HIGHLY ATTENUATED VACCINIA VIRUS DIs AS A POTENTIAL SARS VACCINE

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1. INTRODUCTION

Severe acute respiratory syndrome (SARS) is a newly found infectious disease caused by a novel coronavirus, SARS coronavirus (SARS-CoV).[1,2] DIs strain is a highly restricted host range mutant of vaccinia virus. It does not replicate in and is not pathogenic for mice, guinea pigs, or rabbits, and this strain does not replicate in various mammalian cell lines.[3] Recently, we have established a system for expressing foreign genes.[4] In the present study, we constructed recombinant forms of the DIs containing the gene encoding four structural proteins, envelope (E), membrane (M), nucleocapsid (N), and spike (S), of SARS-CoV either separately or simultaneously. Mammalian cells infected with the recombinant DIs synthesized SARS-CoV proteins that were recognized by SARS patient serum or rabbit antibody raised against synthetic peptides of SARS-CoV proteins in Western blot analyses. Intranasal or subcutaneous inoculations of BALB/c 3T3 mice with the recombinant DIs expressing E/M/S or E/M/N/S proteins elicited neutralizing antibodies to SARS-CoV and protective immunity. Therefore, our study showed that the replication-deficient DIs strain is feasible as a safe and effective SARS vaccine.

2. RESULTS

2.1. Expression of SARS-CoV Structural Proteins by Recombinant DIs

Expression of SARS-CoV N and S proteins were detected by western blotting using monoclonal antibodies.[5] A robust signal was detected at 50 kDa, corresponding to the N

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protein of SARS-CoV as predicted by its genome size.[1,2] A band near 200 kDa appears to correspond to S protein, which is known to be heavily glycosylated. Concerning the M protein, only a smear band in the stacking gel was detected using a polyclonal antibody against synthetic peptide of the M protein,[6] presumably because it formed large ologomers with SDS-resistance in cells. Similar result was observed by the analysis of the M protein of avian coronavirus infectious bronchitis virus.[7]

The subcellular localization of the S, M, and N proteins were analyzed by immunofluorescence microscopy. Cells infected with DIs-M or DIs-N showed that M and N proteins were localized mainly at the Golgi complex, which is consistent with studies with model corona viruses in which it was found that M is retained in the Golgi apparatus.[8] S protein was localized at the Golgi complex but the plasma membrane was also stained, suggesting that some portion of S protein was transported to the plasma membrane. Thus, these results indicate that cells infected with recombinant DIs under the control of the mH5 promoter express significant levels of SARS-CoV proteins with an expected post-translational processing.

2.2. Recombinant DIs Induces Serum IgG Antibody Specific for SARS-CoV

To examine the level of anti-SARS-CoV response in mice after inoculation with recombinant DIs, 4 mice in each group were subcutaneously or intranasally inoculated three times with 10^6 pfu of recombinant DIs that expressed N, M, S, E/M/S, or E/M/N/S. Ten days after the final inoculation, vaccinated mice elicited anti-SARS-CoV IgG antibody in sera at high levels. Mice vaccinated subcutaneously with DIs-E/M/S or DIs-E/M/N/S elicited the highest levels of anti-SARS-CoV IgG.

Whether the immune sera possess the neutralizing activity against SARS-CoV is a crucial aspect of vaccination. We next estimated the neutralizing activity against SARS-CoV of antisera obtained (Table 1). Neutralizing antibodies against SARS-CoV were induced in mice that were either subcutaneously or intranasally injected with DIs-S, -E/M/S, or –E/M/N/S. Among them, the highest level of the neutralizing activity was observed in sera of mice injected subcutaneously with recombinant DIs expressing E, M, N, and S. On the other hand, we could not detect neutralizing activity in sera of mice injected either subcutaneously or intranasally with recombinant DIs expressing M or N proteins. Thus, these results indicate that recombinant DIs induces potent SARS-CoV–specific neutralizing antibodies. It appears that the S protein is prerequisite for eliciting a sufficient level of IgG antibodies with neutralizing activity.

3. DISCUSSION

Highly attenuated vaccinia viruses can express viral and inserted genes at high levels even in nonpermissive cells without showing CPE. rDIs exhibited no replicative ability and produced no infectious virions in these cells, indicating that the DIs strain may have a safety advantage when used as a recombinant vaccine vector.

Efforts directed at vaccine development for SARS-CoV have been carried out by many organizations in variable ways.[8–13] Our results showed that intranasal or subcutaneous inoculations of Balb/c mice with DIs-E/M/S or DIs-E/M/N/S produced

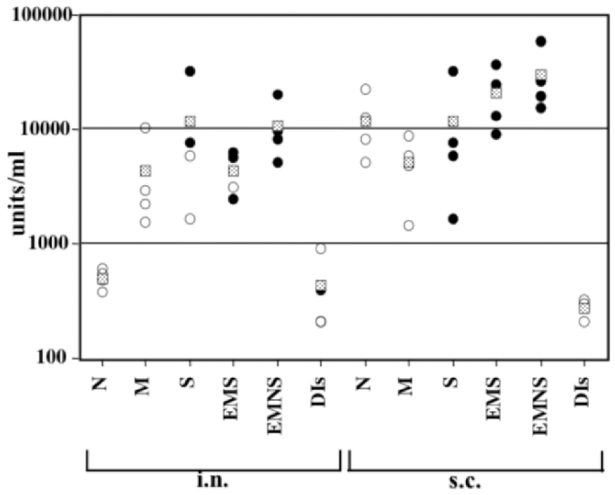

Figure 1. The levels of IgG antibodies against SARS-CoV. Neutralizing-positive sera were shown as closed circles and neutralizing-negative sera were shown as open circles. Averages were shown as dotted boxes.

serum antibodies that recognized the SARS-CoV virion in ELISA and neutralized SARS-CoV *in vitro*. Moreover, DIs-S administered by either route elicited protective immunity, as shown by reduced titers of SARS-CoV in the lungs of mice after challenge. Subcutaneous route appears to be stronger than intranasal rout with respect to the level of anti-SARS-CoV IgG antibody.

In this study, we constructed recombinant forms of the DIs containing the gene encoding four structural proteins of SARS-CoV either separately or simultaneously. Intranasal or subcutaneous inoculations of BALB/c 3T3 mice with the recombinant DIs expressing E/M/S or E/M/N/S proteins elicited neutralizing antibodies to SARS-CoV Therefore our study showed that the replication-deficient DIs strain is feasible as a safe and effective SARS vaccine.

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