VIROLOGY 213, 655–659 (1995)

Replication of Molluscum Contagiosum Virus

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Received July 21, 1995; accepted September 6, 1995

Molluscum contagiosum virus (MCV) infects preadolescent children and sexually active adults, frequently causing a disfiguring cutaneous disease in immunosuppressed HIV-infected individuals. The development of an efficacious treatment regime has been hampered by the failure to replicate the virus in the laboratory. Here we report the first demonstration of MCV replication in an experimental system. In human foreskin grafts to athymic mice, MCV induced morphological changes which were indistinguishable from patient biopsies and included the development and migration of molluscum bodies containing mature virions to the epidermal surface.

Molluscum contagiosum virus (MCV) has the narrowest host-range of vertebrate poxviruses (*1, 2*). It is a natural pathogen of humans and replicates only in the keratinocyte of the epidermis. The infection is observed in children and adults, with spread within this latter group governed in part by sexual practices. Furthermore, MCV is a common opportunistic infection of immunosuppressed human immunodeficiency virus-infected patients (*3–6*). In the immunocompetent individual the disease is long lasting, with the lesions persisting for months or years, but ultimately regressing either spontaneously or in response to trauma, surgical or otherwise (*6, 7*). Disease treatment has been hampered by failure to replicate the virus experimentally in tissue culture or animal models, which prevents the identification of a virus process(es) suitable for the rational targeting of antiviral therapies.

We first attempted to propagate MCV in a differentiated epidermis 5–10 cells thick produced from human foreskin keratinocytes grown on a dermal equivalent at a liquid-air interface ("raft culture"; *8*). Although we produced and maintained for at least 6 weeks a multilayered epithelium that by histological criteria was indistinguishable from authentic human foreskin, no evidence (dot blot hybridization assay) could be obtained for MCV DNA replication at 37° at various m.o.i. from 0.1 to 50 particles per cell and between 2 and 9 days p.i. (data not shown). This result suggested that MCV had an extremely long replication cycle, was terribly fastidious, or the particles, which were harvested from human lesions, had low infectivity and/or lost infectivity on storage.

Where examined, poxvirus virions have been shown to be quite resistant to environmental insults (*9–11*), and the epidemiology of MCV suggests that the MCV virion is also stable on fomites (*1, 2*), although the virion-associated transcriptional machinery was less active than that from vaccinia virus virions (*12*). This suggested to us that it was unlikely that the virions we were using for infection of the "raft" cultures lacked infectivity. A more likely explanation would be that MCV replication requires a condition(s) not provided by "raft" cultures maintained in our hands or in other tissue culture systems (for additional references see *13*). This line of reasoning is reinforced by the recent observation that vaccinia virus intermediate gene transcription has a conditional requirement for a host factor (*14*). If MCV does require a distinct physiological state(s) of the keratinocyte to initiate (and perhaps maintain) the replication cycle, then it could take considerably longer for MCV to replicate than the 12–96 hr observed for most poxviruses. Histopathological observations and *in situ* [^{3}H]thymidine labeling of lesions suggests that MCV replicates during the 9 to 15 days it takes the infected keratinocyte to reach the *stratum granulosum,* with virus specific changes detectable initially in the lower layers of the *stratum spinosum,* becoming progressively more prominent with time and culminating with the formation of a molluscum body (*15–18*). These studies, however, were unable to determine the length of the eclipse period.

