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The Minute Virus of Mice NS2 proteins are not essential for productive infection of embryonic murine cells in utero

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ABSTRACT

The P4 promoter of the autonomous parvovirus Minute Virus of Mice (MVM) drives the production of its non-structural proteins, NS1 and NS2. The NS2 isoforms are without enzymatic activity but interact with cellular proteins. While NS2 is crucial to the viral life cycle in cultured murine cells, NS2-null mutant virus productively infects transformed host cells of other species. In the mouse, sensitivity to MVM infection is age dependent, exhibiting limited subclinical infections in adults, but sustained and potentially lethal infection in embryos. We therefore questioned whether the species-dependent requirement for NS2 function in vitro would be retained in utero. We report here that it is not. NS2-null mutant MVMp is capable of mounting a productive, albeit much reduced, infection of normal embryonic mouse cells in vivo. Based on the data, we hypothesize that NS2 may bear an as-yet undescribed immunosuppressive function.

Introduction

The Parvoviridae are all the small, isometric, non-enveloped DNA viruses that contain linear single-stranded DNA genomes of 4000–6000 nt inside icosahedral capsids. Their genomes terminate in palindromic sequences that can base-pair to form hairpins or other complex forms. These are essential for replication and are the hallmark of the family. The parvovirus subfamily contains the autonomous, vertebrate-specific viruses and includes the prototypic group member, Minute Virus of Mice (MVM) (Crawford, 1966; Kerr and Boschetti, 2006). Among the MVM variants, two, MVMm and MVMc, are common pathogens in mouse animal colonies (Besselsen et al., 2006). Two others – the fibrotropic (MVMp) and the lymphotropic (MVMi) – are the most commonly studied parvoviruses in a research setting (Bonnard et al., 1976; Crawford, 1966; McMaster et al., 1981).

The MVM genome contains two promoters, at map units 4 and 38, driving the expression of overlapping and alternatively spliced RNAs that encode the two major non-structural proteins, NS1 and NS2 (P4), and capsid proteins, VP1 and VP2 (P38). With their minimalist approach, all family members are tightly tuned to the host cell and the natural execution of its functions. None is able to push the cell cycle and each relies almost entirely on the normal host replication machinery (e.g. Deleu et al., 1999). Integration of virus replication into the host machinery is largely executed by the viral non-structural protein 1 (NS1), a pleiotropic 83 kDa (672 amino acid) nuclear phosphoprotein that binds to the viral DNA with sequence specificity. It has ATPase, helicase, nickase, and site-specific endonuclease activities (Cotmore and Tattersall, 1995), and a Mg^{++} binding site (Astell et al., 1987; Cotmore and Tattersall, 1986; Jindal et al., 1994). On the other side of the lifecycle, binding and delivery of the viral genome through the cell and nuclear membranes and into the nucleus are mediated by the VP proteins which exhibit a strikingly diverse set of functions (e.g. Lombardo et al., 2002; Mani et al., 2006; Maroto et al., 2004; Nam et al., 2006).

This leaves NS2. The NS2 protein has three isoforms NS2-P, NS2-Y, and NS2-L, derived from alternative splicing events (Cotmore and Tattersall, 1990) and having identical N-termini but different carboxy ends. They have no known enzymatic activities but are known to interact with a variety of cellular proteins, including members of the 14-3-3 family, SMN, and CRM1 (Bodendorf et al., 1999; Brockhaus et al., 1996; Young et al., 2002; Young et al., 2005). In the setting of its normal cultured murine host cells, NS2 is needed for genome replication (Choi et al., 2005; Naeger et al., 1990) and expression (Mincberg, 2008; Naeger et al., 1993), accumulation of NS1 (Naeger et al., 1990), capsid assembly (Cotmore et al., 1997), and production and nuclear egress of infectious particles (Eichwald et al., 2002; Engelsma et al., 2008; Miller and Pintel, 2002; Naeger et al., 1990). Given this range of interactions and roles, it is not surprising that NS2 is crucial for

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viral reproduction in murine cells (Brownstein et al., 1992; Cater and Pintel, 1992; Naeger et al., 1990; Naeger et al., 1993). And it is surprising that NS2 is not needed for productive infection in transformed host cells of other species (Cater and Pintel, 1992; Naeger et al., 1990) although, even in other species, its loss can certainly reduce propagation of progeny virions (Cotmore et al., 1997).

Although the two research strains of the virus, MVMp and MVMi, display limited and subclinical infections in adult mice in vivo, MVMi infection of neonates can be lethal (Kimsey et al., 1986) and we showed that both mounted productive, sustained and occasionally lethal infections in embryos inoculated during the second trimester in utero (Itah et al., 2004b). It was therefore a natural extension of the curious species-dependent requirement for NS2 to question whether the restriction observed in vitro would be retained in utero. We report here that it is not. NS2-null mutant MVMp is capable of mounting a productive, albeit much reduced, infection of normal mid-trimester mouse embryos in utero.

