Subsequently, we determined in a blinded setting the presence of LGV in a selected group of patients (clinical spectrum and epidemiology described elsewhere [8]) according to C. trachomatis–positive rectal swab (Chlamydia 2SP Collection & Transport Kit [Quelab] by commercially available PCR (COBAS AMPLICOR, Hoffman-La Roche Ltd). By using the 2 reference standard techniques to type C. trachomatis serovars (PCR-based RFLP of the omp1 gene or sequencing the variable segment 2 [VS-2] of the omp1 gene) (9,10) with DNA isolated from rectal swab specimens (standard isopropanol DNA isolation method), we identified 28 of 125 men as LGV-positive. These 28 samples were also positive in both the TaqMan and Rotorgene assays. We also identified 2 additional LGV infections, which were initially typed and then retested as single-strain infections with serovars E and D by both PCR-based RFLP analysis and VS-2 sequencing. This discrepancy is most likely due to a double infection, which will, in most cases, result in the preferential amplification of 1 strain in the omp1 PCR and PCR-based sequencing methods; in the TaqMan and Rotorgene assays, only LGV strains can be amplified. Whether this outbreak is partially technically driven must be assessed in the future by retrospectively investigating the presence of these LGV infections in men who have sex with men and the presence of the L2b strain in the past, since at present only LGV infections from 2003 to 2005 have been investigated.

Servaas A. Morré,*† Joke Spaargaren,†
Johannes S.A. Fennema,†
Henry J.C. de Vries,‡‡
Roel A. Coutinho,‡‡§
and A. Salvador Peña*

*VU University Medical Center, Amsterdam, the Netherlands; †Municipal Health Service, Amsterdam, the Netherlands; ‡Academic Medical Center, Amsterdam, the Netherlands; and §National Institute for Public Health and the Environment, Bilthoven, the Netherlands

References
1. Nieuwenhuis RF, Ossewaarde JM, van der Meijden WI, Neumann HA. Unusual presentation of early lymphogranuloma venereum in an HIV-1 infected patient: effective treatment with 1 g azithromycin. Sex Transm Infect. 2003;79:453–5.
2. Nieuwenhuis RF, Ossewaarde JM, Götz HM, Dees J, Thio HB, Thomeer MG, et al. Resurgence of lymphogranuloma venereum in Western Europe: an outbreak of Chlamydia trachomatis serovar L2 prophylactic in the Netherlands among men who have sex with men. Clin Infect Dis. 2004;39:996–1003.
3. Den Hollander JG, Ossewaarde JM, de Marie S. Anorectal ulcer in HIV patients, don’t forget lymphogranuloma venereum! AIDS. 2004;18:1484–5.
4. French P, Ison CA, Macdonald N. Lymphogranuloma venereum in the United Kingdom. Sex Transm Infect. 2005;81:97–8.
5. Centers for Disease Control and Prevention. Lymphogranuloma venereum among men who have sex with men—Netherlands, 2003–2004. MMWR Morb Mortal Wkly Rep. 2004;53:985–8.
6. van Weel J. Rare sexually transmitted disease hits Europe. Lancet Infect Dis. 2004;4:720.
7. Morré SA, Sillekens P, Jacobs MV, van Aarle P, de Blok S, van Gemen B, et al. RNA amplification by nucleic acid sequence–based amplification with an internal standard enables reliable detection of Chlamydia trachomatis in cervical scrapings and urine samples. J Clin Microbiol. 1996;34:3108–14.
8. Spaargaren J, Fennema HSA, Morré SA, de Vries HJC, Coutinho RA. New Lymphogranuloma venereum Chlamydia trachomatis variant, Amsterdam. Emerg Infect Dis. 2005;11:1090–2.
9. Morré SA, Ossewaarde JM, Lan J, van Doornum GJ, Walboomers JM, MacLaren DM, et al. Serotyping and genotyping of genital Chlamydia trachomatis isolates reveal variants of serovars Ba, G, and J as confirmed by omp1 nucleotide sequence analysis. J Clin Microbiol. 1998;36:345–51.
10. Molano M, Meijer CJLM, Morré SA, Pol R, van den Brule AJ. Combination of PCR targeting the VD2 of omp1 and reverse line blot analysis for typing of urogenital Chlamydia trachomatis serovars in cervical scrape specimens. J Clin Microbiol. 2004;42:2935–9.