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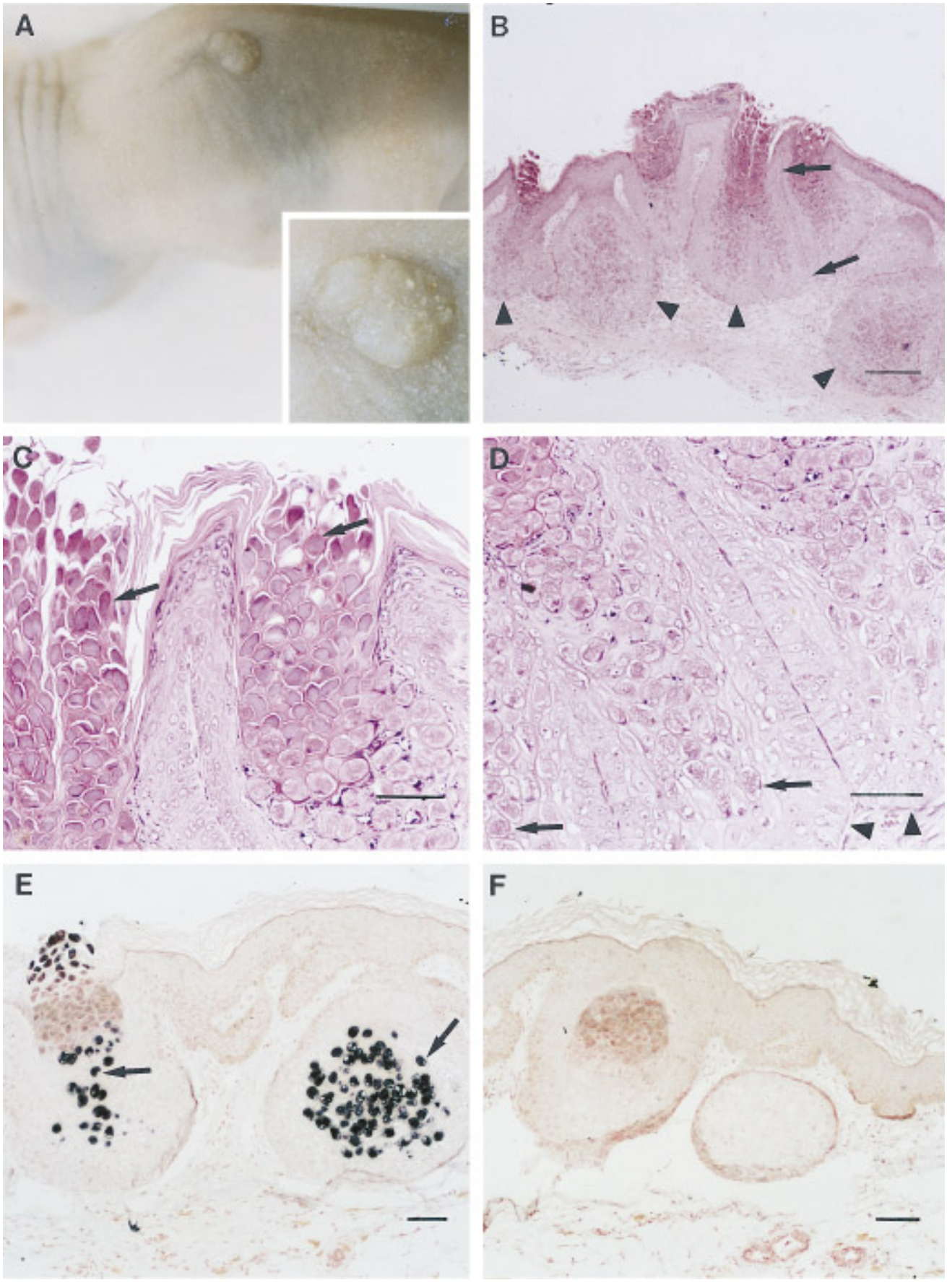
0042-6822/95 $12.00

