Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Mucosal immunisation of African green monkeys (Cercopithecus aethiops) with an attenuated parainfluenza virus expressing the SARS coronavirus spike protein for the prevention of SARS

Alexander Bukreyev, Elaine W Lamirande, Ursula J Buchholz, Leatrice N Vogel, William R Elkins, Marisa St Claire, Brian R Murphy, Kanta Subbarao, Peter L Collins

Summary

Background The outbreak of severe acute respiratory syndrome (SARS) in 2002 was caused by a previously unknown coronavirus—SARS coronavirus (SARS-CoV). We have developed an experimental SARS vaccine for direct immunisation of the respiratory tract, the major site of SARS-coronavirus transmission and disease.

Methods We expressed the complete SARS coronavirus envelope spike (S) protein from a recombinant attenuated parainfluenza virus (BHPIV3) that is being developed as a live attenuated, intranasal paediatric vaccine against human parainfluenza virus type 3 (HPIV3). We immunised eight African green monkeys, four with a single dose of BHPIV3/SARS-S and four with a control, BHPIV3/Ctrl, administered via the respiratory tract. A SARS-coronavirus challenge was given to all monkeys 28 days after immunisation.

Findings Immunisation of animals with BHPIV3/SARS-S induced the production of SARS-coronavirus-neutralising serum antibodies, indicating that a systemic immune response resulted from mucosal immunisation. After challenge with SARS coronavirus, all monkeys in the control group shed SARS coronavirus, with shedding lasting 5–8 days. No viral shedding occurred in the group immunised with BHPIV3/SARS-S.

Interpretation A vectored mucosal vaccine expressing the SARS-coronavirus S protein alone may be highly effective in a single-dose format for the prevention of SARS.

Lancet 2004; 363: 2122–27
See Commentary page 2102

Introduction

Severe acute respiratory syndrome (SARS) emerged in southeast Asia in late 2002 and subsequently spread internationally. To date, it has resulted in more than 8000 cases and 774 deaths. The causative agent was quickly identified as a previously unknown member of the Coronaviridae family. SARS infection of human beings has since been contained through infection-control measures. However, resurgence is still a threat because the causative agent remains in animal reservoirs that are not fully understood and sporadic cases continue to be reported. We have an incomplete understanding of the genesis of SARS coronavirus (SARS-CoV), and there may be the potential for the emergence of variants capable of greater transmissibility. Coronavirus are enveloped viruses with a genome that is a single strand of positive sense RNA of 30 kilobases or more in length. Sequence analysis of the RNA genome of the SARS coronavirus identified 11 open reading frames encoding proteins typical of coronaviruses, including the envelope spike (S) protein of the virus particle. SARS illness in humans is chiefly a pneumonia, notwithstanding the occurrence of systemic disease signs and detection of virus or viral RNA in other organs. The primary mode of transmission of SARS seems to be through mucosal membranes of the eyes, nose, or mouth; faecal-oral transmission has also been suggested but its occurrence and relative importance have not yet been documented. The prominent role of the respiratory tract in SARS transmission and disease suggest that direct immunisation of the respiratory mucosa would be an effective strategy for immunoprophylaxis against SARS. Furthermore, mucosal immunisation of the respiratory tract with live attenuated respiratory virus vaccines efficiently induces systemic as well as local immunity.

As an approach for the development of a vaccine against SARS coronavirus, we took advantage of an existing live attenuated vaccine virus, BHPIV3, that is being developed for intranasal paediatric immunisation against HPIV3 infection and disease. BHPIV3 was derived from bovine (B)PIV3, a closely-related bovine counterpart of HPIV3 that is attenuated in primates because of a natural host range-restriction. It has also been shown to be attenuated and immunogenic in humans, and is a candidate vaccine against HPIV3. BPIV3 was modified previously with recombinant DNA methods to replace its F and HN protective surface antigen genes with their HPIV3 counterparts, yielding BHPIV3. BHPIV3 was an improved HPIV3 vaccine, since it bears protective antigens that exactly match HPIV3.

