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Inhibition of cellular entry of lymphocytic choriomeningitis virus by amphipathic DNA polymers

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Abstract

The prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) represents a powerful experimental model for the study of the basic virology and pathogenesis of arenaviruses. In the present study, we used the LCMV model to evaluate the anti-viral potential of phosphorothioate oligonucleotides against arenaviruses. Our findings indicate that amphipathic DNA polymers (APs) are potent inhibitors of infection with a series of LCMV isolates with IC_{50} in the low nanomolar range. APs target the surface glycoprotein (GP) of LCMV and block viral entry and cell–cell propagation of the virus, without affecting later steps in replication or release of progeny virus from infected cells. The anti-viral action of APs is sequence-independent but is critically dependent on their size and hydrophobicity. Mechanistically, we provide evidence that APs disrupt the interaction between LCMVGP and its cellular receptor, α-dystroglycan. Exposure of LCMV to APs does not affect the stability of the GP virion spike and has no effect on the conformation of a neutralizing antibody epitope, suggesting rather subtle changes in the conformation and/or conformational dynamics of the viral GP.

Keywords: Arenavirus; Anti-virals; DNA polymer; Entry inhibitor

Introduction

Arenaviruses merit significant attention as powerful experimental models and important human pathogens. Infection of the prototypic Old World arenavirus lymphocytic choriomeningitis virus (LCMV) in its natural host, the mouse, illuminated fundamental concepts in immunology and virology that have been extended to other viruses, bacteria, and parasites (Oldstone, 2002). LCMV is also an important pathogen in human pediatric medicine (Jamieson et al., 2006) and has recently caused lethal infections in transplantation patients (Fischer et al., 2006). The closely related Lassa fever virus (LFV) is the causative agent of a severe hemorrhagic fever with high mortality in humans and represents the most important human pathogen among the arenaviruses, responsible for over 300,000 infections and several thousand deaths per year (McCormick and Fisher-Hoch, 2002; Geisbert and Jahrling, 2004).

Arenaviruses are enveloped single-strand RNA viruses with a bisegmented genome in ambisense organization that consists of two single-stranded RNA species: the larger segment encodes the virus polymerase (L) and a small zinc finger motif protein (Z), the smaller RNA segment encodes the virus nucleoprotein (NP) and glycoprotein precursor (GPC). GPC is processed into the peripheral glycoprotein GP1 and the transmembrane glycoprotein GP2. GP1 is implicated in receptor binding (Parekh and Buchmeier, 1986; Borrow and Oldstone, 1992) and GP2 is structurally similar to the fusion active membrane proximal portions of other enveloped viruses (Gallaher et al., 2001; Eschli et al., 2006).

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The initial step of LCMV and LFV infection is the attachment of the virus to specific glycan structures on the cellular receptor α-dystroglycan (α-DG) (Cao et al., 1998; Kunz et al., 2001, 2005a,b; Imperiali et al., 2005; Rojek et al., 2007). Upon attachment, LCMV virions are taken up in smooth vesicles that enter the endocytic pathway and deliver the virus to endosomes where pH-dependent membrane fusion occurs (Borrow and Oldstone, 1994).

Studies in human Lassa fever patients and experimental LFV and LCMV infection in animals showed that the host's control of viral replication is primarily mediated by the anti-viral T-cell response with limited roles of antibodies (Oldstone, 2002; McCormick and Fisher-Hoch, 2002). The strong predictive value of virus concentration in blood for a disastrous disease outcome in human Lassa fever indicates further a close competition between virus spread and the patient's anti-viral immune response. Since rapid viral dissemination critically depends on efficient attachment of the viruses to host cells and subsequent entry, drugs targeting these steps will give the host's immune system an advantage by providing a wider window of opportunity for the generation of an efficient anti-viral immune response. Targeting virus entry also has the advantage that the numbers of virus particles present at early stages of infection tend to be smaller, which allows maximization of the inhibitory effect of an anti-viral drug.

Over the past two decades, phosphorothioate oligonucleotides (PS-ONs) have emerged as potent anti-viral substances that can target early steps of virus infection as illustrated by efficient blocking of receptor binding and fusion of human immunodeficiency virus (HIV) (Matsukura et al., 1987; Stein et al., 1989, 1991; Yamaguchi et al., 1997; Este et al., 1998). These effects were known to be unrelated to the specific nucleotide sequence of PS-ONs but were not clearly elucidated until Vaillant et al. (2006) described the antiviral activity of PS-ONs as being derived from their sequence-independent activity as amphipathic polymers (APs). This unique chemical property allowed the interaction of APs with the alpha-helices in HIV-1 gp41 and neutralized their fusion activity. While HIV and arenaviruses clearly differ in virion structure, cellular receptor use, and mechanism of membrane fusion, recent studies revealed that the cell surface GP of LCMV shares some key features with other fusion-active class I viral glycoproteins of enveloped viruses

