versity of the memory compartment in these subjects.

The most challenging finding of this study[2] is, however, the fact that significant HIV-specific CD4+ memory responses were not detectable in patients with long-term suppression of HIV replication. HIV gag-specific CD4+ memory T cells had already undergone a significant numerical reduction after 24 weeks of effective therapy (taking into account that the study was cross-sectional and not longitudinal). These results are consistent with the previously reported rate of stabilization of the global CD4+ repertoire of T cells[7] and with the reduction in the frequency of HIV-specific CD8+ cells observed after 6 months of highly active anti-retroviral therapy[3] (HAART). Thus, this study conclusively shows that there is indeed a significant loss of CD4+ helper memory, that may lead to inefficient immunological control and successful escape of the virus from the reservoirs. Of course, we cannot infer from the results of Picker et al. whether HIV-specific CD4 memory is completely lost after effective anti-HIV treatment, or whether it merely falls below the detection-threshold of the assay. Indeed, a rebound in the HIV-specific CD4+ cell responses, after interruption of the antiviral treatment and/or resumption of active viral replication, could be expected. It would then be most important to compare levels of cytokine-producing cells before and after treatment and to determine whether HIV immunity can be boosted after one or several periods of stabilization. Interestingly, it was recently shown that after interruption of drug treatment, CD4 cell counts increased before the rebound of viremia[11]. Meanwhile, the data of Picker et al. clearly reveal the impressive silencing of the HIV-specific CD4+ cell response induced by HAART that accompanies, and might even underlie, the previously reported reduction in HIV-specific antibodies[12] and CTL responses[3]. This is not all bad news, as some of these changes may lead to the resolution of several immunological abnormalities[12]. Nonetheless, although combined drug regimens seem to become increasingly more effective, no viral eradication has been achieved so far and more than a little help will probably be needed from the immune system to clear the residual viral load. The findings of Picker et al. provide new evidence to support clinical protocols leading to stimulation of anti-HIV immunity or passive transfer of HIV-specific effectors[13] in association with HAART. No time to rest.

1. Rosenberg, B.D. Walker: HIV type 1-specific helper T cells: A critical host defense. *AIDS Res. Hum. Retroviruses* **14**, S143–S147 (1998).
2. Pitcher, C.J. *et al.* HIV-1 specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. *Nature Med* **5**, 518–525 (1999).
3. Ogg, G.S. *et al.* Quantitation of HIV-1 - Specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* **279**, 2103–2106 (1998).
4. Schmitz, J.E. *et al.* Control of viremia in simian immunodeficiency virus infection by CD8(+) lymphocytes. *Science* **283**, 857–60 (1999).
5. Zajac, A.J. *et al.*. Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188** , 2205–2213 (1998).
6. Rosenberg, E.S. *et al.* Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* **278**, 1447–1450 (1997).
7. Gorochov, G. *et al.* Perturbation of CD4+ and CD8+ T cell repertoires during progression to AIDS and regulation of the CD4+ repertoire during antiviral therapy. *Nature Med* **4**, 215–221 (1998).
8. Jung, T., Schauer, U., Heusser, C., Neumann, C., Rieger, C. Detection of intracellular cytokines by flow cytometry. *J. Immunol. Meth.* **159**, 197 (1993).
9. Gallimore, A. *et al.* Induction and exhaustion of lymphocytic choriomeningitis viru-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J. Exp. Med.* **187**,1383–1393 (1998).
10. Crawford, F. *et al.* Detection of antigen-specific T cells with multivalent soluble class II MHC covalent peptide complexes. *Immunity* **8**, 675–682 (1998).
11. Neumann, A.U. *et al.* HIV-1 rebound during interruption of highly active antiretroviral therapy has no deleterious effect on reinitiated treatment. *AIDS* **13**, 677–683 (1999).
12. Morris, L. *et al.* HIV-1 antigen-specific and -nonspecific B cell responses are sensitive to combination antiretroviral therapy. *J. Exp. Med.* **188**, 233–245 (1998).
13. Bitton, N., Gorochov, G., Debré, P. & Eshhar, Z. Gene therapy approaches to HIV-infection. Immunological strategies: use of T bodies and universal receptors to redirect cytolytic T-cells. *Front. Biosci.* **4**, D386–3D393 (1999).