In our study, BHPIV3 was further modified by the insertion of a transcriptional cassette containing the coding sequence of the full-length S protein of SARS coronavirus. The S protein was chosen because studies with other coronaviruses showed that it is a major viral surface protein important in initiating infection.
Additionally, immunisation of experimental animals with the S protein of other coronaviruses induced virus-specific immunity and, in some cases, conferred protection against subsequent challenge. We investigated the replication, immunogenicity, and protective effects of this experimental BHPIV3/SARS-S vaccine in African green monkeys (Cercopithecus aethiops).

Methods

Construction of BHPIV3/SARS-S and BHPIV3/Ctrl viruses

The Urbani strain of SARS coronavirus was provided by L J Anderson and T G Kaizek of the Centers for Disease Control and Prevention, Atlanta, GA, USA, and propagated in Vero cells. All experiments involving infectious SARS coronavirus were done under approved biosafety level 3 conditions. Viral genomic RNA was isolated and used in RT-PCR to synthesise and amplify a 3768 bp cDNA containing the complete SARS-coronavirus S coding sequence. The cDNA was designed so that the SARS coding sequence was flanked by short BHPIV3-specific transcription signals that are necessary for the foreign gene to be expressed by the transcriptional program of the BHPIV3 vector (sequences of the oligonucleotide primers and details of RT-PCR and DNA construction are available from A Bukreyev).

A PCR product of the expected length was purified and inserted into a NotI restriction endonuclease site that had previously been introduced into a complete cloned cDNA of BHPIV3, and the sequence of the insert and flanking regions was confirmed. In this configuration, the SARS-coronavirus S insert is present as an added gene located between the BHPIV3 P and M genes (figure 1) and would be expressed as a separate mRNA by the BHPIV3 polymerase. Certain idiosyncratic features of HPIV3 molecular genetics were also accommodated: the insert was designed so that the BHPIV3 genome length remained an even multiple of six, which is required for efficient BHPIV3 replication and is thought to reflect a nucleocapsid spacing requirement. In addition, the insert was designed to maintain the spacing of the transcriptional units within this hexamer organisation.

The recombinant BHPIV3/SARS-S virus was recovered and propagated in cell culture as described elsewhere. BHPIV3/SARS-S and BHPIV3/Ctrl were propagated on LLC-MK2 monkey kidney cells, and viral titres were determined by limiting dilution on the same cells using haemadsorption with guinea pig erythrocytes (a property of the HPIV3 HN protein) to detect virus infection. Titres are expressed in units of tissue culture 50% infectious dose (TCID₅₀), which are similar in magnitude to a plaque forming unit.

Serum antibodies specific to SARS coronavirus were quantified by an haemagglutination inhibition (HAI) assay in which dilutions of serum were tested for the ability to block agglutination of guinea pig erythrocytes in vitro by HPIV3, in parallel with known positive and negative control standards. SARS coronavirus was propagated in Vero monkey kidney cells, and viral titres were measured by limiting dilution in the same cells scored by a 50% end point of visible cytopathology.

We immunised four animals with BHPIV3/SARS-S and four with BHPIV3/Ctrl. We used a single combined intranasal and intratracheal inoculation with 10⁶ TCID₅₀ units of SARS coronavirus per well, in parallel with known positive and negative control standards. Each sample involved four wells per dilution to neutralise 100 TCID₅₀ units of SARS coronavirus per well.

Detection of the S protein

LLC-MK2 cells were infected with BHPIV3/SARS-S or the control virus at multiples of 5 TCID₅₀ units of infection per cell, and cells were harvested 18 h post-infection. In addition, the medium overlying other infected cells was harvested at 48 h post-infection, and virus was concentrated by centrifugation at 8000 g at 4°C for 18 h. The viral pellets were resuspended and subjected to centrifugation on a 30–60% w/v discontinuous sucrose gradient at 130 000 g at 4°C for 90 min, after which the banded virus was harvested. Western blot analysis was done with NuPage protein electrophoresis system and WesternBreeze immunodetection kit (Invitrogen, Carlsbad, CA, USA). We detected S protein with serum samples from mice infected with SARS coronavirus and a second antibody of alkaline-phosphatase-conjugated goat anti-mouse IgG (Invitrogen).

