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The tight junction protein JAM-A functions as coreceptor for rotavirus entry into MA104 cells

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ABSTRACT

Several molecules have been identified as receptors or coreceptors for rotavirus infection, including glycans, integrins, and hsc70. In this work we report that the tight junction proteins JAM-A, occludin, and ZO-1 play an important role during rotavirus entry into MA104 cells. JAM-A was found to function as coreceptor for rotavirus strains RRV, Wa, and UK, but not for rotavirus YM. Reassortant viruses derived from rotaviruses RRV and YM showed that the virus spike protein VP4 determines the use of JAM-A as coreceptor.

Introduction

Rotavirus is an important etiologic agent of gastroenteritis in children under 3 years of age (Tate et al., 2012). *In vivo*, this virus infects primarily mature enterocytes in the intestinal polarized epithelium, however, most studies on rotavirus biology have been carried out in nonpolarized cultures of MA104 (epithelial monkey kidney cells), since these are the cells most permissive for replication of the virus in cultured cells. Attachment and entry into the target cell are the first steps of viral infection, and several cell surface molecules have been involved as receptors or coreceptors for rotavirus, including sialic acid, integrins, and hsc70 (Lopez and Arias, 2006), and more recently histo-blood group antigens were also reported to mediate the attachment of some rotavirus strains (Hu et al., 2012; Huang et al., 2012). In a recent siRNA screening we obtained evidence that suggested that the tight junction (TJ) proteins JAM-A, occludin, and ZO-1 might also play an important role during rotavirus RRV entry into MA104 cells (Silva-Ayala et al., 2013).

JAM-A is an integral TJ protein that possesses two extracellular V-type Ig domains, one transmembrane domain and a short carboxy-terminal intracellular domain. JAM-A has been implicated in the entry of reovirus (Barton et al., 2001) and feline calicivirus (Makino et al., 2006). Occludin is a tetraspan membrane protein with two extracellular loops, which are kept apart by a short intracellular loop. Both, the carboxy- and amino-terminal domains are cytoplasmic and are involved in several signaling pathways (Cummins, 2012). Occludin has been involved in the entry of hepatitis C virus and Coxsackie virus B (Coyne et al., 2007; Ploss et al., 2009). ZO-1 is a TJ plaque protein that serves as a bridge between the integral TJ proteins and the actin cytoskeleton, and acts as an adapter for the intracellular proteins involved in signaling processes (Chi et al., 2012; Rodgers et al., 2013). So far, ZO-1 has not been involved in the entry or replication of any virus.

This study addresses the role of JAM-A, occludin, and ZO-1 in the entry process of various rotavirus strains and identifies the viral protein that participates in the interaction of the virus with JAM-A.

Results

Tight junction proteins localize to intercellular contacts in confluent MA104 cell monolayers

Functional TJs are found at the cell–cell contacts in polarized epithelia or in polarized cell cultures. However, since in this work we used non-polarized cells, we first studied the cellular distribution of JAM-A, occludin, and ZO-1 in confluent MA104 monolayers.

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JAM-A was detected in nonpermeabilized cells, with most of the signal showing a typical honeycomb pattern (Fig. 1A), similar to that observed in polarized cells (Iden et al., 2012). Occludin and ZO-1 were also readily detected at the intercellular junctions when the cells were permeabilized, although a large proportion of ZO-1 appeared dispersed in the cytoplasm (Fig. 1B and C).

Different rotavirus strains use tight junction proteins during their entry into MA104 cells

To characterize the role of JAM-A, occludin, and ZO-1 on the infectivity of rotavirus strains Wa (human), UK (bovine), YM (porcine), and RRV (simian), we silenced their expression by RNAi. When the expression of JAM-A was silenced, the infectivity of rotavirus strains UK, RRV, and Wa was reduced by about 50%, but the infectivity of rotavirus YM was not affected (Fig. 2A). Knocking down the expression of JAM-A did not affect the infectivity of these viruses when the replication cycle started from transfected DLPs (Fig. 2A), bypassing the entry step (Silva-Ayala et al., 2013), suggesting that all strains, but YM, use JAM-A to enter MA104 cells.

