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NOTES

Expression of Human MxA Protein in Mosquito Cells Interferes with LaCrosse Virus Replication

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Human MxA protein inhibits LaCrosse virus (LAC virus; family *Bunyaviridae*) replication in vertebrate cells and *MxA*-transgenic mice. LAC virus is transmitted to humans by *Aedes triseriatus* mosquitoes. In this report, we have shown that transfected mosquito cells expressing the human *MxA* cDNA are resistant to LAC virus but permissive for Sindbis virus (family *Togaviridae*) infection.

Expression of the human *MxA* gene is induced by alpha and beta interferons, often in response to viral infection. Interferons induce an antiviral state in surrounding cells. The binding of alpha and beta interferons to specific receptors activates the JAK/STAT signaling pathway, which activates ≥50 genes, including the *Mx* gene (15). Mx proteins belong to the dynamin superfamily of large GTPases found in yeasts, plants, and animals (4). Some Mx proteins exhibit broad-spectrum antiviral activity. For example, human MxA has been shown to inhibit replication of viruses from the families *Orthomyxoviridae*, *Rhabdoviridae*, *Bunyaviridae*, *Paramyxoviridae*, and *Togaviridae* (2, 4, 8).

The human MxA protein inhibits LaCrosse virus (LAC virus) in cell culture and in transgenic mice (3, 5). Alpha/beta interferon receptor knockout mice do not respond to interferons, do not express Mx protein, and are highly susceptible to viral infections despite an otherwise intact immune system. These mice permit evaluation of MxA-induced virus resistance in vivo without the involvement of other interferon-induced gene products. When these knockout mice transgenically express the human *MxA* cDNA, they become resistant to previously lethal virus infections (5). This suggested that expression of MxA in mosquito cells, which do not have an interferon response, would also interfere with LAC virus.

LAC virus is transmitted to humans by *Aedes triseriatus* mosquitoes, which serve as the vector and the reservoir host for LAC virus (9). Thus, the mosquito is a good target for interference strategies to perturb the transmission cycle of LAC virus. In these studies, we demonstrate that mosquito cells expressing the human *MxA* cDNA are resistant to LAC virus but not Sindbis virus (SIN virus) replication.

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Mosquito cells can constitutively express MxA. pIE1-*MxA* was derived from pIE1-3 (Novagen), which contains the *Autographa californica* baculovirus immediate-early (IE1) promoter and hr5 enhancer sequences. pIE1-*MxA* was constructed by PCR amplification of the ~2-kb *MxA* sequence from pHMGMxA (11) using primers (5′-GGATCCGGAAGATGG TTGTTTCCG-3′ and 5′-GGATCCGGACAGAGTGTGGTT AACC-3′) containing *Bam*HI sites flanking the primer sequence. The PCR product was cloned into a TA cloning vector (pCR2.1 TOPO; Invitrogen), excised with *Bam*HI, and cloned into the *Bam*HI site of pIE1-3 (Novagen).

C6/36 (*Aedes albopictus)* cells were transfected with plasmid pIE1-*MxA* using Effectene reagent (Qiagen) according to the manufacturer's suggestions. Forty-eight hours posttransfection, the cells were fixed onto glass coverslips using 4% paraformaldehyde. MxA expression was analyzed by immunofluorescence assay (IFA). A mouse monoclonal antibody to MxA, 2C12 (13), was the primary antibody, and a fluorescein isothiocyanate-linked anti-mouse antibody (Kirkegaard & Perry Laboratories, Inc.) was the secondary antibody. The cells were counterstained with Evans blue. Fluorescence was detected in approximately 25% of cells. MxA-specific fluorescence was localized in the cytoplasm of transfected cells (Fig. 1B) in a punctate pattern similar to that seen in vertebrate cell lines. No MxA-specific fluorescence was seen in nontransfected C6/36 cells (Fig. 1A).

MxA expression inhibits LAC virus replication in mosquito cells. C6/36 cells were transfected with pIE1-*MxA* as described above. Twenty-four hours posttransfection, the cells were challenged with LAC virus (multiplicity of infection, 0.01). At 24 and 48 h postinfection, cells were fixed and analyzed by IFA. LAC virus antigen was detected using rabbit polyclonal hyperimmune serum, and a secondary tetramethyl rhodamine isothiocyanate (TRITC)-linked anti-rabbit antibody (Kirkegaard & Perry Laboratories, Inc.). An Olympus BH-2 fluorescent microscope with a fluorescein isothiocyanate-TRITC filter cube (Chroma Technology Corp.) was used for IFA for MxA and viral antigens. MxA-positive and MxA-negative cells in