Infection and challenge of African green monkeys

We used eight young adult African green monkeys of either sex (bodyweight 3·6–5·9 kg) with a confirmed absence of detectable serum antibodies against HPIV3 or SARS coronavirus. We immunised four animals with BHPIV3/SARS-S and four with BHPIV3/Ctrl. We used a single combined intranasal and intratracheal inoculation with 10⁶ TCID₅₀ units of virus in 1 mL inoculum per site (combined dose of 10¹³ TCID₅₀ units).

Animals were first anaesthetised with ketamine hydrochloride given intramuscularly at a dose of 10 mg/kg, and then placed in dorsal recumbency. Through each nostril, we administered...
after the initial immunisation. These experiments were
lavages were obtained on the same schedule as that used
inoculum at each site. Nasopharyngeal swabs and tracheal
and its absence in the BHPIV3 virus particle
14, and tracheal lavages were done on days 2, 4, 6, 8, 10,
inserted about 3 cm past the epiglottal opening. We used
delivered through a sterile flexible catheter that had been
inoculation, a sterile stainless steel laryngoscope was used
about 3–5 mm into each nostril. For intratracheal
0·5 mL of inoculum with a sterile Luer syringe introduced
analysis.

V107 (F) 6 3·5 3 3·5 9
V103 (F) 6 5·0 5 6·5 9
V117 (F) 7 3·2 1 2·0 8
V104 (F) 5 3·5 0

Table 1: Responses to mucosal immunisation with BHPIV3/SARS-S and BHPIV3/Ctrl

| BHPV3/SARS-S | Nasal swab | Tracheal Lavage |
|--------------|------------|----------------|
| Monkey       | Duration (days) | Peak titre (log$_{10}$ TCID$_{50}$/mL) | Duration (days) | Peak titre (log$_{10}$ TCID$_{50}$/mL) |
| V101 (F)     | 3          | 3-7            | 0              | <0·5*        |
| V104 (F)     | 5          | 3-5            | 0              | <0·5        |
| V117 (F)     | 7          | 3-2            | 1              | 2-0         |
| V191 (M)     | 8          | 5-0            | 3              | 2-5         |

| BHPV3/Ctrl   | Serum Antibodies |
|--------------|------------------|
| Monkey       | HPV3 HAI titre (reciprocal log$_{10}$) | SARS-coronavirus neutralising titre (reciprocal log$_{10}$) |
| V099 (F)     | 9                | 4-5             | 8               |
| V103 (F)     | 6                | 5-0             | 9               |
| V107 (F)     | 6                | 3-5             | 9               |
| 122 (F)      | 10               | 4-2             | 10              |

F=female. M=male. Serum samples taken 1 day before immunisation did not have detectable HAI antibodies (detection limit 1·0 reciprocal log$_{10}$ dilution units) or detectable SARS-coronavirus neutralising antibodies (lower limit of detection =2 reciprocal log$_{10}$). *Lower limit of detection of virus. †Lower limit of detection of serum neutralising antibodies.

Figure 2: Expression of SARS-coronavirus S protein by BHPIV3/SARS-S in cell culture and its absence in the BHPIV3 virus particle

A: western blot analysis shows presence of the SARS-coronavirus S protein in lysates of cells infected with BHPIV3/SARS-S (lane 3) and its absence in lysates from cells infected with BHPIV3/Ctrl (lane 4). S protein was not detectable in purified, concentrated BHPIV3/SARS-S virus particles (lane 1) or in the negative control BHPIV3/Ctrl (lane 2). B: direct Coomassie staining of a gel loaded with replicate samples of purified, concentrated BHPIV3/Ctrl (lane 1) and BHPIV3/SARS-S (lane 2) shows viral protein was present in excess of what should be necessary for detection by western blot analysis.

