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Attenuated Mengo Virus: a New Vector for Live Recombinant Vaccines

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Several features make Mengo virus an excellent candidate for use as a vaccine vector. The virus has a wide host range, including rodents, pigs, monkeys, and most likely humans, and expresses its genome exclusively in the cytoplasm of the infected cell. Stable attenuated strains exist which are deleted for part of the 5′ noncoding region of the genome. Here we report an attenuated Mengo virus recombinant, vLCMG4, that encodes an immunodominant cytotoxic T-lymphocyte epitope of the lymphocytic choriomeningitis virus (LCMV) nucleoprotein. vLCMG4 induced protective immunity against lethal LCMV infection after a single, low-dose immunization in BALB/c mice and elicited an LCMV-specific $CD8^+$ cytotoxic T lymphocyte response. This demonstrates the potential of recombinant Mengo virus vaccines to confer protection against infectious diseases by the induction of cellular immune responses.

With the advent of molecular biology, the engineering of efficacious live recombinant viral vaccines has become possible. These recombinant vaccines are constructed with attenuated strains of otherwise pathogenic viruses, e.g., adenovirus (12, 16) and poliovirus (4, 7, 11), or viruses with relatively mild pathogenicity, such as the poxviruses (17, 24), human rhinovirus (22), or influenza virus (5). The advantage of replicating vectors is that they permit the presentation of foreign antigens via the major histocompatibility complex (MHC) class I pathway and the subsequent induction of cytotoxic T lymphocytes (CTL), which are able to specifically lyse infected cells (31). Requirements for viruses to be used as vectors for live recombinant vaccines are a stably attenuated phenotype, a life cycle not involving integration of the genome, the immunogenic expression of the inserted sequence, easy administration, and failure of the vaccine strain to persist after inoculation.

The pathogenic potential of Mengo virus and the related encephalomyocarditis virus is determined by a poly(C) tract in the 5′ noncoding region of the genome; truncation or deletion of the poly(C) tract leads to a loss of the pathogenic potential (9). Wild-type Mengo virus ($C_{50}UC_{10}$) or encephalomyocarditis virus ($C_{115}UCUC_3UC_{10}$) can cause acute meningoencephalitis in murine hosts, domestic pigs, and baboons (13, 14, 27, 33), whereas disease in humans is rare (8, 25, 28). The Mengo virus genome contains a single open reading frame which codes for a large polyprotein, which is divided into three regions: P1, which is preceded by a 67-amino-acid nonstructural leader peptide, L, of unknown function, is the precursor to the structural proteins; P2 and P3 are the precursors to the nonstructural proteins, including the protease 3C and the polymerase 3D (19). The RNA genomes of Mengo virus and other picornaviruses are expressed exclusively in the cytoplasm of infected cells (20), which makes members of this virus family attractive for the development of vaccine vectors (1–3, 11). Whereas humoral immune responses against foreign sequences have been demonstrated for various recombinant picornaviruses, induction of CTL has been reported only for the Mengo virus system (2). Yet the significance of the immune responses induced relative to protection against foreign pathogens has not been demonstrated to date.

To study the induction of protective immune responses by recombinant Mengo virus vaccines, we used the well-characterized lymphocytic choriomeningitis virus (LCMV; family *Arenaviridae*) model. The LCMV nucleoprotein (NP) contains an epitope which is restricted by the *H-2*^{d}, *H-2*^{q}, and *H-2*^{u} haplotype (18, 30) and which accounts for greater than 96% of the CTL activity induced during an LCMV infection (30). When expressed by a vaccinia virus recombinant, this epitope is able to induce an LCMV-specific protective CTL response in *H-2*^{d} mice but not in *H-2*^{k} or *H-2*^{b} mice (18, 29).

Construction of the Mengo LCMV NP recombinant. The Mengo LCMV recombinant virus vLCMG4 was generated by insertion of the sequence coding for amino acids 117 to 130 of NP into the L-coding region of the Mengo virus cDNA, resulting in plasmid pLCMG4 (Fig. 1). RNA transcripts of pLCMG4 were synthesized with T7 RNA polymerase and transfected into permissive HeLa cells (2, 10). Viable virus, designated vLCMG4, which gave normal size plaques and yielded high virus titers in HeLa cells could be recovered (data not shown). Stocks of vLCMG4 virus were produced by infection of monolayer HeLa cells. The identity of the virus was confirmed by neutralization with Mengo virus-specific antisera and sequencing of the genome through the insert. Recombinant vLCMG4 thus encodes a fusion protein containing 14 amino acids of the LCMV NP inserted into the L peptide.

