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Short communication

RNA immunization can protect mice against foot-and-mouth disease virus

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ABSTRACT

In previous work we have reported the immunization of swine using in vitro-transcribed foot-and-mouth disease virus (FMDV) RNA. With the aim of testing whether RNA-induced immunization can mediate protection against viral infection, a group of Swiss adult mice was inoculated with FMDV infectious transcripts. In most inoculated animals viral RNA was detected in serum at 48-72 h postinoculation. A group of the RNA-inoculated mice (11 out of 19) developed significant titers of neutralizing antibodies against FMDV. Among those animals that were successfully challenged with infectious virus (15 out of 19), three out of the eight animals immunized upon RNA inoculation were protected, as infectious virus could not be isolated from sera but specific anti-FMDV antibodies could be readily detected. These results suggest the potential of the inoculation of genetically engineered FMDV RNA for virulence and protection assays in the murine model and allow to explore the suitability of RNA-based FMDV vaccination in natural host animals.

Foot-and-mouth disease virus (FMDV) is the causative agent of an acute vesicular disease affecting livestock worldwide (Kitching, 2005; Saiz et al., 2002). FMDV belongs to the Picornaviridae family and its genome consists of a single-stranded positive-sense RNA molecule of about 8.5 Kb in length which is translated into the viral structural and non-structural proteins (Martínez-Salas et al., 2008). The FMDV RNA is flanked by two highly structured non-coding regions at its 5′ and 3′ ends, 5′NCR and 3′NCR, respectively harboring the major cis-acting elements required for the viral replication and translation processes (Belsham and Martinez-Salas, 2004). Recently, the replication-defective and attenuated phenotype in swine of FMDV ΔSL-1 viruses carrying a deletion of one of the two stem-loop structures predicted to form the 3′NCR have been reported (Rodriguez Pulido et al., 2009). The finding that delivery of infectious full-length FMDV transcripts was able to generate infectious virus and induce disease in swine as well as the immunization elicited in pigs inoculated with ΔSL-1 transcripts have opened the possibility of RNA-based FMDV vaccination in natural host animals using in vitro-transcribed RNA generated from genetically engineered DNA plasmids (Rodriguez Pulido et al., 2009).

Current FMDV vaccines consist of chemically inactivated virus preparations (Barteling, 2004). This approach has proven useful in preventing the disease but has important constraints as the risk of virus escape and the difficult serological distinction between infected and conventionally vaccinated animals (Doel, 2003; Grubman, 2005). Research focused on the design of new improved vaccines in terms of safety and efficacy has been extensively developed (Beard et al., 1999; Cedillo-Barron et al., 2001; Cubillos et al., 2008; Chinsangaram et al., 1998; Fowler et al., 2008; Pacheco et al., 2005; Sanz-Parra et al., 1999; Wang et al., 2002; Zheng et al., 2006). In this work, the protective capacity of FMDV RNA transcripts in adult mice was evaluated as a first step to RNA vaccination assays in the natural host species.

An RNA vaccine against coxsackievirus B3 has been shown to confer substantial protection in mice against viral challenge (Hunziker et al., 2004). Adult mice are not among natural hosts for FMDV, but are susceptible to infection upon experimental inoculation. The virus is able to replicate for 12–72 h postinoculation (p.i.) and induce neutralizing antibodies (NAbs) that can be detected in infected animals after the decrease of viraemia and remain unchanged up to 30 days (Fernandez et al., 1986). Because of its numerous advantages, the murine model has been widely used for FMDV vaccine development and immune response studies (Barfoed et al., 2006; Borca et al., 1986; Borrego et al., 2006; Collen et al., 1989; Frimann et al., 2007).

We first assayed the feasibility of RNA immunization in mice by inoculation with RNA derived from pO1K/C-S8c1 clone (Baranowski et al., 1998). Resulting O1K/C-S8 transcripts bear the structural proteins of C-S8c1 isolate in the O1K genome, and are infectious for suckling mice and swine (Baranowski et al., 2003; Rodriguez Pulido et al., 2009). Non-replicating O1K-Δ3′NCR RNA

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Table 1
Immunization of mice with in vitro-transcribed FMDV RNA and challenge with infectious virus.

