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Identification of an African Swine Fever Virus Gene with Similarity to a Myeloid Differentiation Primary Response Gene and a Neurovirulence-Associated Gene of Herpes Simplex Virus

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Here we describe an open reading frame (LMW23-NL) in the African swine fever virus genome that possesses striking similarity to a murine myeloid differentiation primary response gene (MyD116) and the neurovirulence-associated gene (ICP34.5) of herpes simplex virus. In all three proteins, a centrally located acidic region precedes a highly conserved, hydrophilic 56-amino-acid domain located at the carboxy terminus. LMW23-NL predicts a highly basic protein of 184 amino acids with an estimated molecular mass of 21.3 kDa. The similarity of LMW23-NL to genes involved in myeloid cell differentiation and viral host range suggests a role for it in African swine fever virus host range.

African swine fever virus (ASFV) is a large icosahedral arbovirus which contains a linear double-stranded DNA genome of 170 to 190 kb and which replicates in the cytoplasm of infected cells (10, 41). It is the causative agent of African swine fever (ASF), a highly significant disease of domestic swine. ASF occurs in several disease forms, ranging from highly lethal infections to subclinical ones, depending on contributing viral and host factors. In the acute or highly virulent form of the disease, the course is short (8 to 10 days) and mortality rates approach 100% (30). Cells of the mononuclear-phagocytic system are major targets for ASFV replication in vivo; viral infections with extensive necrosis of fixed macrophages of the spleen, lymph node, lung, and liver as well as specific lineages of reticular cells are evident following infection with highly virulent virus isolates (8, 12, 20, 29, 30, 32). Strains of ASFV exhibiting moderate virulence also infect these cell types, but the degree of tissue involvement and the resulting damage are much less severe (29, 30). Thus, the abilities of ASFV to replicate efficiently and to induce marked cytopathology in these cell types in vivo appear to be critical factors in ASFV virulence. The nature of the virus-cell host interactions responsible for the differing outcomes of infection with highly virulent, moderately virulent, or avirulent ASFV strains is unknown. Here we describe an ASFV gene, LMW23-NL, that possesses significant similarity to a murine myeloid differentiation primary response gene (MyD116) and the neurovirulence-associated gene (ICP34.5) of herpes simplex virus (HSV). The similarity of LMW23-NL to genes involved in myeloid cell differentiation and viral host range suggests a possible role for it in ASFV host range.

The 11-kb *Sal*I fragment L of the virulent ASFV strain Malawi Lil-20/1 genome (13), which maps near the right terminus of the genome, was subcloned and sequenced in its entirety by a random sequencing strategy employing the dideoxy-chain termination method and an ABI 370A automated DNA sequencer (3, 36). Random sequences were assembled by using the computer programs of Staden (39). Sequences from both strands were obtained for the entire LMW23-NL gene region.

LMW23-NL, an open reading frame (ORF) of 551 bp, begins at position 9828 and ends at position 9277 on the negative strand of the 11,009-bp fragment (Fig. 1). Codon usage bias in an uninterrupted stretch of 128 codons (70% of coding region) coincides with the codon frequency for known ASFV genes that code for p37 (an early structural protein [23]), thymidine kinase (4), p22 (an early structural protein [16]), and p72 (a late structural protein [22]), thus indicating a 99% probability that LMW23-NL codes for protein over this region (38). In addition, transcription from the LMW23-NL ORF was detectable in ASFV-infected porcine macrophages early after infection (Fig. 2). The sequence motif TAAATG, which includes the start codon of the ORF, resembles efficient vaccinia virus late promoters (11, 31). Whether it functions as a promoter here is unknown; however, it has been shown that ASFV and vaccinia virus, the type member of the family *Orthopoxviridae*, are able to utilize each other's promoters (17, 41).

The protein predicted by LMW23-NL is 184 amino acids in length, is hydrophilic in nature (21), and has an isoelectric point of 9.23 and a molecular mass of 21.3 kDa. No evidence for a signal sequence or membrane-spanning regions within the protein was found (42). A search of the Prosite data base (release 7.1) (2) identified six consensus protein kinase C phosphorylation motifs at amino acids 2, 8, 40, 82, 115, and 165 (43); three casein kinase phosphorylation motifs at residues 27, 63, and 107 (34); one tyrosine kinase phosphorylation sequence at residue 126 (9); and one potential Asn glycosylation site at residue 93 within the sequence (27).

FASTA (33) searching of GenBank (release 69.0), Swissprot (release 19.0), and PIR (release 7.0) data bases, as well as BLAST searches of the latter two protein data bases, revealed a striking similarity between LMW23-NL, a myeloid differentiation primary response gene, MyD116 (25), and the neurovirulence-associated protein (ICP34.5) of HSV (6, 7).