Protection studies. BALB/c mice (*H-2*^{d}) were immunized with various doses of vLCMG4, ranging from 10^{-1} to 10^6 PFU, and challenged intracranially with $10^{2.5}$ PFU of the virulent Armstrong strain of LCMV (LCMV-ARM) either on day 10 or 42 postimmunization (p.i.). LCMV-ARM provokes an acute lethal infection in BALB/c mice when inoculated intracranially, whereas when inoculated intraperitoneally, mice become transiently infected and mount a strong and long-lasting pro-

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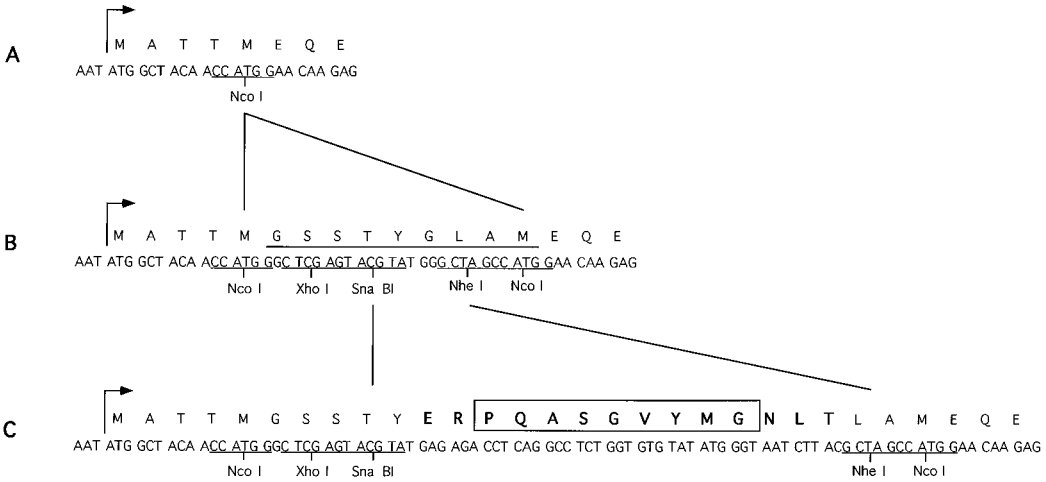

FIG. 1. Construction of vLCMG4. The amino-terminal sequence of the L peptide and the corresponding cDNA sequence are displayed. (A) The pM16 plasmid contains the attenuated Mengo virus cDNA. In the first step, a cDNA cassette containing three unique restriction sites was engineered into the *Nco*I site (nucleotide 729) of pM16, resulting in pMCS (B). Amino acids resulting from linker sequences are underlined. A double-stranded oligonucleotide encoding amino acids 117 to 130 of the NP of LCMV-ARM was inserted into the polylinker of pMCS between restriction sites *Sna*BI and *Nhe*I, resulting in plasmid pLCMG4 (C). LCMV amino acids are given in boldface; the boxed sequence represents the CTL epitope.

tective immune response. Animals immunized with vLCMG4 and challenged at day 10 showed greater than 80% protection from LCMV infection at doses of greater than 10 PFU and partial protection at 1 and 10 PFU (Fig. 2). No protection was observed at 0.1 PFU. Protective immunity induced by vLCMG4 persisted for at least 6 weeks, as demonstrated by the fact that animals were protected when challenged 42 days p.i. (Fig. 2). Long-lasting protective immunity was then observed with doses of vLCMG4 ranging from 10 to 10^6 PFU, but not at 0.1 and 1 PFU. C3H mice ($H\text{-}2^k$) could not be protected from lethal LCMV infection by immunization with 10^6 PFU of vLCMG4 (data not shown), suggesting that the Mengo virus recombinant induced protection in an MHC-restricted manner. This further confirms the results obtained with vaccinia virus recombinants (18, 29), which had previously demonstrated MHC restriction of the NP CTL epitope. In order to verify that LCMV had been eliminated from protected animals, survivors were sacrificed 20 days after challenge and viral antigens were searched for in kidneys by antigen-capture enzyme-linked immunosorbent assay. No virus could be detected in any of the vaccinated animals (data not shown). Furthermore, all surviving animals had high titers of Mengo virus-specific neutralizing antibodies.

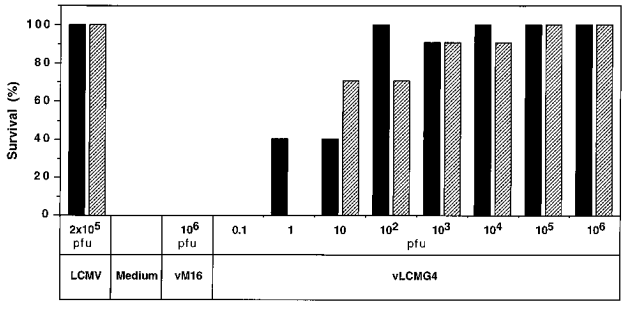

FIG. 2. Protection of BALB/c mice from LCMV infection. Groups of 10 7-week-old, female mice (SPF; purchased from Iffa-Credo, L'Arbresle, France) were immunized intraperitoneally with 0.2 ml of medium, LCMV-ARM, vM16, or vLCMG4 at the indicated doses. Animals were challenged intracranially with $10^{2.5}$ PFU of LCMV-ARM at 10 days (filled bars) or 42 days (striped bars) p.i. Death occurred between day 6 and 8 postchallenge.

