FWY Sin 冼韻儀
SCS Cheng 鄭澤森
KT Chan 陳健德
L Meng 孟　莉
J Yu 愈　靜
Y Xie 謝　雍

Mouse studies of SARS coronavirus–specific immune responses to recombinant replication-defective adenovirus expressing SARS coronavirus N protein

Key Messages

1. A recombinant adenovirus encoding SARS coronavirus (SARS-CoV) nucleocapsid protein (rAd-N) was constructed.
2. The ability of the rAd-N to induce anti–SARS-CoV N antibody production and cellular immune responses was evaluated in an HLA-A2.1/Kb transgenic mouse model.

Introduction

This project aimed to test a new vaccine technology platform using an adenovirus vector to produce a recombinant vaccine against severe acute respiratory syndrome (SARS) infection. A recombinant adenovirus (rAd) encoding SARS coronavirus (SARS-CoV) nucleocapsid protein (rAd-N) was constructed, and the ability of the rAd-N to induce anti–SARS-CoV N antibody production and cellular immune responses was evaluated. Evidence from human gene therapy and vaccine trials has established a good safety record for Ad5- and Ad2-based vectors. Both vectors are safe when administered by intramuscular and intradermal injection, as well as via intranasal inhalation, which may be a particularly important means of inducing mucosal immunity to respiratory infectious diseases such as SARS. Recently, exciting results from human immunodeficiency virus (HIV) vaccine research into an adenovirus type 5 (Ad 5) vector have emerged from primate and human clinical trials.

Materials and methods

This study was conducted from July 2004 to June 2006. SARS-CoV N protein was expressed and purified from bacteria. The DNA fragment encoding the SARS-CoV N protein was generated by a polymerase chain reaction using the following primers: AdN-5' primer (GGAATTCCATATCTCTGATAATGGACCCCAATC) and AdN-3' primer (CATGGGATCCGCCTGAGTTGAATCAGCAG) and cloned into the pET22b(+) vector (Novagen) in fusion with 10 histidines. The sequence of the SARS-CoV N was confirmed, and the resulting plasmid was subsequently transformed into BL21-CodonPlus (DE3)-RIL (Stratagene). Expression of the SARS-CoV N protein in bacterial cells was induced using 0.4 mM IPTG (Calbiochem) for 4 hours at 30ºC. Soluble N protein was purified by His-Bind Kit (Novagen). Anti-N protein monoclonal antibodies were produced using 6-week-old female Balb/c mice (Animal and Plant Care Facility, HKUST). A hybridoma cell line yielding anti-N antibodies was produced and kept in liquid nitrogen for long-term storage.

QBI-HEK 293 cells (Q-biogene) are a strong plastic-attached sub-clone of HEK-293 cells (primary human kidney) containing the E1A and E1B Ad 5 viral genes for the generation and titration of rAd. Human cervix epithelial adenocarcinoma GH329 cells (ATCC) were stably transfected with plasmid carrying Ad 5 E1a and E1b open reading frames and part of the *pIX* gene. The cells were used for propagation of the rAd, and were maintained in culture according to the supplier's specifications.

The rAd-N encoding the SARS-CoV nucleocapsid protein was generated. Briefly, a full-length SARS-CoV N gene was inserted into the Transpose-Ad transfer vector, pCR259 (Q-biogene), to generate pCR-AdN. Integrity of the pCR-AdN was checked using restriction endonuclease cleavage and DNA sequencing. pCR-AdN was subsequently transformed into HighQ-1 Transpose-Ad 294 bacteria to generate a plasmid, p294-AdN. p294-AdN was linearised and transfected into QBI HEK-293 cells for the generation of rAd, rAd-N. rAd-N and rAd-lacZ (provided

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Department of Biology, The Hong Kong University of Science and Technology
FWY Sin, SCS Cheng, KT Chan, L Meng, J Yu, Y Xie

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Principal applicant and corresponding author:
Prof Yong Xie
Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, China
Tel: (852) 2358 7340
Fax: (852) 2358 1559
E-mail: boyxie@ust.hk

by the supplier) were propagated in GH329 cells and purified by an Adeno-X virus purification kit (BD Bioscience). Titres (Tissue culture infectious dose, $TCID_{50}$) of the rAds were determined by their end-point dilutions. For infection, HEK 293 cells were given a multiplicity of infection (MOI=20 for 24 h) with rAd-N and rAd-lacZ. Expression of the SARS-CoV N protein was checked using a Western blot analysis of the infected cell lysate, using a monoclonal antibody against SARS-CoV N protein as described above.