Address for correspondence: Servaas A. Morré, VU University Medical Center, Faculty of Medicine, Laboratory of Immunogenetics, Van der Boechorststraat 7, 1081 BT, Amsterdam, the Netherlands; fax: 31-20-44-48418; email: samorretravel@yahoo.co.uk

SARS Vaccine Protective in Mice

To the Editor: Less than a year after the identification of the severe acute respiratory syndrome coronavirus (SARS-CoV) (1), 3 independent laboratories reported protection from SARS-CoV challenge in animal models using a DNA vaccine or recombinant forms of the modified vaccinia Ankara or a parainfluenza virus, encoding the spike gene (2–4). Their protective efficacies are encouraging because they provide proof that a SARS-CoV vaccine is feasible. However, vaccines based on those technologies are not licensed for human use, and recommendation and licensing will likely take many years. We have developed an inactivated virus vaccine that induces neutralizing antibodies and protects against SARS-CoV challenge.

The vaccine was produced as described elsewhere (5). Briefly, the SARS-CoV (strain FRA, GenBank accession no. AY310120) was grown in Vero cells, inactivated with β-propiolactone (BPL), and complete inactivation was confirmed by 2 consecutive passages on Vero cells. Inactivated virus was purified by column chromatography followed by sucrose gradient centrifugation. The fraction containing virus was dialyzed
against phosphate-buffered saline pH 7.2, and total protein content was determined by using the Micro BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). Immunogenicity of the vaccine was tested by immunizing BALB/c mice at 0, 2, and 4 weeks with 5 µg of inactivated virus combined with the adjuvant MF59, an oil squalene-in-water emulsion (6) approved for human use in Europe for an influenza vaccine. Ten days after the third immunization, serum samples were tested for the presence of SARS-CoV spike protein-specific antibodies by using enzyme-linked immunosorbent assay, and high titers (1–3 × 10⁴) of anti-SARS-CoV spike immunoglobulin (Ig) G antibodies were detected. IgG subclass determination indicated a predominant Th2-type immune response similar to that observed in BALB/c mice vaccinated with a UV-inactivated SARS-CoV plus alum (7).

Efficacy of the inactivated virus vaccine was also assessed in a BALB/c mouse model of SARS-CoV infection (8). Animal studies were approved by the National Institutes of Health Animal Care and Use Committee and were conducted in an animal biosafety level 3 facility. SARS-CoV replicates in the respiratory tract of BALB/c mice following intranasal infection with 10³ 50% tissue culture infectious doses (TCID₅₀) of virus. Generally, virus titers peak within 2 days after infection and are cleared within 7 days (8). BALB/c mice were immunized at 0, 2, and 4 weeks with 5 µg of BPL-inactivated SARS virus with or without the MF59 adjuvant. Mice were also immunized with 4 different control preparations: phosphate-buffered saline, adjuvant MF59 alone, and 5 µg of BPL-inactivated influenza A virus vaccine with or without MF59. Serum samples were collected 2 weeks after each dose, and assayed for their ability to neutralize SARS-CoV (8). After 2 vaccine doses, SARS-CoV neutralizing antibodies were detected only in the group of mice immunized with the BPL-inactivated SARS virus vaccine plus MF59 adjuvant (1:91). Two weeks after the third dose, the BPL-inactivated SARS virus vaccine without MF59 induced neutralizing titers of 1:64, while the adjuvanted vaccine elicited neutralizing titers >1:600 (Table).

