The legacy of Jenner: vaccination past, present and future

Vaccination—the legacy of Edward Jenner—is 200 years old. The Royal Society, The Wellcome Trust and the Royal College of Physicians jointly marked the occasion with a series of symposia and lectures.

Historical session

The historical session was held at the Wellcome Institute on 14 May, and reviewed the historical aspects of vaccine development and distribution, its effectiveness in disease control and its future role in preventive medicine. The morning session was chaired by Professor William F Bynum (Head of the Academic Unit at the Wellcome Institute for the History of Medicine). The afternoon session was chaired by Sir Christopher Booth (Harveian Librarian, Royal College of Physicians).

Smallpox

Professor Roy Porter (Wellcome Institute for the History of Medicine) gave the opening talk entitled ‘Where the statue stood’. His lecture recounted the history of Jenner’s smallpox work as it raised questions about the changing status of Jenner’s historical legacy. Porter began by affirming the value of vaccination. He turned then to Jenner himself, the father of vaccination, presenting a composite description derived from standard biographical accounts. Jenner was portrayed as a true humanitarian, a man who chose to share his knowledge rather than keep it as a secret from which he could make a profit. Icons of the great man, from his favourite chair to a horn supposedly belonging to the cow from which his original cowpox sample derived, had been collected, preserved and displayed, providing further testimony to Jenner’s status. (Reinforcing this point was a Jenner exhibition displayed in the Reading Room of the Wellcome Library, where those attending the conference could glimpse letters in Jenner’s own hand, as well as a first edition of his 1798 Inquiries.) Porter continued the retelling of the Jenner tale with details about the history of variolation, the popular tradition from which vaccination derived, and the discovery, propagation and acceptance by the elite of Jenner’s vaccines.

After this laudatory account, Porter presented the story from the viewpoint of Jenner’s detractors. Some contemporaries thought Jenner a ‘quack’ seeking profit; others argued that his discovery was no discovery at all, the practice already having been used by farmers. There is evidence that vaccine acceptance, production and distribution did not extend as smoothly as early histories had suggested. As the 19th century progressed, anti-vaccination groups became increasingly vocal. Compulsory vaccination laws served as an impetus for these groups to voice their protests in formal texts and even in journals devoted to the opposition of vaccines. So strong was the resistance that not even Jenner’s humanitarian image was enough to quell protests over the placing of his statue in Trafalgar Square in 1858. ‘What business did this man’s contributions have among those of the military great?’ it was argued. Jenner’s statue was moved to Kensington Gardens. Nor was Jenner’s status greatly elevated by the advent of bacteriology. The centenary of his vaccine work was not extensively celebrated; his name attached to a public subscription did not draw significant financial support for the proposed infectious disease institute it was to fund. It, instead, became known as the Lister Institute. Even at his discovery’s bicentennial, Jenner’s legacy revealed its ambiguous status: the Royal Mail rejected a proposal that a Jenner stamp be issued this year. Professor Porter presented this ambiguity as an appropriate context for historical contemplation of vaccination.

Dr Derrick Baxby (University of Liverpool) took up the challenge with his paper ‘Jenner’s role in the introduction of smallpox vaccination’. He opened by...
reminding the audience of the significance of 14 May: on this day in 1796, Jenner inoculated a small boy with cowpox, setting up his demonstration six weeks later that cowpox vaccination protected humans against fully virulent smallpox. Why, Baxby asked, was 14 May chosen from many other, seemingly more likely, dates as ‘the’ occasion for historical celebration of Jenner’s work? The question allowed him to review the various interests involved in promoting or opposing vaccination in 1896, when the centenary date was selected. It also framed the remainder of his paper, which focused on the diffusion of vaccination and how the perception of Jenner polarised some of his contemporaries and affected that diffusion.

Jenner’s famous 1798 Inquiry drew three key conclusions. First, vaccination with cowpox produced only local lesions, unlike smallpox variolation which gave rise to veritable smallpox pustules. Second, cowpox originated in horses, deriving its vaccinating property with cow passage. Third, vaccination worked. Not everyone agreed with his conclusions. Some, including William Woodville who studied 600 cases of vaccination, argued that the procedure frequently gave rise to smallpox pustules, sometimes in quantities large enough to be true smallpox. Jenner countered that ‘true’ cowpox provoked only circumscribed lesions. Vaccination supplies that gave smallpox must have been either contaminated or derived from cows suffering from some other lesion-conferring disease. Still others argued that Jenner had not really ‘discovered’ anything new; that they were in fact responsible for vaccination’s public extension; or, later in the 19th century, that it was attenuation, not cowpox at all, that allowed vaccination. Yet, within the disputed history of vaccination, lies also recognition of its value. Dr Baxby concluded by reminding the audience that the 1980 commission heralding smallpox eradication pointed without hesitation to Jenner’s fundamental role.