Although differing in size, all three proteins contain a centrally located acidic region with a highly conserved,

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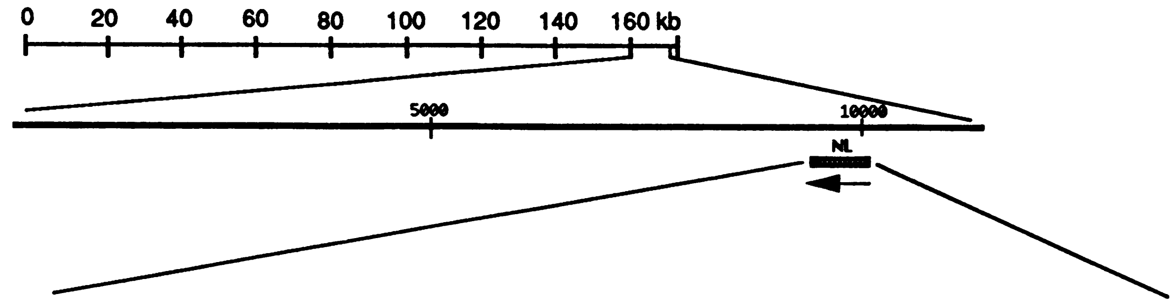


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                                                                                          10000
CAT AAC TTC TAT CTA CAA ACT AAT TAT TTC TAG AAA AAA AAT CAA ATT AAA ATA GTG GAT GTT TCT AAT ACC CAA ATT AAC TTA TTT ATT

                                                                                                               9900
GTA GAA TAT TTA TTG TAA AAT AGA TAT TTT ATT AGA ACT CTT CCC TTC AAA AGC AAT TGC CCA ATC TAT ACT AAA GAA TAA GTT ATC AAA

GTA ATT TAT CAT TAC TAT CTA AAT TTT AAA TAC TCA TGT TAT ATA TCA TAA ATT ATT TAA AAA CTA TAA ATG TCG AGG AGA AAT AAA CGC
                                                                                             M   S   R   R   N   K   R
    9800
AGC CGC CGA CGT CGC AAA AAA CCT CTT AAT ACC ATT CAA CCA GGT CCT TCA AAA CCC TCA GCT CAA GAT GAG CCC ATC AAA TCT GTC TCC
 S   R   R   R   R   K   K   P   L   N   T   I   Q   P   G   P   S   K   P   S   A   Q   D   E   P   I   K   S   V   S
                9700
CAT CAT TCA TCA AAA ATA GGC ACA AAC CCT ATG TTA GCC TTC ATT CTC GGG GGA AAT GAA GAT TTG AGT GAT GAT AGT GAC TGG GAT GAA
 H   H   S   S   K   I   G   T   N   P   M   L   A   F   I   L   G   G   N   E   D   L   S   D   D   S   D   W   D   E
                            9600
GAT TTC TCT CTT GAG AAT ACA TTG ATG CCA TTA AAT GAA GTA TCC CTG AAA GGC AAG CAT GAT TCT AAA CAT TTT AAT AAA GGA TTT GAC
 D   F   S   L   E   N   T   L   M   P   L   N   E   V   S   L   K   G   K   H   D   S   K   H   F   N   K   G   F   D
                                        9500
AAT AAT ACT GCT CTT CAC GAA GTG AAT ACT AAG TGG GAG GCA TTC TAT TCT AGT GTA AAA ATC AGG CAA CGG GAT GTT AAG GTT TAT TTT
 N   N   T   A   L   H   E   V   N   T   K   W   E   A   F   Y   S   S   V   K   I   R   Q   R   D   V   K   V   Y   F
                                                    9400
GCT ACA GAT GAT ATT TTA ATA AAA GTG CGG GAA GCT GAT GAC ATT GAC CGC AAG GGC CCC TGG GAA CAG GCT GCC GTT GAT AGG CTC CGC
 A   T   D   D   I   L   I   K   V   R   E   A   D   D   I   D   R   K   G   P   W   E   Q   A   A   V   D   R   L   R
                                                                9300
TTC CAA CGT CGG ATT GCC GAC ACC GAA AAG ATC CTT TCA GCA GTG CTC CTA CGA AAA AAA TTA AAT CCC ATG GAG CAT GAG TAG AAA TTC
 F   Q   R   R   I   A   D   T   E   K   I   L   S   A   V   L   L   R   K   K   L   N   P   M   E   H   E

ATG GGG CGG AAG GAC GT
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FIG. 1. Nucleotide sequence of ASFV ORF LMW23-NL. LMW23-NL, depicted as a hatched rectangle labeled NL, is located at the right-hand end of the 173-kb ASFV (Malawi Lil-20/1) genome on the 11-kb *Sal*I fragment L (13). An arrow depicts the orientation of the gene. The nucleotide sequence of the coding strand of LMW23-NL is shown here (the coding strand is the negative strand). The numbering is consistent with the location of the gene on the *Sal*I fragment L (LMW23-13). The deduced amino acid sequence is shown beneath the nucleotide sequence in the one-letter amino acid code.

hydrophilic 56-amino-acid domain located at the carboxy terminus (Fig. 3). In addition, LMW23-NL and ICP34.5 both contain a highly basic amino terminus composed of 8 to 10 lysine and arginine residues. In the conserved domain of LMW23-NL (amino acids 118 to 173), there are 25 exact matches and 19 conservative matches with MyD116, which corresponds to 78% overall conservation with 44% identity (based on the Dayhoff Pam-250 symbol comparison table with a 0.5 cutoff [37]). In the same region, comparison of LMW23-NL to HSV ICP34.5 reveals 55% overall conservation and 30% identity. Apart from the sequence similarities noted above, the gene sequences are dissimilar.