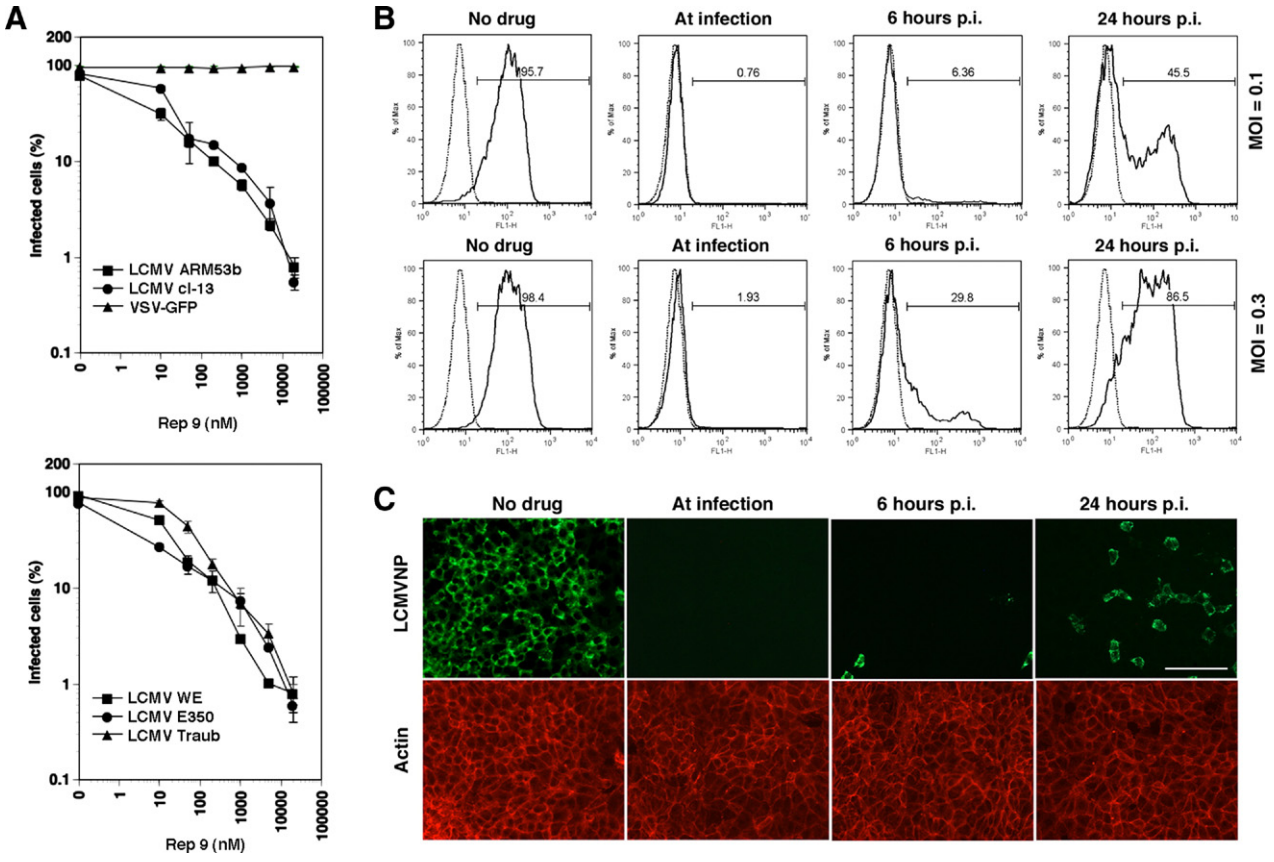

Fig. 1. Blocking of LCMV infection with REP 9. (A) The PS-ON REP 9 blocks infection of cells with LCMV but not VSV: The indicated LCMV isolates and VSV-GFP were pre-incubated with REP 9 for 1 h at 4 °C and added to monolayers of VeroE6 cells (MOI = 3). After 24 h, LCMV infection was detected by intracellular staining for LCMVNP using flow cytometry. Infection with VSV-GFP was detected by direct fluorescence excitation of GFP. Percentages of NP- or GFP-positive cells are given ($n = 3 \pm$ SD). (B) REP 9 blocks viral entry and cell-to-cell propagation: Monolayers of VeroE6 cells were infected with LCMV at the indicated MOI. REP 9 (2 μm) was added at the indicated time points and kept throughout the experiment. After total 48 h, infection was determined as in panel A. In histograms, the *y*-axis represents cell numbers, the *x*-axis fluorescence intensity for NP. The dotted line represents uninfected controls and the solid line infected samples. Percentages of NP-positive cells are given. (C) Detection of virus infection and propagation by immunofluorescence: Monolayers of VeroE6 cells were infected with LCMV ARM53b at MOI of 0.1 and REP 9 added at the indicated time points. After 48 h, cells were fixed, permeabilized and stained with mAb 113 anti-LCMVNP using an FITC-conjugated secondary antibody and phalloidin–rhodamine for counterstaining of actin filaments. Bar = 100 μM.