Available online at www.sciencedirect.com
ScienceDirect
Cellular Immunology 247 (2007) 85–94
Cellular Immunology
www.elsevier.com/locate/ycimm

Recombinant Sendai virus induces T cell immunity against respiratory syncytial virus that is protective in the absence of antibodies

Brigitte Voges [a,1], Simone Vallbracht [b,1], Gert Zimmer [a], Sascha Bossow [c], Wolfgang J. Neubert [c], Kirsten Richter [d], Elias Hobeika [e], Georg Herrler [a], Stephan Ehl [b,*]

[a] *Institut für Virologie, Stiftung Tierärztliche Hochschule Hannover, Bünteweg 17, 30559 Hannover, Germany*
[b] *Zentrum für Kinderheilkunde und Jugendmedizin, Universitätsklinik Freiburg, Childrens Hospital, Mathildenstrasse 1, 79106 Freiburg, Germany*
[c] *Max-Planck-Institut für Biochemie, Am Klopferspitz 18, 82152 Martinsried, Germany*
[d] *Institut für med. Mikrobiologie und Hygiene, Universitätsklinik Freiburg, Hermann-Herder-Strasse 11, 79104 Freiburg, Germany*
[e] *Max-Planck-Institut für Immunbiologie, Stübeweg 51, 71908 Freiburg, Germany*

Received 20 April 2007; accepted 24 July 2007
Available online 29 September 2007

Abstract

Respiratory syncytial virus (RSV) causes severe respiratory disease in infants and a vaccine is highly desirable. The fusion (F) protein of RSV is an important vaccine target, but the contribution of F-specific T cells to successful vaccination remains unclear. We studied the immune response to vaccination of mice with a recombinant Sendai virus expressing RSV F (rSeV F). rSeV F induced protective neutralizing antibody and RSV F-specific CTL responses. T cell immunity was stronger than that induced by recombinant vaccinia virus (rVV F), a well characterized reference vector. Vaccination of antibody-deficient mice showed that vaccine-induced RSV F-specific T cells were sufficient for protective immunity. rSeV F induced T cell immunity in the presence of neutralizing antibodies, which did not impair the vaccine response. Although the F protein only contains a subdominant CTL epitope, vaccination with rSeV F is sufficient to induce protective T cell immunity against RSV in mice.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Virus; T cell; Vaccine; Lung; Cellular immunity

1. Introduction

Human respiratory syncytial virus (RSV) is a major cause of serious pediatric lower respiratory tract infection [1]. Despite intensive efforts, no licensed safe and effective vaccine is currently available. At present, the most promising approach is the development of a live attenuated RSV vaccine generated by targeted manipulation of the RSV genome [2]. It is still unclear, however, whether such a vaccine will be appropriate for all target populations, in particular for infants with maternal antibodies, high-risk infants receiving prophylaxis with RSV-specific monoclonal antibodies or for the elderly who have neutralizing antibodies due to previous exposure [3]. It therefore appears reasonable to further evaluate additional vaccine approaches including recombinant subunit vaccines.

Since both neutralizing antibodies and T cells can contribute to protective immunity, a subunit vaccine should have the potential to induce both types of antiviral immune responses. The F protein of RSV appears particularly well suited for this purpose, because it can elicit neutralizing antibodies that have been shown to be protective in humans [4] and because in addition it induces T cell responses in humans [5] and in mice [6,7]. In the last 20 years, a large number of recombinant viruses expressing RSV F have been studied in animal models [8–18]. These vectors induced variable degrees of protection against RSV, in most cases accompanied by neutralizing antibody

* Corresponding author. Fax: +49 761 270 4481.
E-mail address: stephan.ehl@uniklinik-freiburg.de (S. Ehl).
[1] These authors contributed equally to this work.

0008-8749/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellimm.2007.07.005

responses. Only a few groups have analyzed the induction of RSV F-specific T cells, usually by IFN-γ ELISPOT or ELISA after in vitro restimulation [12,16,18,19]. In none of these studies, however, the question was addressed whether these RSV F-specific T cells are functional in the lung and whether they make a contribution to protective immunity independent of neutralizing antibodies.

Murine parainfluenza virus (Sendai virus, SeV) is currently studied in clinical trials as a vaccine for its closely related human counterpart (human parainfluenza virus type 1) [20]. A reverse genetics system for SeV has been established and a SeV recombinant expressing RSV G has been studied in mice and cotton rats [21,22]. We have recently generated a recombinant Sendai virus expressing the F protein of RSV (rSeV F) [23] and have now constructed an additional virus encoding a soluble form of RSV F (rSeV sF). In the present study, we used these chimeric viruses to analyze the extent and protective capacity of RSV F-specific T cell responses induced after intranasal immunization with a heterologous recombinant virus. Both viruses not only induced neutralizing antibodies, but also a pulmonary antiviral F-specific T cell response that could mediate complete antiviral protection independent of antibodies. This T cell response could also be induced in the presence of pre-existing antibodies.