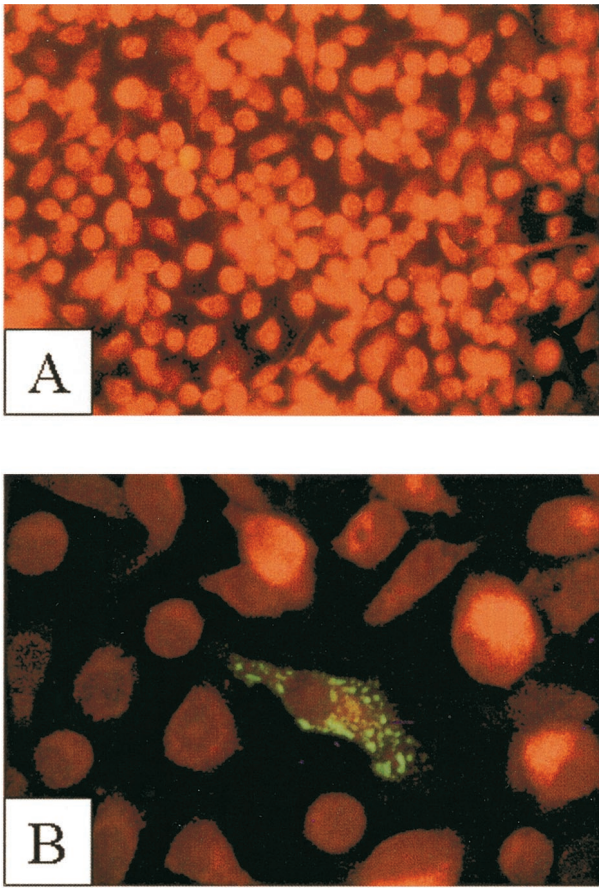

FIG. 1. Expression of human MxA protein in mosquito cell line C6/36. pIE1-*MxA*-transfected cells were analyzed by IFA. (A) Untransfected C6/36 cells (magnification, ×200); (B) C6/36 cells transfected with pIE1-MxA (magnification, ×1,000).

random, nonoverlapping microscope fields were analyzed for LAC virus-specific antigen. Results were statistically analyzed using two-by-two contingency tables with chi-square analysis.

LAC virus antigen was not detected in the majority of MxA-positive cells (Fig. 2B and D). However, dual fluorescence was detected in a small number of cells (Fig. 2C). These cells appeared to have less MxA-specific fluorescence than did cells with no detectable LAC virus antigen, suggesting an Mx dose effect. No LAC virus antigen was detected in uninfected cells (Fig. 2A). Numbers of MxA-positive and MxA-negative cells with or without LAC virus antigen were analyzed (Table 1). LAC virus infection of MxA-positive cells was significantly lower than that of MxA-negative cells at 24- and 48-h time points ($P < 0.0001$). The mean infection rate (Fig. 3) in MxA-negative cells was 67.6%, and the mean infection rate in MxA-positive cells was 7.4%, at 24 h postinfection. These rates were 81.4 and 6.8%, respectively, at 48 h postinfection. Means were determined from three replicates for each group. Infection rates differed statistically by chi-square analysis ($P < 0.0001$).

MxA-expressing mosquito cells are susceptible to infection by SIN virus. C6/36 cells transfected with pIE1-*MxA* were challenged with a recombinant Sindbis virus (SIN virus) which expressed the capsid proteins of *Aedes* densovirus (AeDNV; family *Parvoviridae*) (1). SIN virus replication was assayed us-

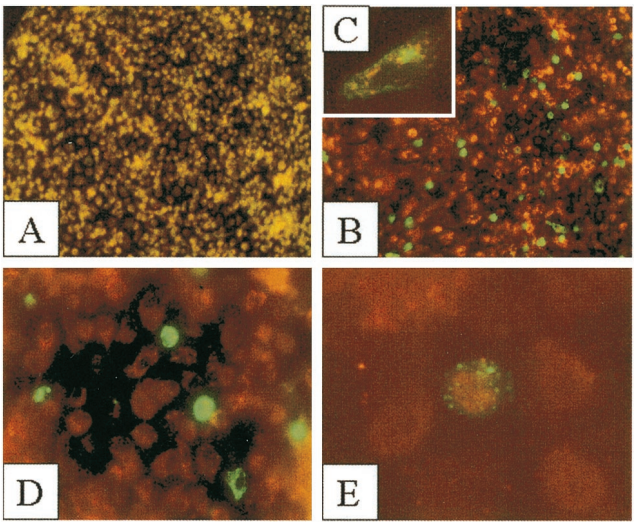

FIG. 2. Analysis of viral infection in MxA-expressing C6/36 cells. Cells that were transformed with pIE1-*MxA* and challenged with LAC virus or a recombinant SIN virus were analyzed by IFA. (A) MxA-negative, LAC virus-negative cells at 24 h (magnification, ×200); (B) MxA-positive, LAC virus-positive cells at 24 h (magnification, ×200); (C) MxA-positive, LAC virus-positive cells at 24 h (magnification, ×1,000); (D) MxA-positive, LAC virus-positive cells at 48 h (magnification, ×400); (E) MxA-positive, SIN virus-positive cells at 24 h (magnification, ×1,000).

ing a rabbit polyclonal antibody to AeDNV and a TRITC-linked anti-rabbit secondary antibody (Kirkegaard & Perry Laboratories, Inc.). Analysis of MxA expression and dual IFA were conducted as described above. AeDNV-specific antigen was detected in a majority of cells expressing MxA. In cells expressing both MxA and AeDNV antigens, MxA-specific fluorescence was seen in the cytoplasm, while AeDNV-specific fluorescence was seen in the nucleus (Fig. 2E). There was no significant difference in the number of cells with or without AeDNV antigen in MxA-positive and MxA-negative cell populations (Table 1; $P = 0.5723$). The mean infection rate in MxA-negative cells was 82.7% and in MxA-positive cells was 80.7% at 24 h postinfection (Fig. 3).