0·5 mL of inoculum with a sterile Luc syringe introduced
about 3–5 mm into each nostril. For intratracheal
inoculation, a sterile stainless steel laryngoscope was used to
observe the epiglottis, and the 1 mL inoculum was
inserted about 3 cm past the epiglottal opening. We used separate sterilised instruments for every animal. Nasopharyngeal swabs were taken on days 0–10, 12, and 14, and tracheal lavages were done on days 2, 4, 6, 8, 10, and 14,21 and titration of BHPIV3/SARS-S and BHPIV3/Ctrl was done as described previously. For tracheal lavage, we used a laryngoscope and cather placed as described above, with a 2 mL wash volume of phosphate-buffered saline that was instilled and aspirated back. For the SARS-coronavirus challenge, the animals were given a similar combined intranasal and intratracheal inoculation with 10$^5$ TCID$_{50}$ units of virus in a 1 mL inoculum at each site. Nasopharyngeal swabs and tracheal lavages were obtained on the same schedule as that used after the initial immunisation. These experiments were done in an approved animal biosafety level 3 facility. Virus titration was done as described earlier. We took serum samples 1 day before immunisation (day –1), 1 day before the challenge (day 27), and 28 days post challenge (day 50), which were analysed for virus-specific antibodies as described above.

The primate study was approved by the National Institutes of Health (USA) Animal Care and Use Committee and was done in a laboratory approved by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Role of the funding source
The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

Results
BHPIV3 was modified to express the envelope spike S protein of SARS coronavirus (figure 1). The recombinant BHPIV3/SARS-S virus was recovered and propagated in cell culture in parallel with the control virus, BHPIV3/Ctrl. The presence of foreign inserts did not affect the efficiency of replication of the vector in vitro. The BHPIV3 genome is a single strand of negative sense RNA of 15·5 kb that contains six non-overlapping genes (figure 1). The virally-encoded polymerase initiates at the left end of the genome and transcribes each gene in turn, including any inserted foreign gene, into a separate mRNA. The correct expression of the S sequence as a separate mRNA was confirmed by northern blot analysis of intracellular RNA isolated from LLC-MK2 cells infected with BHPIV3/SARS-S (data not shown).

Expression of the SARS-coronavirus S protein was confirmed by western blot analysis of lysates of BHPIV3/SARS-S-infected cells with serum of mice that had been infected with SARS coronavirus21 (figure 2A, lane 3). This showed that the S protein migrated as two major, diffuse bands of about 170 and 200 kDa. The two forms of the S protein probably differ by the extent of glycosylation; based on the nucleotide sequence, the

For personal use. Only reproduce with permission from The Lancet.
has 23 potential glycosylation sites.5–7 There was no predicted full length unmodified S protein is 138 kDa and a low level of replication of SARS coronavirus.

response to the antigen present in the virus inoculum or to group after the challenge could be the result of an immune antibody titres 28 days after the challenge are shown in respiratory tract. SARS-coronavirus-neutralising serum coronavirus on any day from either the upper or lower animals immunised with BHPIV3/SARS-S shed SARS coronaviruses. All four monkeys immunised with BHPIV3/SARS-S showed that the peak titre (3·9 log10 TCID50/mL) and duration of shedding (5·8 days) were significantly lower than for the BHPIV3/Ctrl-immunised animals (table 1). Thus, expression of the SARS S protein had an attenuating effect on the replication of the vector, particularly in the lower respiratory tract. Nevertheless, BHPIV3/SARS-S and BHPIV3/Ctrl each induced a moderate titre of HPIV3-specific antibodies as measured by an HAI assay (table 1). In addition, BHPIV3/SARS-S induced a detectable level of serum antibodies that neutralised SARS-S coronavirus in vitro (mean reciprocal titre of 3·9 log2).

Table 2 shows responses to challenges with SARS coronavirus in monkeys immunised with BHPIV3/SARS-S or BHPIV3/Ctrl.

Discussion

We have developed an experimental SARS vaccine using an existing live attenuated HPIV3 vaccine candidate, BHPIV3, as a vector to express the SARS-coronavirus S protein. The use of this respiratory virus as a vector provides for direct immunisation of the respiratory tract, the main site of SARS coronavirus transmission and disease. The monkeys that were vaccinated with BHPIV3/SARS-S had a protective effect against the challenge infection. This finding identified the S protein as a major protective antigen of SARS coronavirus and indicates that vaccines against SARS should include this protein.

Topical immunisation of the respiratory tract also induced detectable SARS-coronavirus-neutralising serum antibodies, evidence of a systemic immune response. However, the post-immunisation titre induced by BHPIV3/SARS-S was almost eight-fold lower than that achieved by SARS-coronavirus infection of the BHPIV3/Ctrl-immunised animals. This difference in immunogenicity might reflect the natural restriction of SARS coronavirus dissemination systemically, at least in non-human primates.4,4 There was no evidence of immune-mediated enhancement of infection or disease, which occurs for one coronavirus, feline infectious peritonitis virus.23 Thus, our results show that one mucosal immunisation with vectored SARS S protein was sufficient to protect against shedding after a large challenge dose of SARS coronavirus.