2. Materials and methods

2.1. Viruses

Recombinant SeV expressing either DsRed or a full-length RSV (Long strain) F protein from an additional transcription unit between the P and M genes was described previously [23]. SeV containing a truncated version of the F gene (sF) lacking the 3′ terminal 50 amino acids was generated accordingly. SeV was propagated on Vero cells in DMEM supplemented with 1 μg/ml of acetylated trypsin. Infection stocks were prepared by centrifugation through a 20% sucrose cushion at 100,000*g* for 90 min at 4 °C, and resuspended in PBS. Titration of SeV was performed as described previously [23]. HRSV A2 (kindly provided by Geraldine Taylor, Institute for Animal Health, Compton, GB) was grown on 16HBE14o$^-$ cells in DMEM/Ham's F12 medium (1:1) supplemented with 5% fetal calf serum (FCS) and non-essential amino acids. RSV titers from lung homogenates were determined as described [24]. Recombinant vaccinia virus expressing the F protein of RSV (rVV F) or the NP protein of LCMV (rVV NP) were kind gifts from Gail Wertz (University of Alabama, Birmingham, USA) and Rolf Zinkernagel (Institute for Experimental Immunology, Zürich, Switzerland), grown on BSC-40 cells and kept at −80 °C.

2.2. Western blot analysis

Vero cells grown on 6-well plates were infected with RSV (strain A2) or rSeV with an m.o.i. of 0.1. Two days post-infection, twofold-concentrated SDS sample buffer was added to the supernatant of non-infected and infected cells. Cells were lysed with NP40 lysis buffer (50 mM Tris/HCl, pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP40, protease inhibitors) as described [25]. Samples were separated under non-reducing conditions on 10% SDS–polyacrylamide gels and transferred to nitrocellulose membranes. A mouse anti-RSV F antibody (Dianova, Hamburg, Germany) was used for immunostaining as described previously [23].

2.3. Mice and infection

Specific-pathogen-free BALB/c mice were obtained from Charles River (Sulzburg, Germany) and Taconic (Ry, Denmark) and used at 6–12 weeks of age. Ig$\alpha^{-/-}$ BALB/c mice of the strain *Mb-1 MerCreMer* were generated by insertion of a cDNA encoding for a tamoxifen-inducible Cre (MerCreMer) into the *mb-1* gene locus endoding for Ig-alpha [26]. B lymphocytes of these mice do not express Ig-alpha and cannot mature beyond the pro-B cell stage. Mice were kept in a venti-rack (BioZone, Kent, UK). For immunization studies, animals were lightly anaesthetised with diethylether or with ketamine and xylazine and inoculated intranasally with 4×10^5 pfu of rSeV DsRed, rSeV F or rSeV sF in 80 μl serum-free medium. Alternatively, mice were immunized intravenously or intranasally with 2×10^6 pfu or with 4×10^5 pfu rVV F or rVV NP. Three weeks after immunization, mice were challenged i.n. with 1×10^6 pfu of RSV A2. Passive immunization was performed with RSV hyperimmune serum (HIS) generated by infecting naive BALB/c mice 5 times in 3-week intervals with 5×10^5 pfu of RSV. Mice were injected i.v. with 150 μl of HIS (with a neutralizing antibody titer of 1:20480) or control serum obtained from naïve mice.

2.4. Antibody assays

Mice were bled through the tail vein and clarified sera were heat-inactivated at 56 °C for 30 min. For ELISA, 96-well plates (Maxisorp; Nunc, Wiesbaden, Germany) were coated with 50 μl of a 2 μg/ml solution of sucrose purified RSV and incubated overnight at 4 °C. Virus was removed, plates were blocked with 1% BSA in PBS and incubated for 1 h at 37 °C. Wells were washed three times with PBS containing 0.05% Tween 20. Mouse sera were diluted in PBS containing 0.1% BSA. Twofold serial dilutions were performed in 96-well plates (U bottom). 50 μl of each dilution was added to the antigen coated wells and incubated for 2 h at 37 °C. Following three washes with PBS/0.05% Tween, horseradish peroxidase-conjugated rabbit anti-mouse Ig (Dako, Hamburg, Germany) diluted in PBS/0.1% BSA (1:1000) was added to each well (50 μl) and plates were incubated for 2 h at 37 °C. After additional three washes, bound secondary antibody was detected by adding 50 μl ABTS (Roche, Mannheim, Germany) to each well. Plates were incubated for 15 min at

Download English Version:

https://daneshyari.com/en/article/2168014

Download Persian Version:

https://daneshyari.com/article/2168014

Daneshyari.com