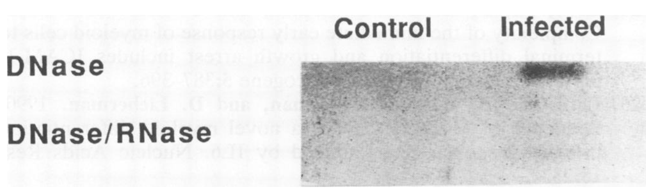

FIG. 2. LMW23-NL is transcribed in ASFV-infected porcine macrophages. Macrophage cultures were infected with ASFV (Malawi Lil-20/1) (multiplicity of infection, 20) and harvested at 2 h postinfection (1, 15). Total cell RNA was prepared as previously described (5). RNA samples (10 μg) were treated with either DNase I (1 μg/ml) or DNase I and RNase A (1 μg/ml), blotted to a nylon membrane, and hybridized at high stringency (0.5 M $Na_2H_2PO_4$ [pH 7.2], 1% bovine serum albumin, 1 mM EDTA, 7% sodium dodecyl sulfate 65°C) with a single-stranded ^{32}P-labeled DNA probe complementary to the entire LMW23-NL coding region (19, 35). A mock-infected control culture was treated in an identical manner.

Recently, McGeoch and Barnett have reported the similarity between MyD116 and HSV ICP34.5 (28). LMW23-NL represents an additional viral gene belonging to this increasingly interesting group. The HSV ICP34.5 gene, although nonessential for growth in cell culture, is strongly associated with HSV neurovirulence in mice and appears to be a host range gene required for viral replication and virulence in fully differentiated neural tissue (6, 7, 40).

MyD116, a murine myeloid differentiation primary response gene, is expressed in leukemic myeloblasts immediately following induced differentiation with interleukin-6 or granulocyte/macrophage colony-stimulating factor and is expressed at high levels in terminally differentiated cells of the myeloid lineage. The function of MyD116 is unknown; however, because it is expressed early in cellular differentiation and in terminally differentiated cells, it may be involved in regulating the differentiation process or in maintaining the differentiated state in myeloid cells (24, 25).

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   hsv1 186 tpatpaRVrFsphvrvrhLV.VWasAArVARaGsWareraDRaRFrRRVAEaEaVIGPcLGpeararalar 255
   hsv2 166 .......VcFsTrvqvrhLV.AWetAArVARaGsWareraDRdRFrRRVAaaEaVVGPcLepearararar 229
 myd116 541 IPlKArKVHFAEKvTvhfLa.VWaGPAQaARRGPWEQfArDRsRFARRIAQAEekLGPyLtpdsrarawar 611
lmw23nl 118 IRqRDvKVYFATDdI...LIkVR.EADDIDRKGPWEQaAVDRlRFQRRIADTEkILSAvLlrkklnpmehe 184
```

FIG. 3. Conserved carboxy-terminal domains of LMW23-NL, MyD116, and HSV ICP34.5. The sequences shown are ICP34.5 from HSV type 1 (6, 7), the ICP34.5 homolog from HSV type 2 (28), MyD116 (25), and ASFV ORF LMW23-NL. Residues identical to LMW23-NL are shown as uppercase bold letters, while conservative amino acid substitutions are indicated by uppercase letters. The numbering of LMW23-NL is consistent with the amino acid sequence shown in Fig. 1. The other proteins are numbered as they appear in the citations.

Interestingly, ASFV targets differentiated cells of the mononuclear-phagocytic system. Viral pathogenesis studies indicate that fully differentiated tissue macrophages and reticular cells are major viral targets in vivo (8, 12, 20, 29, 30, 32). In addition, macrophages, obtained from peripheral blood following differentiation of blood monocytes in culture, are highly susceptible to ASFV infection in vitro (14, 18, 26). Although the function of LMW23-NL in ASFV infection is unknown, its similarity with genes involved in myeloid cell differentiation and viral host range suggests a possible role for it in ASFV host range. It is tempting to speculate that LMW23-NL's function may involve regulation or manipulation of monocyte/macrophage differentiation states in such a way that it increases cell permissiveness to viral infection.

Nucleotide sequence accession number. The GenBank accession number for LMW23-NL (Fig. 1) is M95672.

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