Some cynomolgus monkeys (Macaca fascicularis) infected with SARS coronavirus have been reported to develop clinical disease signs including a transient rash, respiratory distress, and lethargy.24,8 We have investigated three species of monkeys, namely cynomolgus, rhesus (Macaca mulatta), and African green monkeys, for permissiveness for SARS-coronavirus replication and possible disease (unpublished data). Of the three, African green monkeys supported the highest levels of replication as measured by virus shedding and hence were used in this study. None of the 12 animals in any of the three species developed overt disease signs, and we did not note disease signs in this study. Mice, ferrets, and cats have been shown to support high levels of pulmonary SARS-coronavirus replication, and some of the infected ferrets became lethargic.25,26 We did not assess BHPIV3/SARS-S in mice because the HPIV3 vaccine vector is severely restricted for replication in this animal, and the cat and ferret models were only recently reported. Thus, although our study showed complete protection against shedding of challenge virus, we could not assess whether protection would be afforded.

For personal use. Only reproduce with permission from The Lancet.
against clinical disease signs. This study limitation is not unusual. With respect to human viruses, experimental animals rarely provide faithful models of the infection and disease that is observed in humans, and disease signs often are minimal, altered, or absent. The measurement of infectious challenge virus in secretions or other samples from experimental animals is a general standard for measuring the efficacy of viral vaccines. The assessment of an experimental vaccine in a non-human primate is especially important in view of the phylogenetic and anatomical similarity to humans, and is an appropriate last step before clinical trials.

Attenuated versions of HPIV3 are under active development as vaccines for intranasal immunisation of infants and young children, as are HPIV3-based vectored vaccines that also express protective antigens of respiratory pathogens such as measles virus, respiratory syncytial virus, and metapneumovirus.11,13,15 HPIV3 efficiently infects the respiratory tract but does not spread far beyond it, which is an important safety factor. HPIV3-based vectors have proven effective in inducing local and systemic immunity against a number of foreign antigens.12,13,15 Furthermore, safe intranasal administration of attenuated HPIV3 and related viruses has been shown possible.16,20,27 An additional safety feature is that RNA recombination is almost non-existent in nature for the family of viruses represented by HPIV3, whereas recombination is extremely frequent in coronaviruses, and the potential for recombination with circulating human coronavirus would be a concern for a live-attenuated SARS-coronavirus vaccine virus.

As currently constructed, the BPIV3/3/SARS-S vector is an excellent candidate for clinical testing as a vaccine that is likely to be highly attenuated, safe, and effective against both HPIV3 and SARS for infants and young children, in whom the vector would replicate efficiently. This vaccine would be especially useful if a more transmissible version of SARS coronavirus emerges and immunisation of infants and children is needed. However, any replicating viral vector bearing the protective antigens of a common human pathogen, such as adenovirus or HPIV3, is unlikely to replicate sufficiently in adults to be immunogenic because of a prevalence of neutralising antibodies to such pathogens.20,27 Fortuitously, para-
influenza viruses have been amenable to swapping of the surface proteins without loss of infectivity in vitro or in vivo.19,20 Therefore, it should be possible to replace the HPIV3 HN and F surface proteins with those of an antigenically-distinct parainfluenza virus for which the general population lacks immunity—in particular one of the many avian parainfluenza viruses such as an attenuated strain of Newcastle disease virus. The resulting vector would be a useful SARS vaccine for mucosal immunisation of the entire human population.

Contributors
A Bukreyev participated in the design of the constructs, construction and in-vitro characterisation of the recombinant viruses, the design of the monkey experiment, SARS-coronavirus titrations, and interpretation of the results. E Lamirande participated in the construction and in-vitro characterisation of the recombinant viruses, SARS-coronavirus titrations, and serum neutralisation assays. U Buchholz gave useful recommendations on BPIV3 recovery, did internal blot analysis, and participated in SARS-coronavirus titrations. L Vogel prepared challenge virus and viralisation assays. W R Elkins and M St Claire supervised the animal experiments. B Murphy participated in planning of the experiments and interpretation of the results. K Subbarao prepared SARS-coronavirus RNA and mouse SARS-coronavirus-immune serum, established the African green monkey model for SARS coronavirus, and participated in the planning of the experiments and interpretation of the results. P Collins participated in design of the constructs, planning of the experiments and interpretation of the results, and providing general guidance for the project.