Results

Pattern of MVMp-NS2null infection in developing mouse embryos

Our initial approach to characterizing a requirement for NS2 activity during infection in utero was to repeat with the mutant virus a time course infection study (Itah et al., 2004b). 5×10^5 pfu of MVMp-NS2null virus or MVMp-wt virus were injected into 13.5 dpc C57BL/6 embryos. After a further 24, 48, 72, or 96 h gestation, embryos were removed and processed for immunohistochemistry. At least 3 injected embryos and 2 non-injected littermates from each time point were serially sectioned and the distribution of viral capsid was determined using an anti-capsid primary

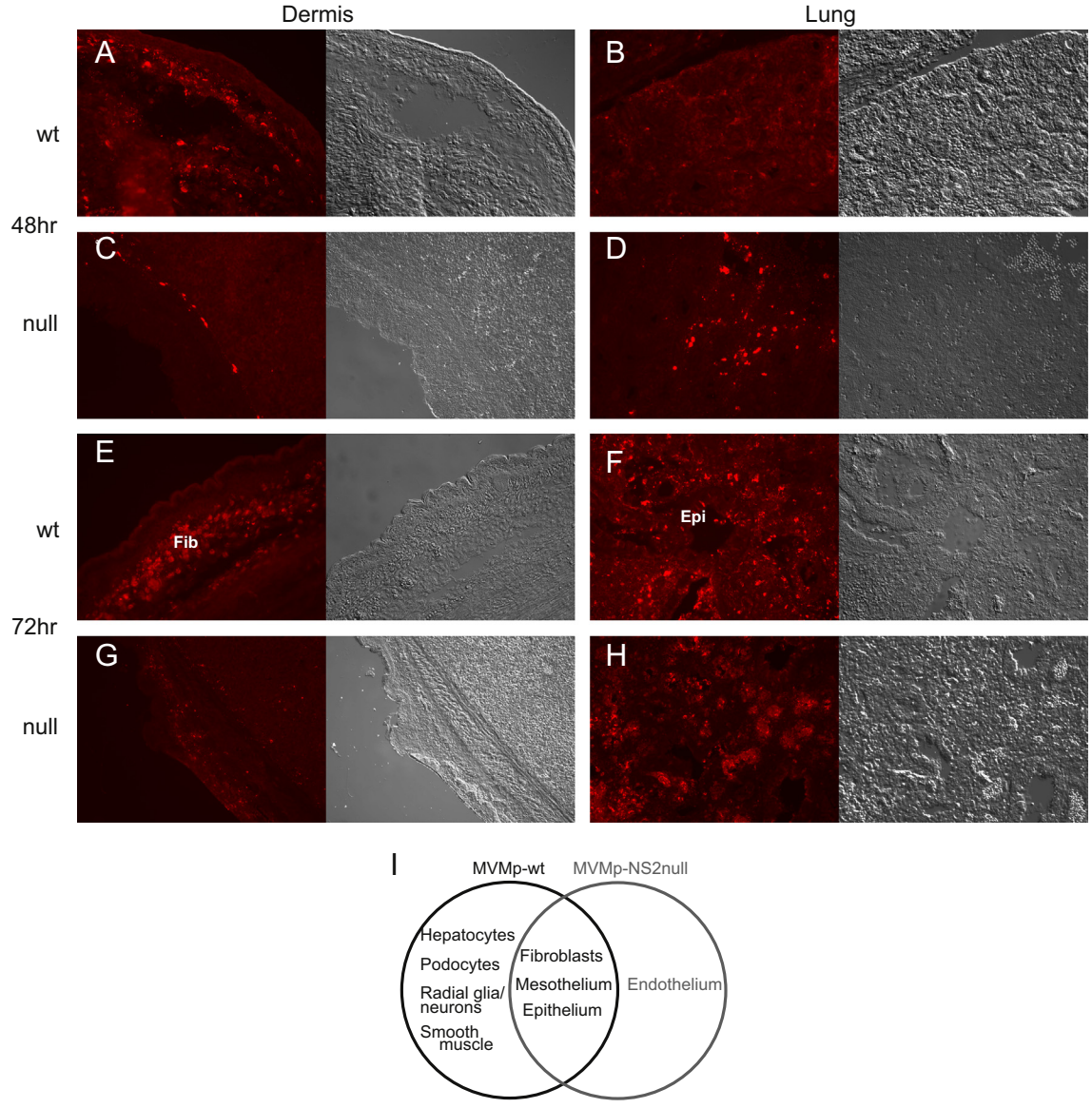

Fig. 1. MVMp-NS2null infection in C57BL/6 embryos. C57BL/6 embryos were inoculated with 5×10^5 pfu of MVMp-NS2null or MVMp-wt at 13.5 dpc by injection in utero (*Materials and methods*). Gestation was left to continue for another 24, 48, 72, or 96 h. Embryos were collected, sectioned and subjected to immunohistochemistry using a polyclonal anti-MVMp capsid primary antibody and Cy3-labeled secondary antibody (*Materials and methods*). Viral coat protein distribution is shown in image pairs (A–H): immunofluorescence (left) and DIC (right). Two representative tissues showing widespread infection, dermis (A, C, E, G) and lung (B, D, F, H), were selected for illustration. Panels are 48 h post-inoculation (A–D) or 72 h post-inoculation (E–H). Inoculum was either wild type MVMp (A, B, E, F) or NS2 null mutant virus (C, D, G, H). (I) Tropism of MVMp-NS2null virus vs. MVMp wt virus. Host cell types were determined by histology and immunohistochemistry at 24, 48, 72, and 96 h post-infection. Fib – fibroblasts, and Epi – epithelia.