Typhoid

Dr Anne Hardy (Wellcome Institute for the History of Medicine) provided another example of contested inoculations in her paper ‘Straight back to barbarism: military and civilian application of anti-typhoid inoculation, 1890–1920’. Dr Hardy framed her paper with what sounded to be the objective voice of scientific medicine in 1914, quoting William Osler’s and Almroth Wright’s independent championing of anti-typhoid inoculations for soldiers. Indeed, Wright warned that failure to inoculate would lead the civilised world ‘back to barbarism’. Underlying the scientific rhetoric and dire warnings was a crisis in public health that pitted immunology against the traditional focus on hygiene and individual rights embraced by British liberalism. It was on the events leading up to this crisis and the significance of its resolution that Dr Hardy concentrated.

An anti-typhoid vaccine, developed by Wright amongst others, was introduced in 1896. The centenary of Jenner’s smallpox vaccination witnessed protests against the new vaccine by those who thought compulsory vaccination infringed on individual rights. The anti-vaccinationists further asserted that, in a civilised country, hygiene alone would protect the vigilant citizen against disease—particularly a disease like typhoid. In 1899, Wright used the Boer War as an opportunity to prove his vaccine by testing it on humans. Unfortunately, his testing was disorganised and premature. His group did not keep proper records and he had not developed a dose limit, which led to the illness and even death of several of his subjects. Moreover, the existence of paratyphoid A and B was then unknown, making his vaccine seem ineffective when in fact the disease subsequently suffered was a different kind of infection. In all, Wright’s work discouraged further uptake of the vaccine.

The early 20th century witnessed the gradual re-establishment of faith in the vaccine mainly through the efforts of Leishman, who established a precise dose and perfected the vaccine further. World War I raised the contested question of compulsory vaccination for soldiers. Osler and Wright spoke (dis)passionately for the plan. Others, remembering the consequences of Wright’s earlier tests, continued to advocate hygiene and to oppose vaccines. And, while many soldiers elected to be vaccinated, the military never made the anti-typhoid vaccination compulsory. According to Dr Hardy, this turn of events indicated the failure of immunology and its vaccines. The public had connected barbarism not with lack of hygiene, but with lack of liberty. Immunology was thus seen to be an ‘uncivilised’ infringement on individual rights. Public resistance to preventive medicine continued in Britain into the 1950s.

Polioymielitis

The afternoon session opened with a paper by Mr Anthony Gould (freelance writer, London), ‘The influence of the National Foundation for Infantile Paralysis on the development of the polio vaccines’. In poignant reference to Dr Hardy’s talk, Mr Gould mentioned that he had contracted polio in 1959 while in the British Army, which did not vaccinate against polio. He then turned to the 1930s and the American story of the development of the polio vaccine under the auspices of the National Foundation for Infantile Paralysis.

Though initiated by President Franklin D. Roosevelt, the National Foundation was a voluntary establishment. Led by Basil O’Connor and Thomas Rivers, the Foundation supported both basic research and all polio-related needs. They raised money with ideas such as the ‘March of Dimes’, which asked the public to send their dimes—and dollars—directly to the President in an effort to stop polio. Meanwhile,
scientific research on polio, financially aided by the National Foundation, continued. John Enders had discovered that the polio virus could be grown and cultivated in non-nervous tissues, with implications for understanding the course of transmission and for vaccine development. Vaccine development had two paths available: live, attenuated virus; or killed virus. Enders and Albert Sabin focused on the live variety; Jonas Salk on the killed.