We then chose to examine a second experimental system used by others to propagate another fastidious virus, human papillomavirus (*19, 20*). Kreider and colleagues had shown previously that athymic nude mice grafted with skin from the rabbit, cow, or human would support replication of Shope papillomavirus, bovine papillomavirus, and human papillomavirus, respectively. Accordingly, human foreskin tissue was infected with MCV and grafted under the renal capsule of seven athymic mice using conditions that resulted in 100% transformation with human papillomavirus type 11 by 90 days p.i. Mice carrying the MCV-treated grafts were sacrificed at 105, 130, 153 and 174 days p.i. No evidence of MCV replication was obtained in any of the grafts (R. M. L. Buller, J. Burnett, and J. Kreider, unpublished results). To more closely mimic the natural conditions of MCV replication, MCV-treated human foreskins were grafted orthotopically on the dorsolateral thorax of athymic mice. In the first experiment, 12 foreskin grafts were infected with MCV. Seven grafts failed to take and the remaining grafts began rejection at approximately 50 days p.i. Interestingly, 1 graft showed what appeared to be an early MCV lesion of 5–6 cells in the basal area of the epidermis. This suggested that if the grafts could be maintained longer we might be able to observe more vigorous virus replication. The extremely long eclipse period suggested by this experiment was unanticipated as experimental infections of volunteers first by Juliusberg (*21*) and then by Wiles and Kingery (*22*) found gross lesion development 50 and 14 to 25 days p.i., respectively. In efforts to reduce the rate of graft failure, healthy grafts were first established and then infected with MCV. Using these conditions, 24 grafts were inoculated and 16 were rejected over the course of the experiments due to graft failure. Of the remaining 8 grafts, 3 developed varied gross morphological changes that resembled authentic MCV lesions by 74 (mouse 7), 108 (mouse 10), and 142 (mouse 23) days p.i. Figure 1A is a photograph of the graft on mouse 7 on the day of sacrifice, 123 days p.i., with the inset clearly showing the lesion projecting above the epidermis. Light level microscopy of hematoxylin-eosin-stained paraffin-thick sections from formalin-fixed tissues from mice 7, 10, and 23 revealed classic MCV histopathology (*15–18*). As shown for mouse 7 in Figs. 1B, 1C, and 1D hypertrophied and hyperplastic epidermis extended down into the dermis without breaching the basement membrane and projected up and above the surface of the skin. By analogy to vaccinia virus, the hyperplasia associated with the MCV lesions may be mediated in part by an epidermal growth factor-like activity encoded by the virus (*23, 24*). The basal cells and their nuclei were enlarged. Beginning in the lower cells of the *stratum spinosum,* intracytoplasmic inclusion bodies were detected (Fig. 1D, arrow). As the infected cells migrate through the *stratum granulosum,* the inclusion bodies became larger, ultimately occupying a major portion of the cell and relegating the nucleus to the periphery. It is this cellular structure that is referred to as a molluscum body and is pathogenomic for molluscum contagiosum. The remaining 5 mice were sacrificed at 142 days p.i., and tissue was examined by light-level microscopy. One exhibited MCV pathology, while the remaining 4 specimens were negative. Thus out of a total of 8 long term grafts, 4 or 50% showed evidence of MCV pathology.

Although the pathological changes observed by light level histology of hematoxylin-eosin-stained sections was diagnostic of MCV replication, we sought to confirm this finding through *in situ* hybridization with biotinylated MCV genomic DNA and rule out the involvement of other poxviruses as unlikely as this might seem. As illustrated in Fig. 1, the regions of the graft which showed histologic changes typical of MCV replication also reacted specifically with the MCV (Fig. 1E) but not to an EV (Fig. 1F) DNA probe. Electron microscopy further confirmed that

