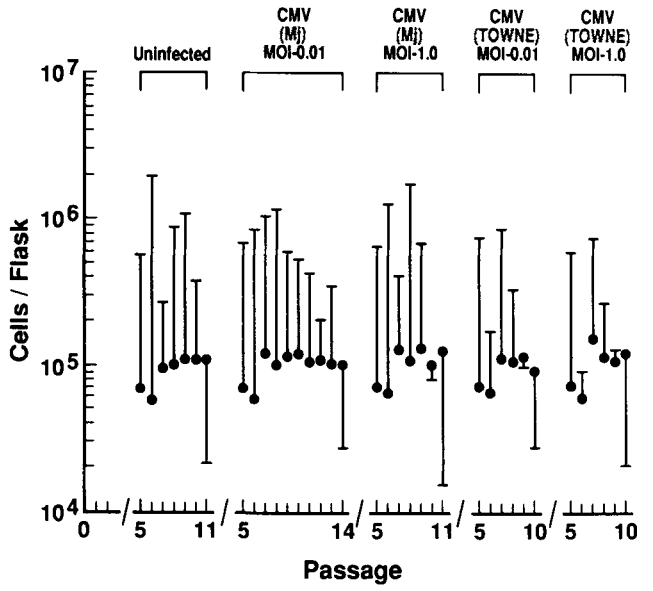

FIG. 1. Increase or decrease in cell number during successive passages of uninfected and infected SMCs from donor #12. *Dot* indicates the initial density in each passage.

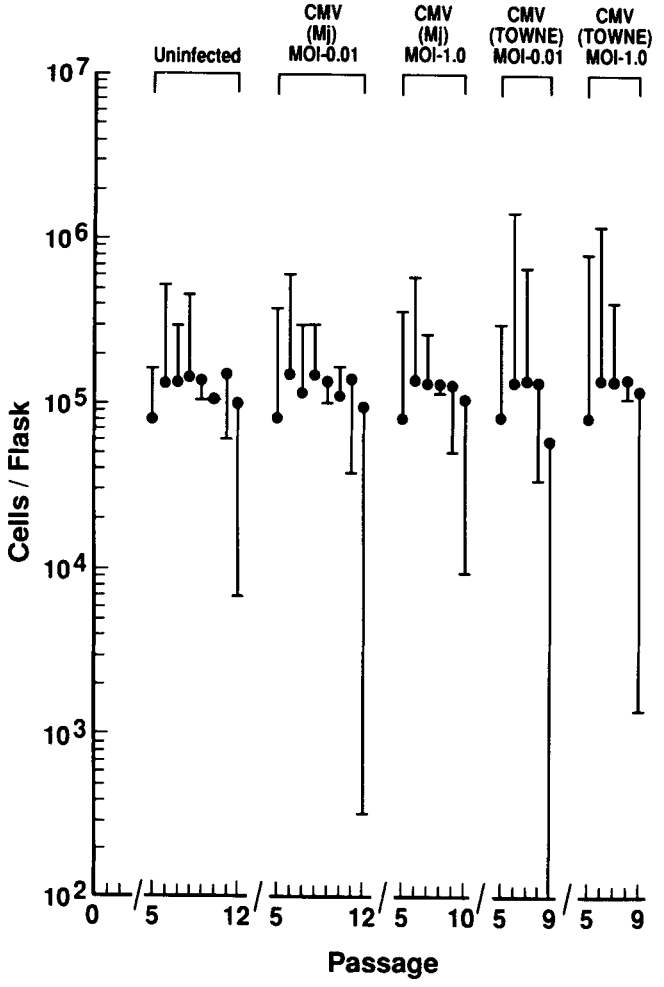

FIG. 2. Increase or decrease in cell number during successive passages of uninfected and infected SMCs from donor #12. Passage 5 was a mixture of SMCs predominantly in Passage 5 with a small proportion in Passage 6.

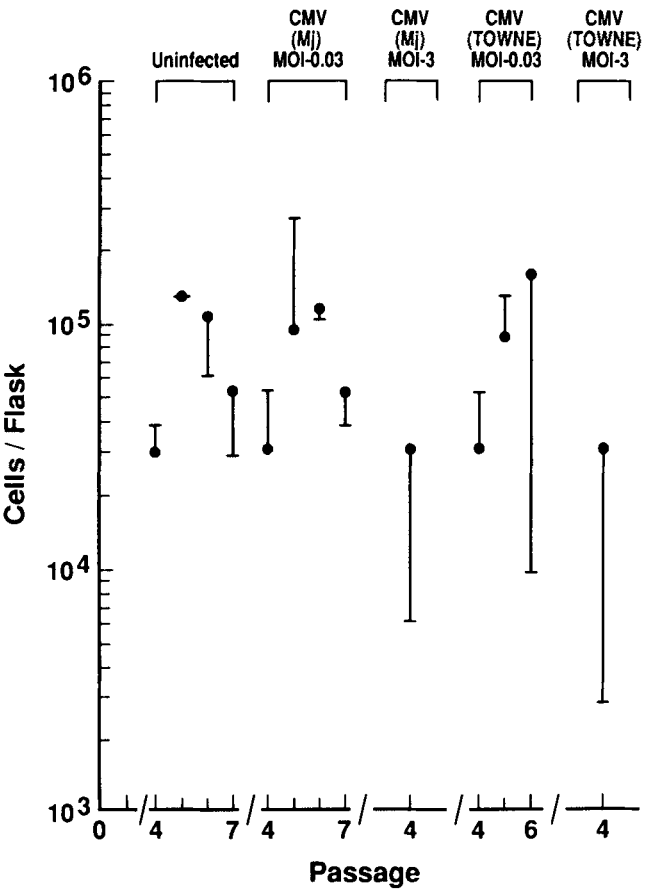

FIG. 3. Increase or decrease in cell number during successive passages of uninfected and infected SMCs from donor #40.

However, delay in termination may not be characteristic of repair after all insults disruptive of vessel wall architecture.

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