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Myxoma virus M128L is expressed as a cell surface CD47-like virulence factor that contributes to the downregulation of macrophage activation in vivo

C.M. Cameron[a,b], J.W. Barrett[a], M. Mann[a], A. Lucas[c], Grant McFadden[a,b,*]

[a]*BioTherapeutics Research Group, Robarts Research Institute, 1400 Western Road, London, ON, Canada N6G 2V4*
[b]*Department of Microbiology and Immunology, University of Western Ontario, London, ON, Canada N6A 5C1*
[c]*Vascular Biology Research Group, Robarts Research Institute, P.O. Box 5015, 100 Perth Drive, London, ON, Canada N6A 5K8*

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Abstract

The M128L myxoma virus gene expresses a five-membrane spanning cell surface protein with significant amino acid homology to the cellular CD47 proteins. CD47, also called integrin-associated protein (IAP), is associated with the modulation of leukocyte adhesion, motility, activation, and phagocytosis. Creation of an M128L-deletion mutant myxoma virus strain and subsequent infection of the European rabbit demonstrated that M128L is necessary for the production of a lethal infection in susceptible rabbits, while it is fully dispensable for virus replication in vitro. Secondary sites of infection developed on the majority of rabbits infected with the M128L-deletion mutant (vMyx128KO), demonstrating that the M128L protein is nonessential for the dissemination of virus within the host. Although the size and severity of the primary lesions on vMyx128KO-infected rabbits were comparable to rabbits infected with the wild-type virus at the early stages of disease progression, by day 7 the reduced virulence of the vMyx128KO virus was clearly evident and all of the animals recovered from infection by the M128L-knockout virus. Histological analysis of the tissues of vMyx128KO-infected rabbits revealed greater activation of monocyte/macrophage cells in infected and/or lymphoid tissues when compared to those of wild-type myxoma-infected rabbits. We conclude that the M128L protein is a novel CD47-like immunomodulatory gene of myxoma virus required for full pathogenesis of the virus in the European rabbit and that its loss from the virus results in increased activation of monocyte/macrophage cells during infection.

Keywords: Poxvirus; Myxoma virus; CD47; Virulence factor; Pathogenesis; Antiviral immune response; Integrin-associated protein

Introduction

Myxoma virus is a member of the poxvirus family and the prototypic example of the Leporipoxvirus genus, and is the causative agent of myxomatosis in European rabbits, a severely debilitating disease characterized by profound systemic cellular immunosuppression and virtually 100% rate of mortality in this host (Fenner, 1983; Fenner and Ratcliffe, 1965). The complete 161.8-kb dsDNA genome of myxoma virus (strain Lausanne) was sequenced and analyzed to reveal a spectrum of potentially novel immunomodulatory genes (Cameron et al., 1999). Bioinformatic analyses of the predicted protein products of the 159 unique identified open reading frames revealed several host-related proteins, including a family of proteins with multiple ankyrin-repeat domains, a CD200-like protein, three serpins, a chemokine binding protein, several cytokine receptor homologs, a growth factor, a complement inhibitor, and a number of proteins with domains closely related to diverse host immune regulatory proteins (Barrett et al., 2001; Cameron et al., 1999). The M128L gene was one of the newly identified myxoma genes with the potential for an immune-related function based on its predicted immuno-

* Corresponding author. Department of Microbiology and Immunology, Robarts Research Institute and University of Western Ontario, London, ON, Canada N6G 2V4. Fax: +1 519 663 3715.
E-mail address: mcfadden@robarts.ca (G. McFadden).

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globulin domain and its sequence similarity to the cellular CD47 molecule.

The predicted myxoma virus M128L gene product shares approximately 23% identity with bovine CD47 under optimal pairwise alignment conditions and over the entire length of the protein (Cameron et al., 1999). Other CD47 homologues have been identified within the genomes of many, but not all, of the chordopoxvirus subfamily members sequenced to date; members of the orthopoxvirus, capripoxvirus, leporipoxvirus, suipoxvirus, and yatapoxvirus genera as well as deerpox, an unclassified chordopoxvirus (Afonso et al., 2005), possess CD47-like genes (Seet et al., 2003). CD47-like genes have not been identified to date in the sequenced members of the parapoxvirus, avipoxvirus, or molluscipoxvirus genera. CD47 homologs have not been described in the sequenced members of the entomopoxvirus subfamily, nor have they been recognized as occurring in other virus families, such as herpesviridae, which are large dsDNA viruses known to encode multiple immunomodulatory proteins.

