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Long-lasting balanced immunity and protective efficacy against respiratory syncytial virus in mice induced by a recombinant protein G1F/M2

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Abstract

Respiratory syncytial virus (RSV) is the primary cause of serious lower respiratory tract illness in young children. We have engineered a recombinant candidate vaccine G1F/M2, consisting of a cytotoxic T lymphocyte (CTL) epitope of RSV-M2 protein and a domain of RSV-G protein. In this study, the long-term immunogenicity and protective effect were evaluated. In G1F/M2-immunized mice, special antibodies lasted for more than 19 weeks, and the IgG1/IgG2a ratio remained a balanced level till the end of the study, suggesting mixed Th1/Th2 type of responses. Concomitantly, G1F/M2 elicited long-lived RSV-specific CTL activity that was detectable at 12 weeks after the final immunization. Stronger CTL responses were induced with immunization once more at 13 weeks after the last immunization in G1F/M2-primed mice than those in F/M2-primed mice. These results suggest that G1F/M2-induced long-lasting balanced humoral and cellular immunity responses, and immunological memory in mice. Furthermore, following RSV challenge, long-term protective efficacy was observed. RSV replication in lungs of G1F/M2-primed mice elicited also mixed Th1/Th2 responses, a property that is considered advantageous for the safety of an RSV vaccine. Therefore, G1F/M2 is a promising RSV subunit vaccine.

Keywords: Respiratory syncytial virus (RSV); Recombinant vaccine; Long-lasting protection

1. Introduction

Respiratory syncytial virus (RSV) is the primary cause of serious lower respiratory tract illness in young children; nearly all children have been infected with RSV by age 2 [1]. RSV may also cause significant disease in the elderly and those with compromised cardiac, pulmonary or immune systems [2,3]. Outbreaks of RSV occur each year. RSV infections cause hospitalization of nearly 125,000 children and 1800 deaths in the United States annually, resulting in an estimated expenditure of US$ 500 million per year [4]. The rate of hospitalization due to RSV infections worldwide is even higher with mortality rates approaching 5%. Despite three decades of effort, there is no licensed vaccine to protect children against RSV infection. Development of a safe and effective RSV vaccine has become a priority.

Vaccine development has been slowed by the catastrophic consequences seen with an older RSV vaccine. Studies of a formalin-inactivated vaccine (FI-RSV) observed more severe lower respiratory illness after exposure to natural RSV infection among naive vaccinees compared with controls [5]. This enhanced pulmonary disease may have been associated with an imbalance in the immune response favoring an exaggerated Th2-type response. The Th2 polarization has been related to the absence of a Th1-promoting RSV-specific CTL response after immunization with FI-RSV [6,7]. We have recently engineered a recombinant candidate vaccine G1F/M2, consisting of a CTL epitope (M2: 81–95) of

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RSV-M2 protein and a domain of RSV-G (G: 125–225, G1) [8]. The two epitopes were chosen for several reasons: (1) the G protein is one of the alterprinciple RSV immunogens in humans and rodents [9]; (2) G1 fragment of RSV G protein includes residues (164–173, 163–190, 160–189) those are completely conserved in all known RSV isolates [9–11]; (3) DsbA-G1-induced strong RSV specific IgG antibody and neutralizing antibody in BALB/c mice [8,12]; (4) M2: 81–95 is a strong CTL epitope [24]. G1 and F/M2: 81–95 gene fragments were ligated into a bacterial expression plasmid pET-His. Expressed fusion protein G1F/M2 in *E. coli* was efficiently purified by affinity chromatography on Ni^{+}-Sepharose. In BALB/c mice, fusion protein G1F/M2 induced not only humoral immunity but also RSV-specific CTL response. In addition, interestedly, G1F/M2 elicited balanced IgG1/IgG2a responses, suggesting a mixed Th1/Th2 type of response [8]. Although CTLs play an important role in protective immunity against RSV [13], the longevity of CTL responses in vivo is usually very short. There are evidences to show that induction of long-lasting CD8+ CTL responses in vivo often requires the concomitant participation of CD4+ Th1 cells during immune induction [14–17]. Th1 cells, characterized by secretion of IFN-γ and TNF-α, are primarily responsible for activating and regulating the development and persistence of CTL, and important in the maintenance of CD8+ T cell memory and facilitate their survival [18–20]. Since G1F/M2 induced both mixed Th1/Th2 and CTL responses in mice, the CTL response should be long-lived.