Vaccination of the HLA-A2.1/Kb transgenic mice: HLA-A2.1/Kb transgenic mice (Mutant Mouse Regional Resource Centers, US) were bred and maintained at the Animal Care Centre (HKUST). All procedures were performed in accordance with the international guidelines for experimental animals. Three groups (five mice per group) of 6-to-8-week-old female HLA-A2.1/Kb transgenic mice were used for vaccination via intraperitoneal injection according to the following scheme: group 1 was immunised with PBS using a dosage of 0.1 mL/mouse; group 2 was immunised with 1×10^8 $TCID_{50}$ rAd-N using a dosage of 0.1 mL/mouse; group 3 was immunised with 1×10^8 $TCID_{50}$ rAd-LacZ using a dosage of 0.1 mL/mouse. The mice were killed 3 weeks after immunisation, and examined for both humoral and cellular immune responses using the enzyme-linked immunosorbent assay (ELISA) and IFN-γ ELISPOT assay, respectively.

Results and discussion

SARS-CoV N protein was expressed in BL21-CodonPlus (DE3)-RIL after IPTG induction. Purified soluble N protein was checked using SDS-PAGE. After IPTG induction, an additional band of 49kDa was found in the total cell lysate, which is consistent with the predicted size of the His-tagged recombinant N protein. After purification, a protein preparation consisting of a single band of the ~49kDa recombinant N protein was obtained (Fig 1a). Expression of the SARS-CoV N protein from the rAd was demonstrated by infecting the HEK 293 cells with rAd-N and rAd-lacZ at MOI=20 for 24 h. After viral infection, cell lysate was prepared and the expression of the SARS-CoV N protein was confirmed by Western blot. A specific band of the N protein was clearly observed in the rAd-N infected HEK-293 cells by using monoclonal antibodies against the SARS-CoV N protein, whereas no N protein band was found in the parental cells and the cells infected by the rAd-lacZ (Fig 1b). The result indicated that infection of the rAd-N is sufficient to mediate the SARS-CoV protein expression.

To assess the humoral immune response upon vaccination with rAd-N, sera from mice immunised with either 1×10^8 $TCID_{50}$ rAd-N, rAd-lacZ or PBS, were collected and antibodies against SARS-CoV N protein were measured by ELISA. The result revealed a significant increase in antibody level against the SARS-CoV N protein, suggesting that immunisation of rAd-N was able to induce the production of anti-N protein antibodies in vivo (Fig 2).