The only viral CD47-like gene which has been previously characterized is vaccinia virus Western Reserve strain A38L, which shares 28% amino acid (aa) identity in the N-terminal immunoglobulin-variable (IgV)-like domain region with that of mammalian CD47 (Parkinson et al., 1995), and 16% aa identity with M128L. The A38L gene was found to encode a membrane-associated and glycosylated 33-kDa protein that was expressed at low levels during late times of virus infection, but not as a component of IMV or EEV. Loss of the A38L gene from vaccinia virus resulted in a small reduction in virus plaque size, but had no consequences regarding the production of intracellular mature virus (IMV) or extracellular enveloped virus (EEV) particles in tissue culture cells. The A38L-deletion mutant virus did not exhibit a loss of virulence in the murine intranasal model, and therefore it was concluded that the virulence and virus particle production of this strain of vaccinia were not dependent upon A38L protein expression (Parkinson et al., 1995). Further investigation of A38L function was carried out using a recombinant vaccinia virus capable of inducibly overexpressing the A38L protein, and demonstrated that A38L overexpression results in profound observed differences in the characteristics of infected cells in terms of their adhesion, permeability, and morphology (Sanderson et al., 1996). Infected cells overexpressing the A38L protein underwent cellular necrosis, increases in membrane permeability, and finally Ca2+-dependent lysis. It was therefore suggested that the A38L protein plays a role in plasma membrane pore formation, enabling Ca2+ influx and resulting in necrosis. In addition, it was noted that the A38L overexpressing virus had a smaller plaque size, as did the A38L-deletion mutant, and resulted in the creation of fewer particles in vitro (Sanderson et al., 1996).

Mammalian CD47 is a unique member of the immunoglobulin superfamily, possessing a single extracellular N-terminal immunoglobulin domain, five transmembrane domains, and a relatively small cytoplasmic domain (Brown and Frazier, 2001). Alternatively spliced isoforms of CD47 exist, with all splice variants mapping in the intracytoplasmic domain (Lindberg et al., 1993). CD47 is ubiquitously expressed as a 50-kDa cell-surface protein, with one of its four major isoforms found on most cell-types including erythrocytes (Reinhold et al., 1995). CD47 was initially discovered as a surface protein that copurifies with the integrin αvβ3, but has subsequently been shown to associate with other integrins as well, such as α2β1 and αIIbβ3 (Green et al., 1999; Wang and Frazier, 1998). This physical association is biologically functional. Treatment of cells with activating antibodies directed against CD47 results in the identical signaling cascade to that induced by the activation of the coupled integrin, while treatment with blocking antibodies to the CD47 molecule is able to inhibit integrin signaling (Brown et al., 1990; Lindberg et al., 1993). Importantly, CD47 has been shown to also act independently of integrins and interact with a variety of ligands which activate it directly (Barazi et al., 2002; Brown and Frazier, 2001).

CD47 is a well-documented regulator of the vertebrate immune response and exerts multiple functions on various immune cell types (Brown, 2001; Brown and Frazier, 2001; Pettersen, 2000). CD47 was shown to play a critical role in host defense by Lindberg et al., who demonstrated that CD47-deficient mice are more susceptible to bacterial infection than heterozygous $CD47^{(+/-)}$ littermates (Lindberg et al., 1996). The polymorphonuclear cells of CD47-deficient mice were found to have defects in migration and activation during *E. coli* infection, both in vivo and in vitro. In terms of the host response to an invading pathogen, the ability of effector leukocytes to become activated is ineffective if these same cells cannot reach the site of infection. CD47 and its receptor, SIRPα (CD179a), appear to regulate human polymorphonuclear cell chemotaxis (Liu et al., 2002), and antibodies to CD47 augment the proliferation of primary human T cells and increase their expression of CD25 and IL-2 (Reinhold et al., 1997; Ticchioni et al., 1997). In fact, CD47 ligation on a mixed population of naive human mononuclear cells has been shown to foster the development of anergic T cells, with defects in IL-2 and cytokine production, proliferation, and ability to respond to IL-2, IL-4, or IL-12 (Lindberg et al., 1996).

It has also been shown that activation of CD47 on the surface of monocytes inhibits proinflammatory cytokine release, specifically that of TNF-α, IL-12, and IFN-γ (Armant et al., 1999; Hermann et al., 1999). Similarly, mAb-induced activation of CD47 at the surface of immature dendritic cells inhibits the production of TNF-α, IL-12, GM-CSF, and IL-6 (Demeure et al., 2000). CD47 has also been shown to play a role in determining the destiny of activated T cells through a novel apoptotic pathway not dependent on CD3, CD4, CD45, or p56lck (Avice et al., 2001; Mateo et al., 1999; Pettersen et al., 1999). These findings make evident