Identification of LCMV-specific CTL. In order to study the nature of the effectors of the protective immunity, we analyzed the anti-LCMV cytotoxic activity in splenocytes from mice immunized with vLCMG4 (Table 1). CTL activity against LCMV was tested in the spleens of BALB/c ($H\text{-}2^d$) mice essentially as previously described (32). No CTL activity could be detected when heterologous L929 ($H\text{-}2^k$) target cells were used. However, when the assay was performed with LCMV-infected J774 target cells labelled with ^{51}Cr, splenocytes recovered from mice immunized with LCMV or vLCMG4, but not those from vM16-immunized mice, exhibited LCMV-specific cytotoxic activity. Depletion of $CD8^+$ lymphocytes, with the anti-CD8 monoclonal antibody 31M (21) and Low-Tox-complement (Cederlane, Hornby, Ontario, Canada), abolished this activity, showing that the cytotoxic cells were $CD8^+$ T lymphocytes (Table 1). Thus, Mengo virus allowed the efficient processing of the LCMV antigen and the induction of CTL in an MHC-restricted manner.

The results presented here demonstrate the ability of Mengo virus recombinants to induce a protective CTL immune response after a single low-dose immunization in mice, thus making Mengo virus a primary candidate as an expression vector for different heterologous antigens comprising CTL epitopes in vivo. In fact, human immunodeficiency virus (HIV)-specific CTL have been demonstrated in mice that have been immunized with an HIV recombinant Mengo virus, which codes for 147 amino acids of the HIV type 1 gp120 (2). This is encouraging, as cellular immune responses to HIV are involved in the initial control of HIV replication in primary infections, and CTL might play a major role in protective immunity against HIV in vivo (6, 15).

The RNA nature of the Mengo virus genome and the fact that it induces a self-limiting infection, as well as the stably attenuated phenotype of the poly(C)-deleted Mengo viruses, are properties that meet important safety considerations for its use as a vaccine vector. Given the efficacy of the protective response it elicited and its wide host range in animals, we propose the use of Mengo virus as a novel vector for the

TABLE 1. Evidence for MHC-restricted LCMV-specific $CD8^+$ CTL in vLCMG4-immunized mice

Immunization[a]	Effector/target ratio	% Cytotoxicity[b]			
		L929 ($H\text{-}2^k$) targets	J774 ($H\text{-}2^d$) targets, with the following treatment of effector cells[c]:		
			Medium	Complement	MAb 31M + complement
vM16	50	2 (7)	7 (4)	10 (5)	0 (2)
	25	3 (5)	8 (3)	6 (5)	0 (2)
	12.5	2 (3)	0 (4)	0 (7)	2 (5)
	6.25	0 (3)	0 (4)	0 (7)	0 (5)
vLCMG4	50	1 (4)	22 (2)	35 (0)	2 (6)
	25	2 (3)	15 (0)	17 (2)	3 (0)
	12.5	2 (6)	9 (2)	12 (3)	1 (0)
	6.25	2 (2)	6 (0)	6 (0)	1 (0)
LCMV-ARM	50	1 (1)	34 (4)	37 (6)	4 (3)
	25	1 (4)	20 (6)	22 (4)	4 (2)
	12.5	1 (7)	13 (0)	12 (6)	2 (0)
	6.25	1 (3)	7 (3)	7 (4)	1 (2)

[a] Effector splenocytes from groups of five female BALB/c mice, immunized intraperitoneally with vM16 (1×10^6 PFU), vLCMG4 (1×10^6 PFU), or LCMV-ARM (2×10^5 PFU), were stimulated in vitro for 3 days with phorbol myristate acetate (1 ng/ml) and ionomycin (100 ng/ml) prior to the CTL assay (23, 26).

[b] Percent cytotoxicity was calculated according to the following formula: (experimental release − spontaneous release)/(maximum release − spontaneous release), where spontaneous release was determined in the absence of effector cells and maximum release was determined in the presence of 1% Triton X-100. Results are expressed as percent cytotoxicity with LCMV-infected target cells and, in parentheses, with uninfected target cells. MAb, monoclonal antibody.

[c] Efficiency of depletion was controlled by FACScan analysis (11a).

engineering of live recombinant vaccines for use in animals and possibly in humans.

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