Mice were challenged intranasally at this point with 10⁴ TCID₅₀ SARS-CoV (Urbani strain, GenBank accession no. AY278741). Nasal turbinates and lung tissues were analyzed for infectious virus 2 days later (Table). SARS-CoV titers in mice from the control groups were =10⁶ TCID₅₀ virus/g of lung tissue and =10³ TCID₅₀ virus/g of nasal turbinate tissue. Complete protection from virus replication was observed in mice that received the MF59 adjuvanted SARS-CoV vaccine. Immunization with the nonadjuvanted vaccine resulted in complete protection of the upper respiratory tract and a significant reduction (30,000-fold) of viral titers in the lower respiratory tract compared to the control groups. The incomplete protection of this group was attributed to a single animal that contained detectable infectious virus in the lung.

Accelerated or enhanced virus replication or disease in immunized persons is a concern in developing any vaccine. This may be particularly true for SARS-CoV vaccines since adverse effects have been reported for one animal coronavirus vaccine, feline infectious peritonitis virus (9). Additionally, some in vitro experiments were performed with pseudotyped lentiviruses that expressed the spike glycoprotein derived from SARS-like virus isolated from civets. In these experiments, the presence of antibodies that neutralized most human isolates of SARS-CoV

| Immunogen*                                                                 | 2 wk post 1st dose | 2 wk post 2nd dose | 2 wk post 3rd dose | Virus replication upon challenge† | Lungs | Nasal turbinates |
|---------------------------------------------------------------------------|--------------------|--------------------|--------------------|----------------------------------|-------|-----------------|
| PBS                                                                       | <1:8               | <1:8               | <1:8               |                                 |       |                 |
| MF59                                                                      | <1:8               | <1:8               | <1:8               |                                 |       |                 |
| Influenza A (5 µg)                                                        | <1:8               | <1:8               | <1:8               |                                 |       |                 |
| Influenza A (5 µg) + MF59                                                | <1:8               | <1:8               | <1:8               |                                 |       |                 |
| BPL-SARS-CoV (5 µg)                                                       | <1:8               | <1:8               | 1:64               |                                 |       |                 |
| BPL-SARS-CoV (5 µg) + MF59                                                | <1:8               | 1:91               | 1:645              |                                 |       |                 |

*The indicated immunogens or control preparations were administered to mice by subcutaneous injection on 3 occasions 2 weeks apart; PBS, phosphate-buffered saline.
†Neutralization titers were determined as described (8).
‡Mice were challenged with 10⁴ 50% tissue culture infectious doses (TCID₅₀) SARS-CoV intranasally.
§Virus titers are expressed as log₅₀ TCID₅₀/g of tissue.
¶p<0.00001 in a 2-tailed Student t test, compared to titers seen in mice that were immunized with PBS.
#Indicates the titer of a single animal. The remaining 3 mice had no detectable levels of virus.
**Virus not detected; the lower limit of detection of infectious virus was 1.5 log₅₀ TCID₅₀/g in a 10% v/v suspension of lung homogenate and 1.8 log₅₀ TCID₅₀/g in a 5% v/v suspension of nasal turbinates.
††p = 0.025 in a 2-tailed Student t test, compared to titers seen in mice that were immunized with PBS.
demonstrated enhanced entry into renal epithelial cells (10). In our studies, we did not find enhanced virus replication in the respiratory tract of vaccinated mice upon SARS-CoV challenge. However, since mice are a model of SARS-CoV infection but not disease, the issue of disease enhancement will have to be carefully evaluated if and when an appropriate animal model in which this phenomenon can be demonstrated becomes available.

In summary, an inactivated SARS-CoV vaccine, produced with a technology that has a safety record established by immunizing hundreds of millions of persons, protects mice from challenge with SARS-CoV. The vaccine adjuvanted with MF59 elicits neutralizing antibodies (titer 1:91) after only 2 doses. We conclude that the vaccine described here has desirable properties, and our data support further development and plans for clinical trials.

Acknowledgments

This study was supported by the Fonds der Chemischen Industrie, the 6th Framework Program of the European Commission (FP6-511065), and Chiron Vaccines.