RNA inoculation					Viral challenge	
RNA[a]	Animal	Viral RNA detection[b]		Neutralizing activity[c]	Virus isolation[d]	FMDV antibodies[e]
		48 h p.i.	72 h p.i.			
O1K/C-S8 (10 μg)	1	+	+	25	6.0	+
	2	+	+	50	<1.0	–
	3	+	+	55	6.0	+
	4	+	–	55	<1.0	+
	5	+	+	63	<1.0	+
	6	+	+	30	<1.0	–
	7	+	+	50	<1.0	–
	8	–	+	0	6.0	+
	9	–	ND	40	6.0	+
	10	+	+	0	6.0	+
	11	+	+	0	>4.5	+
	12	+	–	0	>4.5	+
	13	+	+	45	2.5	+
O1K/C-S8 (50 μg)	14	–	+	100*	<1.0	+
	15	–	–	0	>4.0	+
	16	ND	–	40	>3.5	+
	17	+	–	0	6.0	+
	18	–	+	0	6.0	+
	19	+	+	0	<1.0	–
O1K-Δ3′NCR (100 μg)	20–25	–	–	0	ND	ND
–	26–30				2.5–6.0	+
	31				<1.0	–

ND, not determined. In all cases sera were obtained after clotting of blood collected from the tail at the indicated days.

[a] O1K/C-S8 and O1K-Δ3′NCR RNAs were generated from the corresponding plasmids, as described (Saiz et al., 2001). A group of naive non-inoculated animals was included as control for the challenge.

[b] The presence of FMDV RNA in sera was detected by amplification of a conserved region in the 3Dpol gene by RT-PCR (Saiz et al., 2003).

[c] Neutralizing activity is expressed as the percentage of plaque reduction (Mateu et al., 1987) using a 1:10 dilution of sera collected at day 15 p.i. and heat-inactivated relative to pre-immune sera. Values are the average of two or three independent experiments.

[d] Virus isolation from sera collected at day 2 p.ch. Titers ($TCID_{50}$) are expressed as the reciprocal of the highest serum dilution assayed (log_{10}) inducing CPE in 50% of the wells.

[e] Sera samples collected at day 6 p.ch. were used for detection of anti-type C virus antibodies by ELISA (Rodriguez Pulido et al., 2009). Samples were scored as positive when the OD values were at least twice the OD of the corresponding pre-immune sera.

[*] For mouse 14, 50% of plaque reduction was achieved at a serum dilution of 1/250.

bearing a complete deletion of the 3′NCR was inoculated as well as a negative control (Saiz et al., 2001).

Swiss adult mice between 8 and 10 weeks of age were injected into the tibialis anterior muscle with 10, 50 or 100 μg of in vitro-transcribed RNA suspended in sterile phosphate-buffered saline (PBS) in a volume of 50 μl. Sera from blood samples collected at 48 and 72 h after RNA inoculation were assayed for the presence of viral RNA by RT-PCR and used for viral isolation in IBRS-2 cells, as described (Rodriguez Pulido et al., 2009). FMDV RNA could be detected in most animals at both times assayed (Table 1). However, no virus could be isolated from any serum sample from RNA-inoculated mice even after two blind passages (not shown), consistent with the higher sensitivity of the RT-PCR assay used (10^{-2} plaque forming units (pfu) for O1K virus) (Saiz et al., 2003) compared to viral isolation assays. None of the 6 mice inoculated with O1K-Δ3′NCR RNA developed a detectable viraemia.