In this study, we investigate the long-term immunogenicity and protective effect of G1F/M2 in BALB/c mice. G1F/M2 induced a strong, long-term balanced IgG1/IgG2a (Th2/Th1) and long-lived CTL responses, and showed long-lasting protection against RSV. In addition, following RSV challenge, balanced Th1/Th2 responses were exhibited in G1F/M2-primed mice, unlike FI-RSV inducing the polarizing Th2 immune response in mice or vaccinees [5–7].

2. Materials and methods

2.1. Respiratory syncytial viruses preparation

RSV-A (long strain) was propagated in HEp-2 cells as previously described [21]. Viruses were harvested after 48–72 h by scraping attached cells into the medium, centrifuging the suspension at $460 \times g$ for 15 min, and collecting the supernatant purified by a discontinuous sucrose gradient as previously described [22,23]. RSV was stored at $-70\,^{\circ}$C until use. The pellet was resuspended in PBS (pH 7.4) to prepare viral protein ELISA antigen and stored at $-20\,^{\circ}$C. Approximately 1 μg of RSV antigen, diluted in carbonate–bicarbinate buffer pH 9.6, was applied per well in a 96-well polystyrene ELISA plate. Uninfected HEp-2 cells were prepared in a similar manner as cell control ELISA antigens.

2.2. Production and purification of G1F/M2 molecule

Gene assembly, vector constructions, expression, and first-step protein purification of G1F/M2 were undertaken as previously described [8]. G1F/M2 containing fractions visualized by SDS-PAGE were pooled individually and freeze-dried. Protein content was determined by BCA method. Proteins were analyzed for purity and antigenicity by SDS-PAGE on a 12% homogeneous gel and Western blots using RSV-A-specific serum, respectively.

2.3. Mice

Female BALB/c mice, aged 6–9 weeks, were purchased from Beijing Laboratory Animal Center (Beijing), housed and manipulated according to the Care and Use of Laboratory Animals (Beijing), and kept under specific pathogen-free conditions. They were confirmed seronegative to RSV before inclusion in the studies.

2.4. Immunizations and RSV challenge

Mice were immunized with 1.7 nmol doses of G1F/M2, G1, F/M2, or PBS alone, intraperitoneally (i.p.) with a total 200 μl volume, in PBS containing 20% alhydrogel (v/v) or i.n. instillation with 10^{5} plaque form unit (pfu) RSV-A. An interval of 10 days was invariably employed for second, third and subsequent immunizations. Serum at 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 23 weeks after the first immunization were tested for anti-RSV-A antibody titers. Mice were challenged 2, 11 or 19 weeks after the last immunization with 10^{6} pfu/50 μl RSV-A by i.n. instillation, and sacrificed 3, 5, 7 or 12 days post-challenge, respectively to determined IgG subclass. In order to detect the length of immunoprotective ability, animals were challenged 3, 7, 11, 15, or 19 weeks with 10^{6} pfu of RSV-A after the last immunization, sacrificed 5 days post-challenge, which corresponds to the peak of viral growth, and their lungs were removed for RSV viral titers (viral titration) post-challenge. To determine CTL activity, half of BALB/c mice immunized with G1F/M2, F/M2 or PBS were sacrificed at 1 or 12 weeks after the last immunization, and the other half were immunized once more, and were sacrificed at 1 week after the fifth immunization. Spleens were removed, and splenocytes were tested for CTL activity.

2.5. ELISA

Anti-virus antibody titers in serum samples were assessed by standard ELISA protocol [21]. Briefly, microtiter plates were coated overnight at $4\,^{\circ}$C with 50 μl per well of RSV-A or HEp-2 cell protein preparations. The plates were washed thoroughly with PBS + 0.05% of Tween-20 (PBST) and then blocked with 200 μl of PBS containing 1% BSA for 2 h at $37\,^{\circ}$C. Twofold serial diluted serum samples were allowed to react with coated plates at $37\,^{\circ}$C for 2 h, followed by