Konrad Stadler,‡
Anjeanette Roberts,†1
Stephan Becker,†1 Leatrice Vogel,†
Markus Eickmann,‡
Larissa Kolesnikova,‡
Hans-Dieter Klenk,‡
Brian Murphy,‡ Rino Rappuoli,* Sergio Abrignani,* and Kanta Subbarao‡

*Chiron Vaccines, Siena, Italy; †National Institutes of Health, Bethesda, Maryland, USA; and ‡Institut für Virologie, Marburg, Germany

†These authors contributed equally to this work.

References

1. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med. 2003;348:1953–66.
2. Yang ZY, Kong WP, Huang Y, Roberts A, Murphy BR, Subbarao K, et al. DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature. 2004;428:561–4.
3. Bisht H, Roberts A, Vogel L, Bukreyev A, Collins PL, Murphy BR, et al. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. Proc Natl Acad Sci U S A. 2004;101:6641–6.
4. Bukreyev A, Lamirande EW, Buchholz UJ, Vogel LN, Elkins WR, St Claire M, et al. Mucosal immunisation of African green monkeys (Cercopithecus aethiops) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS. Lancet. 2004;363:2122–7.
5. Song HC, Seo M-Y, Studler K, Yoo BJ, Choo Q-L, Coates S, et al. Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. J Virol. 2004;78:10328–35.
6. Podda A, Del Giudice G. MF59-adjuvanted vaccines: increased immunogenicity with an optimal safety profile. Expert Rev Vaccines. 2003;2:197–203.
7. Takakura N, Fujii H, Takahashi Y, Kasai M, Morikawa S, Tamura S, et al. A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. Int Immunol. 2004;16:1423–30.
8. Subbarao K, McAlilffe J, Vogel L, Fahle G, Fischer S, Tatti K, et al. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. J Virol. 2004;78:3572–7.
9. Vennema H, de Groot RJ, Harbour DA, Daldrup M, Gruluyff-Jones T, Horzinek MC, et al. Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus vaccination. J Virol. 1990;64:1407–9.
10. Yang ZY, Werner HC, Kong WP, Leung K, Traggiai E, Lanzavecchia A, et al. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. Proc Natl Acad Sci U S A. 2005;102:797–801.

Echinococcosis, Ningxia, China

To the Editor: Ningxia is the smallest provincial autonomous region on the Loess Plateau in central China, with a population of 7.5 million persons composed of 35 ethnic and various religious groups. The natural environmental conditions and socioreligious ethnic status in Ningxia, particularly the southern region, are conducive to sheep farming, an important part of the agricultural economy. Both cystic echinococcosis (CE) and alveolar echinococcosis (AE) (1) are endemic in the northwest part of China; high prevalences have been reported in several provinces (1), including Gansu (2,3) and the Xinjiang Uigur Autonomous Region (4). Little information is available about the extent of human echinococcosis in Ningxia; this is the first report of a provincial investigation for both human CE and AE there.

We conducted a retrospective survey of clinical records from 7 local county hospitals and 4 other hospitals in Yinchuan to determine the epidemiology of human echinococcosis in southern Ningxia. All surgical and clinical records were checked, and diagnoses were confirmed based on imaging, surgical reports, and histopathologic reports. Data concerning age, sex, domicile, ethnicity, occupation, year of diagnosis, cyst or lesion numbers and anatomical location, type and duration of anthelmintic treatment (if given), and the nature and number of surgeries performed for echinococcosis were recorded for each confirmed CE and AE patient.

From 1985 to 2002, a total of 2,216 cases of echinococcosis were recorded, most of which were due to CE (96%). The incidence of combined CE and AE from 1994 to 2001 was 7/100,000 persons for southern Ningxia, compared with 1/100,000 persons for Yinchuan in the north

Addresses for correspondence: Sergio Abrignani, Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy; fax: 39-0577-243-564; email: sergio_abrignani@chiron.com