Dept. of Cellular Immunology
CERVI, UMR CNRS 7627
Hôpital Pitié-Salpêtrière
83 Bvd. de l'Hopital
73013 Paris, France
email: guy.gorochov@psl.ap-hop-paris.fr

The Holy Grail of influenza virologists

High throughput sequencing now makes it likely that many complete viral genomes will be sequenced. This raises the question of whether it is necessary to study live virus for infectious diseases that have been eradicated or viruses from past epidemics.

THE US NATIONAL Academy of Sciences Institute of Medicine established a committee to decide the fate of the two remaining stocks of smallpox (*Variola* virus). Last month it reached its conclusion: a recommendation to retain stocks of live virus. This represents a brave decision, given the evocative nature of the disease and its medical and social effect[1]. These scientific discussions revolved around a central issue: do we need to keep a viable virus frozen at –70 °C when the complete genome sequence is available for study? There seems to be a consensus that much more can still be learned from studies of the virus replication in culture, its structure and the ways in which it interacts with cells to avoid

JOHN S. OXFORD[1] &
ROD S. DANIELS[2]

the host immune surveillance system[2] than ever could be conceived by a mere perusal of the genome sequence. Moreover, although there is still a need to develop an animal model for smallpox, an actual virus rather than a virtual one will be required to develop new antiviral drugs in preparation for the possible re-emergence of the virus.

There is a virus which in a single year of frenetic activity spread around the entire globe and killed even more individuals than did smallpox in 100 years—Spanish influenza. The revised estimate is that 100 million persons (1% of the then human population) died of Spanish flu between 1918 and 1919 (ref. 3). Yet this remarkable outbreak, probably the largest of any infectious disease in human history, occupies only a minuscule niche in the human memory. (Crosby[4] called the outbreak the "Forgotten Plague"). But for a small group of aficionados, interest in the so-called Spanish influenza of 1918 has been raised again. In the early 1950s, two groups in the US attempted to recover infectious virus from the lungs of Spanish influenza victims buried in permafrost in Alaska. These attempts were unsuccessful, but the pathologist who initiated the first search in 1951 considered the event

as 'unfinished business' and, having read Taubenberger's preliminary analysis of influenza RNA extracted from the formalin-fixed piece of lung of a young soldier who had died in 1918 (ref. 5), he decided to re-exhume at the same burial site in 1997. This decision raised important issues of safety as well as awakening public and scientific awareness of a serious gap in our knowledge. What is the genetic nature of a most virulent and lethal virus? Were there special social and demographic circumstances in 1918–1919 with so many young people returning homewards from the war? With even more people travelling the world today, could such a virus rise again?

Viruses are very unpredictable. Although it is unlikely that an enveloped myxovirus, such as influenza, would survive at –15 °C for 80 years, no one can really know the answer until the experiment is done. And if it could survive these conditions, it is possible that the virus could re-infect. An international expedition to Spitsbergen last year to exhume the bodies of influenza victims—involving a team of eleven scientists with expertise in geography, geology, microbiology, pathology and virology—concluded that the chance of infection at the site of an exhumation of frozen influenza victims from 1918 would be negligible, provided sensible safety precautions were taken to avoid aerosols. We experimented with a series of hollow cutting or coring devices that could, as in the manner of coal miners or geologists, take low-speed core samples from frozen lungs. Indeed, the devices were engineered to allow, if needed, slow boring through the wood of a coffin and solid ice to a depth of 20 inches.

It also seems possible that either from Taubenberger's fixed lungs, Hultin's freshly fixed frozen lung[6], or the samples from the six Spanish influenza victims in Spitsbergen, the complete genome of the 1918 influenza can be retrieved. If so, what would the complete sequence tell us? Could the virulence of the virus have been polygenic and thus could small nucleotide changes scattered across more than one of the organism's eight genes have provided the key to the subsequent human disaster, and could these scattered changes be missed or misinterpreted?

Detailed biological analysis of virulent strains of avian influenza, H5 and H7 subtypes, backed up with sequencing studies, have shown that virulence can be associated with alterations in the virus hemagglutinin (HA), a virus-surface glycoprotein responsible for initiating infection. Possession of either a polybasic amino-acid sequence at the cleavage site between HA1 and HA2 (ref. 7) or the loss of a potential glycosylation site at position 23 of HA1 (ref. 8), which maps close to the cleavage site such that carbohydrate could interfere with the cleavage process, have each been associated with an increase in virulence. Sequencing studies of the 1918 H1 HA gene predict that neither of these virulence-determining motifs are present[5,6], suggesting that more subtle and not yet understood mutations within the gene and its HA product may account for the virulence of the 1918 virus. Live 1918 influenza virus would allow examination of receptor binding capabilities, of possible spread of the virus beyond the respiratory system in animal models, studies of antiviral drugs *in vitro* and the development of reassortant viruses that could be used as future vaccine strains. While undoubtedly an exciting prospect, the total genome sequence of the 1918 influenza virus is, by itself, unlikely to reveal the cause(s) of the virus' extreme virulence. For the full story, there is a need to study live virus and the most likely source is virus in permafrost-preserved tissues of 1918 influenza victims.