The knockdown of occludin reduced in 50–60% the infectivity of all four rotaviruses, and this effect was also exerted at the virus entry level, as shown by the lack of inhibition when DLPs were transfected (Fig. 2B). Silencing the expression of ZO-1 also reduced the infectivity of all strains in 50–70% (Fig. 2C). Since ZO-1 plays an important role in the formation and maintenance of TJs in polarized cells (Fanning et al., 1998; Ikenouchi et al., 2007; Umeda et al., 2006), we determined the cellular localization of JAM-A, and occludin, in ZO-1-silenced cells. The subcellular localization of both proteins was altered under these conditions. JAM-A mostly disappeared from the cell–cell junctions and its overall abundance decreased significantly, suggesting a possible degradation of the protein, while the distribution of occludin shifted, from a mostly junctional localization to a punctuated cytoplasmic signal (Fig. 2D). These observations strongly suggest that the localization of both JAM-A and occludin in nonpolarized MA104 cells is maintained by ZO-1 and that this localization is necessary for virus entry. This complicates the evaluation of the effect that silencing ZO-1 might have *per se* on the infectivity of the virus. The effect of the various siRNAs used was specific for rotaviruses, since the replication of SV40, whose infectivity has not been reported to require TJ proteins, was not affected by the siRNA treatments (Fig. 2A–C).

Since YM behaves differently from the other rotavirus strains tested, we evaluated if its phenotype was associated with its porcine origin. For this, we characterized the effect of silencing the TJ proteins on the infectivity of the porcine rotavirus strain

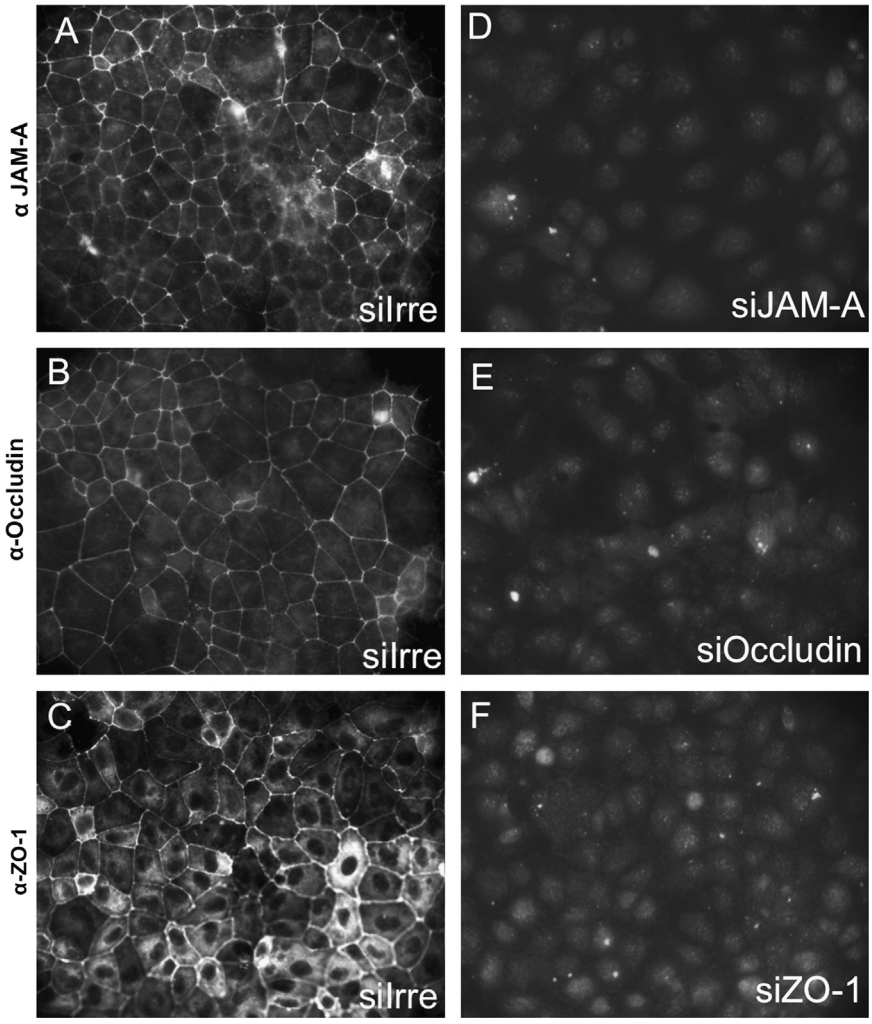

Fig. 1. Tight junction proteins localize to intercellular contacts in MA104 cells. MA104 cells on glass coverslips were transfected with the indicated siRNAs using a reverse method (Silva-Ayala et al., 2013), and 72 hpt cells were fixed and permeabilized (anti-occludin and anti-ZO-1) or not (anti-JAM-A) and stained for indirect immunofluorescence as indicated under *Materials and methods* using a monoclonal antibody (MAb J10.4) to the distal membrane extracellular domain of JAM-A, or polyclonal antibodies to ZO-1, or to the intracellular N-terminal cytoplasmic tail of occludin, as indicated, followed by incubation with secondary Alexa-conjugated antibodies. siIrre stands for an irrelevant siRNA, which in this immunofluorescence assays was an siRNA against luciferase.