FIG. 1. Molluscum contagiosum virus-induced pathological changes in human foreskin grafts. Foreskins were surgically removed from healthy newborn infants and stored at 4°C in minimum essential medium. Within 24 hr of surgery, split-thickness grafts (approximately 1 cm^2) were placed orthotopically onto the dorsolateral panniculus carnosus of athymic nude mice and secured with Vaseline gauze, and an occlusive dressing of gauze pads and adhesive tape. After 1 week the dressings were removed and after a further 3 weeks the healthy grafts were lightly scarified with a 25-gauge needle and inoculated with 30 μl of a 1:6 dilution of a MCV stock solution which contained 2×10^8 MCV particles as estimated by electron microscopy using a known concentration of latex spheres. The stock virus suspension was prepared within 24 hr of the surgical removal of several MCV lesions (which were kept on ice) from a single patient. The virions were released from the lesion material by disruption in 0.5 ml of 0.15 *M* NaCl using a hand-held, conical glass tissue grinder. The mice with infected grafts were housed in an isolator bubble until sacrifice for microscopic examination of gross pathological changes or due to graft rejection. The major cause of graft rejection appeared to be the infiltration of the graft by mouse leukocytes which correlated with graft dwindling and disappearance. (A) At 123 days p.i., mouse 7 was sacrificed and the graft was photographed. The gross pathological changes of this graft were the most extensive of all of the grafts studied. (B) Tissue from the MCV-treated foreskin graft on mouse 7 was fixed in Tellyesniczky's acetic acid–ethanol–formalin solution, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin–eosin before being examined with the light microscope. Arrowheads denote the junction between the dermis and epidermis. Note the hyperplasia associated with the lesion which causes severe invagination of the epidermis but without loss of integrity of the basal cell layer (arrows; line represents 100 μm). (C) A higher magnification of the area in B containing the upper arrow. Note the molluscum bodies devoid of any cellular architecture exiting the surface of the epidermis (arrows; line represents 25 μm) (D) A higher magnification of the area in B containing the lower arrow. The epidermis/dermis boundary is indicated by arrowheads. MCV-associated changes are noted in the cells just above the basal layer (arrows; line represents 25 μm). (E/F) Graft tissue from mouse 10 was embedded, sectioned at 4 μm, deparaffinized, and hybridized at room temperature for 2 hr with a biotinylated MCV (E) or ectromelia virus (F) DNA probe. DNA–DNA hybrids were detected with an avidin–alkaline phosphatase developing system (arrows), and counterstained with nuclear Fast Red (line represents 25 μm).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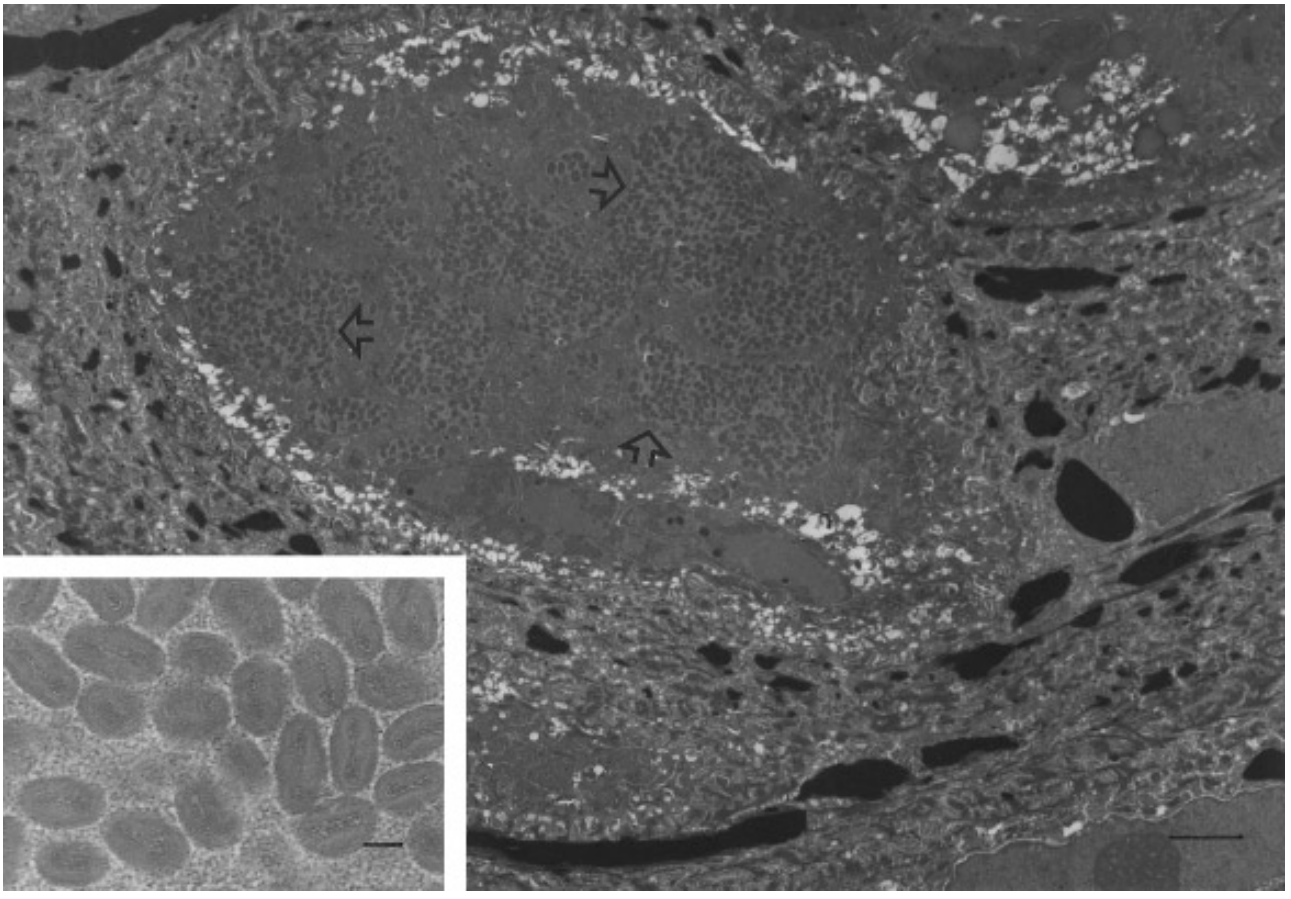