The cellular immune response is a major component

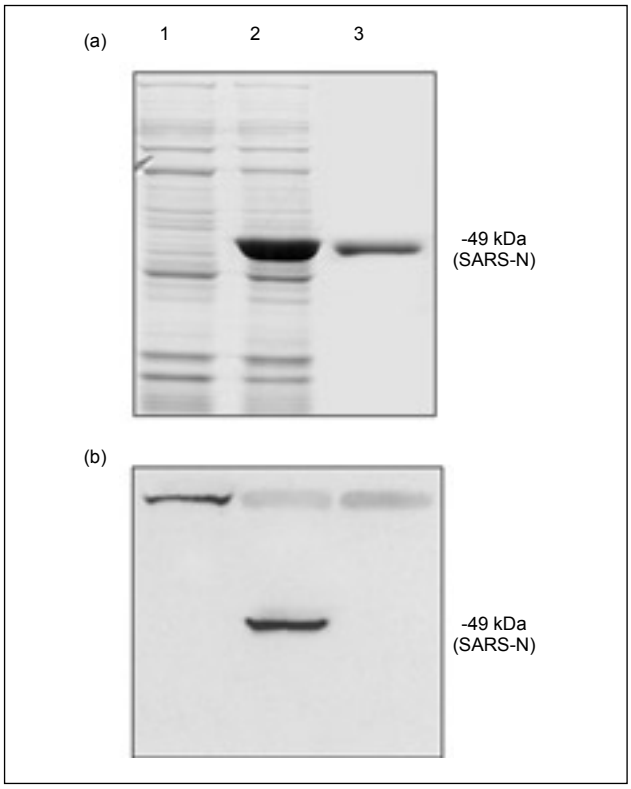

Fig 1. (a) Expression of SARS-CoV N protein in BL21-CodonPlus (DE3)-RIL
Coomassie blue staining: lane 1, total cell lysates before IPTG induction; lane 2, total cell lysates after 4 h IPTG induction at 30°C; lane 3, purified recombinant N protein
(b) Expression of SARS-CoV N protein in infected HEK-293 cells
Lane 1, uninfected cell lysates; lane 2, rAd-N infected cell lysates; and lane 3, rAd-LacZ infected cell lysates

of the immune system's weaponry against viral infection. The specific cellular response elicited by the rAd-N after immunisation was assessed by the secretion of IFN-γ by mouse splenocytes stimulated in vitro with recombinant SARS-CoV N protein for 40 h. The results clearly showed that rAd-N immunisation stimulated a substantial elevation of the IFN-γ-secreting T-cell response upon N protein stimulation compared with rAd-LacZ and PBS immunisation (Fig 3). This suggests that immunisation with rAd-N was able to trigger a SARS-CoV–specific T-cell response in the HLA-A2.1/Kb transgenic mice.

An effective vaccine must be able to raise a protective response from B and T cells after exposure to the viral agent. Different approaches have been used to produce a vaccine against SARS-CoV infection, including the use of inactivated SARS-CoV particles,[1] recombinant virus-like particles,[2] DNA,[3] recombinant proteins,[4] and recombinant viruses.[5]

In this study, a recombinant replication-defective adenovirus encoding the SARS-CoV nucleocapsid protein, rAd-N, was constructed (Fig 1) and its ability to induce the production of anti-SARS CoV N antibodies and cellular

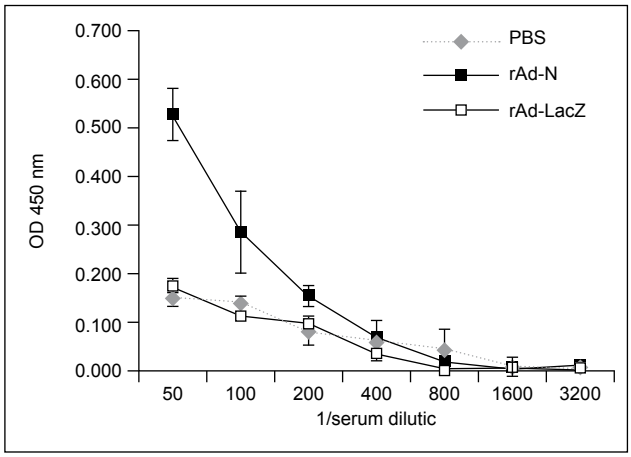

Fig 2. Antibody response against SARS-CoV N protein induced by rAd-N immunisation
Sera obtained from mice immunised either with 1×10^8 $TCID_{50}$ rAd-N, rAd-LacZ or PBS at 3 weeks after immunisation. The presence of anti-SARS-CoV N protein antibodies was detected by ELISA. Values represent the mean OD values measured from the sera of three individual mice

