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Protection from SARS coronavirus conferred by live measles vaccine expressing the spike glycoprotein

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ABSTRACT

The recent identification of a novel human coronavirus responsible of a SARS-like illness in the Middle-East a decade after the SARS pandemic, demonstrates that reemergence of a SARS-like coronavirus from an animal reservoir remains a credible threat. Because SARS is contracted by aerosolized contamination of the respiratory tract, a vaccine inducing mucosal long-term protection would be an asset to control new epidemics. To this aim, we generated live attenuated recombinant measles vaccine (MV) candidates expressing either the membrane-anchored SARS-CoV spike (S) protein or its secreted soluble ectodomain (Ssol). In mice susceptible to measles virus, recombinant MV expressing the anchored full-length S induced the highest titers of neutralizing antibodies and fully protected immunized animals from intranasal infectious challenge with SARS-CoV. As compared to immunization with adjuvanted recombinant Ssol protein, recombinant MV induced stronger and Th1-biased responses, a hallmark of live attenuated viruses and a highly desirable feature for an antiviral vaccine.

Introduction

Severe acute respiratory syndrome (SARS) is a newly emerged, human infectious disease that first appeared in China in late 2002. Between November 2002 and July 2003, the virus spread to 29 different countries on 5 continents and was responsible for 8096 clinical cases, leading to 774 deaths (WHO, 2004). WHO case management guidelines and restricted travel advices allowed to bring SARS under control by July 2003. The etiological agent of SARS was identified as a novel coronavirus, named SARS-associated coronavirus (SARS-CoV) (Drosten et al., 2003; Ksiazek et al., 2003) that is genetically distinct from previously characterized members of the *Coronaviridae* family (Rota et al., 2003). During the 2002–2003 outbreak, SARS-CoV has been isolated in Chinese civets and racoon dogs (Guan et al., 2003) from which the virus was likely introduced into the human population (Kan et al., 2005; Song et al., 2005). Other SARS-CoV-like viruses sharing more than 88% nucleotide identities with SARS-CoV have been isolated from Chinese horseshoe bats, which have therefore been proposed to represent a natural reservoir host of SARS-CoV (Li et al., 2005). To date, endemic bat SARS-CoV-like viruses have also been detected in Africa and Europe (for review: Balboni et al., 2012), and reemergence of a SARS-like disease from an animal reservoir remains a credible public health threat. An efficient vaccine would be the most effective way to control a new epidemic.

Similar to other coronaviruses, SARS-CoV is an enveloped, positive-stranded RNA virus whose replication takes place in the cytoplasm of infected cells. Viral particles are composed of four major structural proteins: the nucleoprotein (N), the small envelope protein (E), the membrane protein (M), and the large spike protein (S). The spike protein is a type-I transmembrane glycoprotein of 1255 amino acids. It assembles into homotrimers at the surface of viral particles, and gives the virion its crown-like appearance (Neuman et al., 2006). Each monomer (180 kDa) is composed of a signal sequence (a.a. 1–14), a large ectodomain (a.a. 15–1190) with 23 potential N-glycosylation sites, a transmembrane domain (a.a. 1191–1227), and a short cytoplasmic tail of 28 a.a.

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(Ksiazek et al., 2003; Rota et al., 2003). The S protein is responsible for viral entry, binds to the cellular receptor ACE2 (Li et al., 2003) and mediates fusion between the viral and cellular membranes (Petit et al., 2005; Simmons et al., 2005). Structurally, the N-terminal globular head (S1 domain, a.a. 1–680) contains the receptor-binding region (Wong et al., 2004), and the membrane-anchored stalk region (S2 domain, a.a. 727–1255) mediates oligomerization and fusion (Petit et al., 2005). Similarly to other coronaviruses, cleavage of the S protein by proteases into its S1 and S2 subunits is required for activation of the membrane fusion domain following binding to target cell receptors (Matsuyama et al., 2010; Simmons et al., 2005).

Due to its critical involvement in receptor recognition, as well as virus attachment and entry, the S protein is the most promising and studied candidate antigen for SARS-CoV vaccine development. It is the major target for neutralizing antibodies in human patients (He et al., 2005; Nie et al., 2004) and in animal models (Buchholz et al., 2004; Tripp et al., 2005). Passive transfer of IgG from convalescent SARS patients enhanced the recovery of acute phase patients when administered within 15 days after the onset of symptoms (Cheng et al., 2005; Yeh et al., 2005). Administration of S-specific antibodies, including monoclonal antibodies, to naïve animals conferred protection against a subsequent SARS-CoV infection, demonstrating that the antibodies alone can protect against SARS in mice (Bisht et al., 2004), hamsters (Roberts et al., 2006), ferrets (ter Meulen et al., 2004) and *Rhesus macaques* (Miyoshi-Akiyama et al., 2011). Accordingly, several candidate vaccines relying on the induction of spike-specific neutralizing antibodies, including DNA vaccines (Callendret et al., 2007; Yang et al., 2004), live viral vectors (Buchholz et al., 2004; Chen et al., 2005; Kapadia et al., 2005), live attenuated vaccines (Lamirande et al., 2008), subunit vaccines (Bisht et al., 2005; He et al., 2006; Zhou et al., 2006) and inactivated virus vaccine (Stadler et al., 2005; Zhou et al., 2005), have been reported to induce a protective immune response in various animal models. Only a few of them have been evaluated in phase I clinical trials and, lacking a natural challenge, there is no data on efficacy in humans (Roberts et al., 2008; Roper and Rehm, 2009).