FIG. 2. The presence of MCV virions in a MCV-inoculated human foreskin graft. Graft tissue from mouse 10 was fixed for 3 hr in 2.5% glutaraldehyde in Millonig's sodium phosphate buffer (pH 7.9) and prepared for electron microscopy by standard protocols first using toluidine blue-stained 4-μm sections to locate grossly the areas of MCV-induced pathology. Arrowheads denote pockets of mature virions (line represents 2 μm). The inset shows the characteristic structure of the intracellular poxvirus virion (line represents 100 nm).

specimens showing MCV-like histopathology and reactivity with MCV-DNA probes, also contained the characteristic mature, intracellular brick-shaped virions associated with other poxvirus infections (Fig. 2; arrow). Of interest is the failure to observe large numbers of extracellular poxvirus virions (*25*) either in these experimental infections or biopsy material (R. M. L. Buller, data not shown). A failure of MCV to produce efficiently extracellular virions, in combination with a restricted tissue host-range (i.e., perhaps MCV lacks functional homologues of orthopoxvirus host-range genes such as CHOhr (*26, 27*), C7L (*28*), and/or K1L (*29, 30*)), may explain the restriction of MCV replication to the human epidermis.

To our knowledge this is the first demonstration of the experimental propagation of MCV. It is not clear why the incubation period of MCV in this system differs so markedly from that obtained by limited experimental infections of volunteers; however, this model now provides a means to verify the infectious nature of MCV virions from biopsy material, a necessary first step to the development of an *in vitro* tissue culture system. Also, this animal model can currently be used to test the efficacy of antiviral treatments which are needed for chronic MCV infections of AIDS patients. For example, we have previously shown that nitric oxide is a potent antiviral for the replication of poxviruses vaccinia and ectromelia (*31*), and we now plan to test the efficacy of nitric oxide donor compounds for anti-MCV activity using this animal model.

ACKNOWLEDGMENTS

We are grateful to Ms. Sue Sulkey for editorial assistance; and Ms. Helina Rubinstein, Sue Patrick, Pat Welsh, and Lynn Budgeon for technical assistance. This work was supported in part by Dr. Kreider's Grants NIAID NO1-AI35138 and 2RO1CA47622 and The Jake Gittlen Memorial Golf Tournament.

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