Conflict of interest statement
None declared.

Acknowledgments
We thank Kim-Chi Tran for technical assistance and Kathryn Hanley for assistance with statistical analysis. This project was funded as part of the NIAID Intramural Program.

References
1 WHO. Communicable disease surveillance and response: severe acute respiratory syndrome (SARS) http://who.int/csr/sars/en/ (accessed Dec 31, 2003).
2 Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1953–66.
3 Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. N Engl J Med 2003; 348: 1955–65.
4 Drosten C, Günther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003; 348: 1967–76.
5 Rota PA, Oberste MS, Monroe SS, et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 2003; 300: 1394–99.
6 Zeng FY, Chan CW, Chan MN, et al. The complete genome sequence of severe acute respiratory syndrome coronavirus strain HKU-39849 (HK-39). Exp Biol Med (Maywood) 2003; 228: 866–73.
7 The complete genome of the SARS associated Coronavirus. Gene Bank accession number AY274256.
8 Kuiken T, Fouchier RA, Schutten M, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003; 362: 263–70.
9 Peiris JS, Yuan KY, Osterhaus AD, Stohr K. The severe acute respiratory syndrome. N Engl J Med 2003; 349: 2431–41.
10 Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonias: a prospective study. Lancet 2003; 361: 1767–72.
11 Schmidts A, McAuliffe J, Huang A, et al. Bovine parainfluenza virus type 3 (BPIV3) fusion and hemagglutinin-neuraminidase glycoproteins make an important contribution to the restricted replication of BPIV3 in primates. J Virol 2000; 74: 8822–29.
12 Schmidts AC, McAuliffe JM, Murphy BR, Collins PL. Recombinant bovine/human parainfluenza virus type 3 (B/HPIV3) expressing the respiratory syncytial virus (RSV) G and F proteins can be used to achieve simultaneous mucosal immunisation against RSV and BPIV3. J Virol 2001; 75: 4594–603.
13 Dubin AP, Skidmore-Moore MH, McAuliffe JM, Rigs JM, Surman SR, Collins PL, Murphy BR. Human parainfluenza virus type 3 (PIV3) expressing the hemagglutinin protein of measles viruses provides a potential method for immunization against measles virus and PIV3 in early infancy. J Virol 2000; 74: 6821–31.
14 Murphy BR, Collins PL. Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics. J Clin Invest 2002; 110: 21–27.
15 Tang RS, Schickli JH, MacPhail M, et al. Effects of human metapneumovirus and respiratory syncytial virus antigen insertion in two 3’ proximal genome positions of bovine/human parainfluenza virus type 3 on virus replication and immunogenicity. J Virol 2003; 77: 10189–28.
16 Karron RA, Makhene M, Gay K, Wilson MH, Clements ML, Murphy BR. Evaluation of a live attenuated bovine parainfluenza type 3 vaccine in two- to six-month-old infants. Pediatr Infect Dis J 1996; 15: 650–54.
17 Callebaut P, Pensaat M. Expression and immunogenicity of the spike glycoprotein of porcine respiratory coronavirus encoded in the E3 region of adenovirus. Adv Exp Med Biol 1995; 380: 265–70.
18 Daniel C, Talbot PJ. Protection from lethal coronavirus infection by affinity-purified spike glycoprotein of murine hepatitis virus, strain A59. Virology 1990; 174: 87–94.
19 Tuboly T, Nagy E, Dennis JR, Derbyshire JB. Immunogenicity of the S protein of transmissible gastroenteritis virus expressed in baculovirus. Arch Virol 1994; 137: 55–67.
20 Subbarao K, McAuliffe J, Vogel L, 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: 5872–77.
21 Kolakofsky D, Pelet T, Garcon D, Hausmann S, Curran J, Roux L. Paramyxovirus RNA synthesis and the requirement for hexamer genome length: the rule of six revisited. J Virol 1998; 72: 891–99.
22 Crowe JE, Jr, Collins PL, London WT, Chanock RM, Murphy BR. A comparison in chimpanzees of the immunogenicity and efficacy of
live attenuated respiratory syncytial virus (RSV) temperature-sensitive mutant vaccines and vaccinia virus recombinants that express the surface glycoproteins of RSV. Vaccine 1993; 11: 1395–1404.