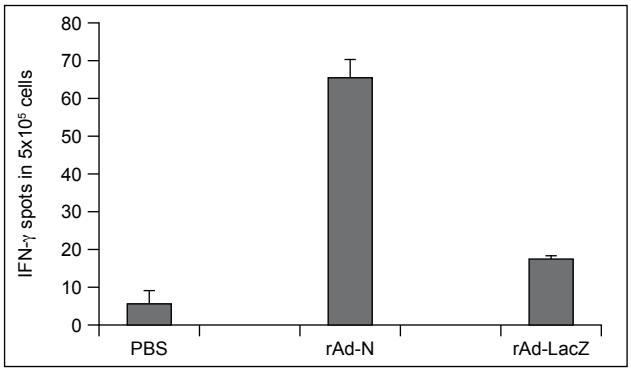

Fig 3. Cellular immune response induced by rAd-N immunisation
Splenocytes obtained from mice previously immunised either with 1×10^8 $TCID_{50}$ rAd-N, rAd-LacZ or PBS, were cultured in the presence of recombinant N protein. Secretion of IFN-γ was measured by ELISPOT. Results represent the mean±standard deviation (n=3, 5 mice per group)

immune responses was evaluated. Upon infection with the rAd, the infected HEK-293 cells expressed the SARS-CoV protein (Fig 1). The protein expressed within the infected cells was subjected to proteasome digestion and the resulting peptide was transported into the endoplasmic reticulum via the TAP protein, and eventually loaded onto the MHC class I molecule to elicit a cytotoxic T-cell response. Effective presentation of the viral derived peptides depends mainly on the high affinity binding of the MHC-I molecules towards the immunogenic peptides. Although previous studies have shown that the SARS-CoV protein expressed using adenoviral vectors is able to elicit an immune response against SARS protein, their assays were performed using normal murine models including C57, Balb/c and 129S6/SvEv. Since the peptide binding patterns of the MHC molecules from different animal species are so disparate, using normal murine models for such studies cannot reflect the T-cell response exhibited by humans. Our study used the HLA-A2.1/Kb transgenic mouse model which expresses human MHC-I molecules, and this model has been used for studying the immunogenicity of class I–restricted $CD8^+$ cytotoxic T lymphocyte (CTL) responses. In a previous study, the CTL response elicited by HLA-A2.1/Kb transgenic mice after infection with the influenza virus A/PR/8/34 was directed against the same dominant epitope recognised in humans expressing the HLA-A*0201 antigen. Subsequent studies using a panel of 38 different epitopes demonstrated that there is a good correlation between the CTL repertoire of these transgenic mice and HLA-A*0201–positive human individuals. Therefore, the model we used to investigate the immunogenicity of the rAd, rAd-N, is a more appropriate animal model for assessing the potency of a viral vaccine. The IFN-γ ELISPOT assay demonstrated that vaccination with rAd-N induces IFN-γ production from T cells upon SARS-CoV stimulation (Fig 3). Activation of T cells not only resulted in the elimination of infected cells, but the production of IFN-γ could also block or even lead to the elimination of virus from infected cells. Our ELISA data clearly show that the recombinant N protein can induce a strong humoral response against SARS-CoV N protein in vivo (Fig 2). Although the N protein is enclosed within the virus particle and expresses fewer neutralisation-mediated determinants, the antibody against the N protein can promote a T-cell response by forming an immune-complex through cross-presentation. Our results show that rAd-N can generate strong SARS-CoV–specific humoral and cellular immunity and may potentially be used as a SARS-CoV vaccine. Pre-existing immunity to wild-type human adenovirus in adult humans may affect the efficacy of Ad5-based vaccines against SARS-CoV. Nonetheless, modifying the adenovirus vector's surface chemically or with formulations able to mask antigenic determinants that can be recognised by neutralising antibodies may circumvent this problem. Also, the existence of approximately 50 identified adenovirus serotypes in humans and many other adenovirus of animal origin provides a great degree of genetic flexibility for designing sequential vaccine vectors. Overall this study provides valuable information aiding development of anti-SARS vaccines and other vaccine candidates for disease prevention in the future.

Acknowledgement

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