MxA protein inhibited LAC virus in mosquito cells, which do not have the alpha/beta interferon pathways. There was a significant reduction in LAC virus replication in mosquito cells expressing MxA (Table 1 and Fig. 3). Similarly, the MxA-positive, alpha/beta interferon receptor knockout mice were also protected from a lethal challenge with LAC virus (5).

Most of the viruses susceptible to MxA inhibition have negative-sense RNA genomes (4); however, Semliki Forest virus

TABLE 1. Comparison of LAC virus- or SIN virus-infected cell numbers in MxA-positive and MxA-negative cell populations

Cell population	No. of cells negative or positive for virus at time					
	LAC virus (24 h)		LAC virus (48 h)		SIN virus (24 h)	
	−	+	−	+	−	+
MxA^-	207	433	56	245	54	258
MxA^+	358	30	221	16	35	146

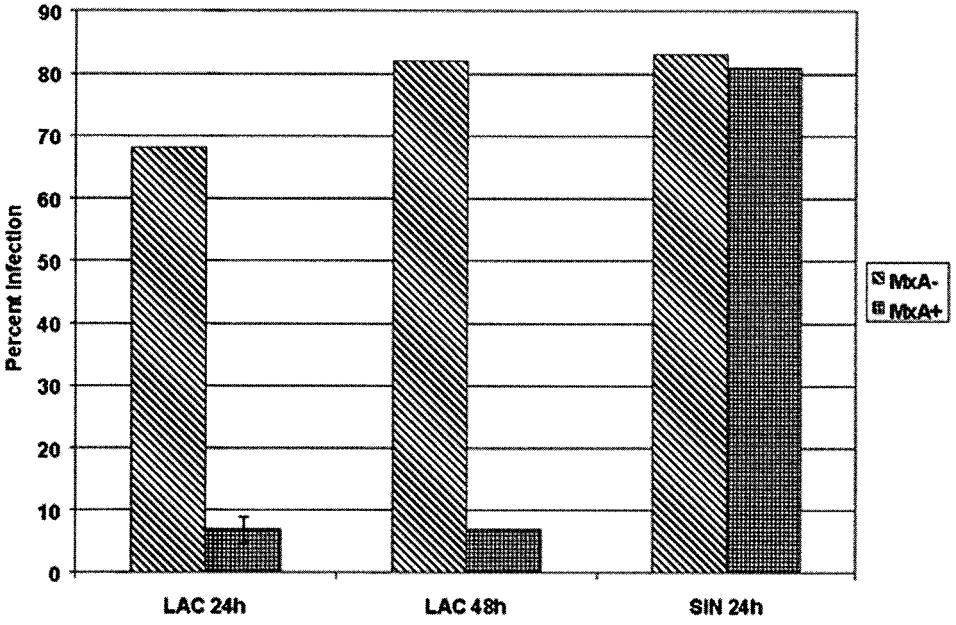

FIG. 3. Comparison of LAC virus and SIN virus infection rates in MxA-negative and MxA-positive cell populations at 24 and 48 h postinfection. LAC virus infection rates differed statistically by chi-square analysis ($P < 0.0001$). SIN virus infection rates did not differ statistically by chi-square analysis.

(family *Togaviridae*), which has a positive-sense RNA genome, is inhibited by MxA (8). However, MxA-expressing mosquito cells were susceptible to challenge (Fig. 2 and 3 and Table 1) with SIN virus (family *Togaviridae*). Different susceptibilities to MxA have been seen with similar viruses and with the same virus in different cell types. For example, MxA inhibits measles virus in human but not in mouse cells (4). The spectrum of antiviral activity of MxA in mosquitoes needs to be determined. If it is broad, MxA expression in mosquitoes may be an effective means to combat a number of mosquito-borne viruses.

The mechanism of MxA-specific interference with LAC virus replication is currently unknown, but there is evidence that MxA interferes with transcription and replication of LAC virus RNA (3). MxA also inhibits vesicular stomatitis virus transcription (12, 14), and mouse Mx1 inhibits influenza virus transcription (7, 10). Alternatively, MxA may inhibit LAC virus replication by binding to ribonucleoprotein complexes, thereby preventing their transport and budding through Golgi membranes. MxA has been shown to bind Thogoto virus, thereby preventing its transport into the nucleus and inhibiting subsequent replication (6). It will be interesting to determine the molecular basis for the antiviral activity of MxA protein in mosquito cells.

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