We estimate that the chances of recovering 1918 Spanish influenza from the poorly preserved tissue of the coal miners in Spitsbergen are remote. However, as our safety analysis clearly warned of the dangers of virus amplification in either mammalian cells or embryonated hens' eggs, the recovered samples are now being studied in a containment IV laboratory. Although recovery of infectious influenza virus is unlikely, the recovery of measles virus RNA from badly decayed seals has been described[9], so we remain optimistic as to the recovery of influenza RNA.

A surprising result, given the more usual rapid evolution of influenza when it enters a new host[10], is the high degree of sequence conservation in the HA genes of samples derived from the two American soldiers and the Inuit woman[6]. Although phylogenetic analysis of the HA gene sequences shows them to be most closely related to mammalian strains of H1 influenza, they have many HA characteristics resembling viruses of avian origin. The most plausible explanation of this is that an avian H1 influenza entered the human population early in 1918, developed its pathogenic potential therein and circulated, in North America at least, as a genetically homogeneous virus 'quasi-species'. The Spitsbergen samples could add greatly to our knowledge of the 1918 influenza, because all the autopsy samples that have yielded information on the virus so far have been located in North America.

Sequence analysis of other influenza genes contained in the American samples, with those in the Spitsbergen samples and any other fixed or permafrost-preserved specimens related to influenza of avian and mammalian origin from the early part of this century, will shed new light on what surely must be considered the likely future global spread of this infection. But whilst there is clearly a need for more samples to analyze, ideally from geographically dispersed sites, without an infectious virus we may never obtain the full picture of what happened in 1918–1919.

1. Fenner, F., Henderson, D.A., Arita, I., Jezek, S. & Ladnyi, I.D. in *Smallpox and its Eradication* (World Health Organization, Geneva, 1988).
2. Smith, G.L., Symons, J.A., Khanna, A., Vanderplasschen, A. & Alcami, A. Vaccinia virus immune evasion. *Immunol. Rev.* **159**, 137–154 (1997).
3. The Spanish 'Flu 1918-1919. (Intl. Conf. Cape Town, 12–15 September, 1998).
4. Crosby, A. *America's Forgotten Pandemic* (Cambridge Univ. Press, Cambridge, UK, 1989).
5. Taubenberger, J.K., Reid, A.H., Krafft, A.E., Bijwaard, K.E. & Fanning, T.G. Initial genetic characterization of the 1918 "Spanish" influenza virus. *Science* **275**, 1793–1796 (1997).
6. Reid, A.H., Fanning, T.G., Hultin, J.V. & Taubenberger, J.K. Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. *Proc. Natl. Acad. Sci. USA* **96**, 1651–1656 (1999).
7. Horimoto, T. & Kawaoka, Y. Reverse genetics provides direct evidence for a correlation of hemagglutinin cleavability and virulence of an avian influenza virus. *J. Virol.* **68**, 3120–3128 (1994).
8. Kawaoka, Y., Naeve, C.W. & Webster, R.G. Is virulence of H5N2 influenza viruses in chickens associated with loss of carbohydrate from the hemagglutinin? *Virology* **139**, 303–316 (1984).
9. Osterhaus, A.D.M.E. *et al.* Morbillivirus in monk seal mass mortality. *Nature* **388**, 838–839 (1997).
10. Webster, R.G. 1918 Spanish influenza: The secrets remain elusive. *Proc. Natl. Acad. Sci. USA* **96**, 1164–1166 (1999).

[1]*Academic Retroscreen Virology,*
Department of Medical Microbiology
St. Bartholomew's and The Royal London
School of Medicine & Dentistry,
64 Turner Street, Whitechapel,
London E1 2AD, UK
[2]*Virology Division, The National Institute for*
Medical Research, The Ridgeway, Mill Hill,
London NW7 1AA, UK