23 Weiss RC, Scott FW. Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. Comp Immunol Microbiol Infect Dis 1981; 4: 175–89.

24 Fouchier RA, Kuiken T, Schutten M, et al. Aetiology: Koch’s postulates fulfilled for SARS virus. Nature 2003; 423: 240.

25 Martina BE, Haagmans BL, Kuiken T, et al. Virology: SARS virus infection of cats and ferrets. Nature 2003; 425: 915.

26 Clements ML, Belisle RH, King J, et al. Evaluation of bovine, cold-adapted human, and wild-type human parainfluenza type 3 viruses in adult volunteers and in chimpanzees. J Clin Microbiol 1991; 29: 1175–82.

27 Karron RA, Wright PF, Newman FK, et al. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in healthy infants and children. J Infect Dis 1993; 167: 1445–50.

28 Tao T, Skiadopoulos MH, Davoodi F, Riggs JM, Collins PL, Murphy BR. Replacement of the ectodomains of the hemagglutinin-neuraminidase and fusion glycoproteins of recombinant parainfluenza virus type 3 (PIV3) with their counterparts from PIV2 yields attenuated PIV2 vaccine candidates. J Virol 2008; 74: 6448–58.

29 Tao T, Durbin AP, Whitehead SS, Davoodi F, Collins PL, Murphy BR. Recovery of a fully viable chimeric human parainfluenza virus (PIV) type 3 in which the hemagglutinin-neuraminidase and fusion glycoproteins have been replaced by those of PIV type 1. J Virol 1998; 72: 2955–61.

Uses of error

Immediate and delayed learning

Seth Powsner

Mistakes made during training are easier for me to acknowledge. An implicit excuse is provided by the training role. Memories of three mistakes remain particularly vivid.

Internship. A general medicine ward. A geriatric patient, almost recovered. Little work remained. However, custom mandated a lumbar puncture (LP) for any patient who had a positive blood test for syphilis without recorded treatment. Positive spinal fluid meant more penicillin. I had come to regard LPs as benign, bedside procedures: just check for bulging optic discs. I mastered a technique for one handed opening and closing of screw-top specimen tubes. I could do LPs on my own, with no senior resident or nurse. So, I was working alone in my patient’s room at the end of the hallway. I maneuvered a long needle into the vertebral interspace. I removed the stylet. Out flowed bright red blood. Undiluted. Freely flowing. I fought my panic, replaced the stylet, fought my panic, removed the needle, fought my panic, pressed the site. No call button in reach. Too embarrassing to yell. Much time passed. A nurse finally happened along. Lesson learnt: Beware of overconfidence and always arrange backup.

Residency. A modern psychiatric ward. A depressed grandmother. This was an opportunity to do daily psychotherapy. My attempt comprised questions such as “Tell me your feelings. What is on your mind? What do you remember?” During the sixth session, she backed away in her chair. She was trying to get away even though her chair was stuck in a corner. There was no place to go. She literally began pushing herself up the wall. I stopped.

In retrospect, her childrens’ suicides were clear markers of inherited affective illness. I was rubbing salt in old wounds. Electroconvulsive treatments worked perfectly. Lesson learnt: Beware of preconceived notions of illness and treatment, and shift paradigms as needed.

Internship. A general pediatrics ward. A pretty, young teenager admitted for an excision. Her family was pleasant, unsophisticated, of modest means, grateful that neurofibromatosis had only required small excisions over the years. Unfortunately, this procedure revealed sarcoma in her thigh. Off to our library. Small chances for 5-year survival, assuming hip disarticulation, according to case series of the time. Attending physicians promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be promised discussion later, went to a trusted consultant. This oncologist sided with the others: “try for the best chance.” I closed ranks. I endorsed treatment—only to be