An ideal vaccine against SARS should induce long-lasting protective responses after a single administration, be produced at low cost and scaled up to millions of doses. Live attenuated vaccines are particularly appropriate for mass vaccination as they are inexpensive to manufacture and induce a strong immunity and long-term memory after a single injection. To evaluate such a vaccine approach, we previously developed a vector derived from the live-attenuated Schwarz strain of measles virus (MV) (Combredet et al., 2003). MV vaccine is a live-attenuated negative-stranded RNA virus proven to be one of the safest and most effective human vaccines. Produced on a large scale in many countries and distributed at low cost through the Extended Program on Immunization (EPI), this vaccine induces life-long immunity to measles after one or two injections. We previously showed that MV vector stably expressed different proteins from HIV and flaviviruses and induced strong and long-term transgene-specific neutralizing antibodies and cellular immune responses, even in the presence of preexisting immunity to MV (Brandler et al., 2007, 2013; Despres et al., 2005; Guerbois et al., 2009; Lorin et al., 2004). In the present study, we evaluated the immunogenic potential of recombinant MV-SARS vectors expressing either the full-length or the secreted ectodomain of the spike glycoprotein of SARS-CoV. In a mouse model of MV infection, MV-SARS recombinant viruses induced neutralizing antibodies against SARS-CoV and fully protected immunized animals from intranasal challenge with SARS-CoV. Antibody responses induced by MV-SARS vectors were quantitatively and qualitatively compared to responses induced by a prototype subunit vaccine prepared from alum-adjuvanted recombinant Ssol protein.

Results

Recombinant MVSchw-SARS viruses express the SARS-CoV spike glycoprotein, secrete its soluble ectodomain, and replicate efficiently

We synthesized human codon-optimized genes encoding the full-length, membrane anchored SARS-CoV spike (S) protein and its entire ectodomain (residues 1–1193, hereafter designed as Ssol), which is expressed in mammalian cells as a soluble and secreted polypeptide (Callendret et al., 2007, Callendret et al., unpublished results). Their length respects the "rule of six", which stipulates that the total number of nucleotides into the MV genome must be a multiple of 6 (Calain and Roux, 1993). MV editing- and polyadenylation-like sequences were mutated (Lamb and Kolakofsky, 2001; Schneider et al., 1997). Both S and Ssol sequences were inserted as an additional transcription unit (ATU) into MV vector (pTM-MVSchw plasmid), which contains an infectious MV cDNA corresponding to the anti-genome of the Schwarz vaccine strain (Combredet et al., 2003) (Fig. 1A). The resulting pTM-MVSchw-S and pTM-MVSchw-Ssol plasmids were transfected into helper 293-T7-MV cells as previously described (Combredet et al., 2003). The corresponding recombinant measles viruses MV-S and MV-Ssol were successfully rescued as indicated by the formation of syncytia, and then propagated in Vero cell culture. We analyzed the replication of MV-S and MV-Ssol viruses on Vero cells by using the same MOI (0.01) than for standard MV stock production (Fig. 1B). The growth of MV-Ssol was only slightly delayed, compared with that of parental empty Schwarz MV (MVSchwarz). The final yield, routinely obtained at 60 h post-infection, was high and identical to that of parental MVSchwarz (10^6 TCID50/ml). Viral growth and yield of MV-S were more affected than that of MV-Ssol. This may be due to reduced MV budding because of the insertion of full length S at the surface of the infected cells, as already observed for MV expressing membrane-anchored forms of HIV1 gp160 (Lorin et al., 2004).

We analyzed the expression of SARS-CoV spike antigens by indirect immunofluorescence (IFA) of Vero cells infected by recombinant viruses and by immunoblotting of infected-cell lysates. At 48 h post-infection, IFA performed on permeabilized cells using an anti-S hyperimmune mouse ascitic fluid revealed a strong expression of both full-length S and entire ectodomain Ssol of the spike protein along the compartments of the secretory pathway in measles-induced syncytia (Fig. 1C, upper panels). When infected cells were not permeabilized before labeling, only the cell surface of MV-S induced syncytia was readily stained, indicating that the membrane-anchored S is efficiently transported to the surface (Fig. 1C, lower panels). Western blot analysis of cell lysates and supernatants using rabbit anti-S polyclonal antibodies confirmed the expression of S and Ssol proteins in recombinant MV-infected Vero cells with the expected apparent molecular mass of ~180 kDa (Fig. 1D, left panel). Under reducing SDS-PAGE conditions the full length S protein migrates as a doublet, which was described as two differentially glycosylated forms (Song et al., 2004). The lighter product was suggested to be an ER-resident form of the glycoprotein and the heavier a Golgi-processed form containing complex carbohydrates. Other bands of lower molecular weight were also observed that probably correspond to minor degradation fragments, since they were not present at earlier time points (not shown). Expression levels were similar in lysates of MV-infected Vero-NK cells and SARS-CoV infected VeroE6 cells. As expected, the full-length S protein was only detected in cell lysates. In contrast, Ssol was clearly detected both in lysate and supernatant of MV-Ssol infected Vero cells at 40 h after infection (Fig. 1D, right panel), indicating an efficient secretion. Consistently, the Ssol protein secreted in the cell culture medium was heavier than the Ssol observed within cell lysates, which is in agreement with this glycoprotein being synthesized in an immature form in the ER prior to transfer to the Golgi, from which it is secreted.