NAbs are the major protective component in swine and ruminants against FMDV (McCullough and Sobrino, 2004). However, protection in the absence of detectable NAbs has been reported in swine (Cedillo-Barron et al., 2001; Garcia-Briones et al., 2004) and mice (Borrego et al., 2006; Kamstrup et al., 2006). The presence of NAbs in sera of RNA-inoculated mice was analyzed at day 15 p.i. Eleven out of 19 (58%) of the animals inoculated with O1K/C-S8 transcripts were immunized and significant neutralizing activity could be detected in serum (Table 1). Inoculation of the higher RNA dose did not seem to increase the ratio of immunized animals as 70% of mice (9 out of 13) inoculated with 10 μg seroconverted, while only 2 out of 6 animals injected with 50 μg of transcripts developed detectable NAbs. Interestingly, one of the mice inoculated with 50 μg of RNA (mouse 14) developed high titers of NAbs, similar to those detected in mice infected with FMDV or vaccinated with inactivated virus (Borrego et al., 2006). The big variation observed between the groups inoculated with 10 or 50 μg, second including a lower number of animals, might be due to the use of an outbred non-syngeneic mouse strain, with a MHC diversity comparable with those of natural host's populations. Although no relevant differences were observed in pigs inoculated with FMDV transcripts in a range of 150–500 μg (Rodriguez Pulido et al., 2009), a putative activation of the antiviral innate response triggered by high amounts of RNA can not be ruled out and will be investigated in the future using a higher number of animals and a wider range of RNA doses. Our results suggest that FMDV RNA inoculation can generate infectious virus in adult mice and elicit a specific immune response inducing detectable NAbs. On the contrary, delivery of O1K-Δ3′NCR viral transcripts, unable to replicate in cell culture and suckling mice, did induce neither viraemia nor specific antibodies (Table 1). Positive RT-PCR amplification but negative NAbs detection was found in some cases (mice 8, 10–12), suggesting that a replication threshold is required for seroconvertion.

Once proven the immunizing capacity of the FMDV transcripts, the protective efficacy of O1K/C-S8 RNA delivery was tested. All mice inoculated with the infectious transcript (mice 1–19) were challenged with FMDV 98 days after RNA immunization by injection of 10^3 pfu of C-S8c1 virus into the footpad (Borrego et al., 2006). A group of six naive mice (mice 26–31) was included as a control for the efficacy of the challenge. Serum samples from blood collected at days 2 and 6 postchallenge (p.ch.) were used for virus assay and for detection of anti-type C virus antibodies by ELISA, respectively.

ELISA-negative sera at day 6 p.ch. correlated in all cases (mice 2, 6, 7 and 19) with negative results for viral isolation at day 2 p.ch., time reported as yielding the peak of viraemia after FMDV infection (Borrego et al., 2006), indicating that viral challenge had failed in those four mice. On average, the efficacy of FMDV C-S8 infection in Swiss mice was around 75–85% (Table 1 and unpublished data). Animals with positive sera in ELISA but negative for virus isolation (mice 4, 5 and 14) were considered protected as capable of blocking viral replication by effective immunization achieved upon RNA injection. At day 98 p.i. antibodies against FMDV are not expected to be detectable in RNA-inoculated mice unless they have been effectively boosted by viral challenge. This was confirmed by the lack of detection of antibodies in sera from mice 2, 6 and 7, all showing neutralizing activity (Table 1) and giving positive results in ELISA at day 15 p.i. (not shown). Indeed, ELISA titers of sera from protected mice at day 6 p.ch. were about 3 times higher than those detected at day 15 after RNA inoculation (not shown). Consistently, in all three protected animals, induction of NAbs against FMDV had been detected prior to challenge (Table 1). This group included mouse 14 which developed the highest neutralization titers among all the RNA-inoculated animals.

In summary, we have shown in a first experimental approach that adult mice can be immunized by in vitro-transcribed FMDV RNA inoculation. Moreover, this RNA-induced immunization, that will benefit from technical improvement aimed to increase efficiency, can mediate protection against FMDV challenge. These results provide a new tool for virulence studies in the murine model and open the possibility of new experimental approaches for the assay of FMDV attenuated genotypes as RNA-based vaccines in the natural host.

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