The story of why Salk's vaccine received preference in the US was, at this juncture, directly linked to the National Foundation. Having met Enders and Salk on the ship returning from a polio conference in Copenhagen, O'Connor elected to support Salk's work. The Foundation could no longer be thought of as an impartial advocate of polio research. Fearing rising competition from commercial laboratories, O'Connor pressed the Vaccine Advisory Council to conduct a trial of the Salk vaccine. Thomas Francis directed the trial. Francis's positive conclusions led to a media frenzy and a government order for, and purchase of, the vaccine. Several vaccine-related deaths, however, led the Surgeon General to withdraw the vaccine. By the early 1960s, Sabin's oral vaccine, having been proven safe and effective in trials in the USSR, was rapidly replacing the Salk vaccine, and the March of Dimes had dropped polio from its agenda. Mr. Gould suggested that American resistance to the Welfare State and the American Medical Association's resistance to any free vaccination effort as 'socialist' were key factors in polio vaccination in the 1960s. Still, he concluded, Salk's vaccine had helped not only to lessen the incidence of polio but also to lessen fear of the disease. By the time Sabin's vaccine was implemented, it functioned primarily to clear up the remaining cases. Within this context, the National Foundation offers insight into social intervention in the fight against disease.

Hepatitis B

Dr. Jennifer Stanton (London School of Hygiene and Tropical Medicine) spoke next on 'Vaccine viability: the shifting fortunes of hepatitis B immunisation'. It is generally believed that the 20th century has witnessed the decline of infectious disease through the combined efforts of vaccination and higher standards of living. Smallpox provided an example of what could be accomplished by the combined efforts of an effective vaccine and an effective system of delivering that vaccine. Hepatitis B, on the other hand, has provided an illustration of what happens when one has a viable vaccine but lacks an effective delivery system. The substance of Dr. Stanton's paper was an account of the variety of blocks to distribution of hepatitis B vaccine in both the developed and the developing worlds.

A cost-benefit analysis conducted in Ireland provided the example of social constraints against the vaccine in Britain. Conducted on health workers, the trial concluded that the low incidence of hepatitis B did not justify the cost of vaccinating workers. Hidden beneath the rhetoric of immediate cost was concern for a larger price to pay: testing would reveal individuals who had contracted the disease earlier in their work and who would thus have to be compensated for the multiple long-term consequences of their infection. Only in the 1980s, with changes brought about by the development of a cheaper vaccine in combination with HIV-related work, were the hepatitis B test and vaccine more commonly used. Still, connections between HIV and hepatitis B screening led doctors to resist compulsory screening, arguing that it infringed their liberties.

In the Third World, hepatitis B was a far more extensive problem. Still, governments were unenthusiastic about the vaccine. The transfer of vaccine technology was blocked by cost: early vaccines cost several times the annual national health budgets per citizen in these nations. The later, cheaper vaccine did not help much as it still cost about a third of the annual budget per person. Hepatitis B was a serious problem, but could not be given priority to the extent of justifying such a high cost. Vaccine viability did not necessarily lead to universal vaccination. One needed to understand the social and political dimensions of alliances and disease concepts in order to develop an effective vaccination program.

Malaria

Dr. Mary Dobson (Wellcome Unit for the History of Medicine, University of Oxford) gave the session's final paper 'Waiting for malaria eradication: why the resistance?' The history of efforts to 'conquer' malaria shows a shifting emphasis between control or eradication, and a shifting public mood between optimism and pessimism. The geographical boundaries of malaria are now as narrow as they can be; still, the disease kills three million children each year. General optimism hinges on hopes for a synthetic vaccine developed in 1989 and currently undergoing trials. What can the historian of medicine tell those who will be in charge of distributing the vaccine if it is viable—or who will return to control efforts if it is not?

The development of DDT allowed those who had attempted to control malaria in the 1950s by eliminating the adult mosquito to shift their focus to the destruction of larvae, and their mind-set to eradication. DDT was seen as a kind of 'magic bullet', a metaphorical vaccine against malaria that would act as did penicillin. To illustrate the war-like eradication mind-set, Dr. Dobson showed a short film from the 1950s demonstrating how DDT was working to eradicate malaria throughout the world. In the 1960s, malaria appeared to be yielding. By 1973, however, it was clear that eradication efforts had been thwarted by the combined difficulty of disease complexity and social/political resistance. Malaria was back in ascen-
dancy, and the World Health Organisation (WHO) dropped DDT from its policy. Those currently responsible for developing policy for malaria control must, Dr Dobson concluded, attend to the history of malaria control, which points to the interrelated concerns of politics, culture and profit, if they hope to develop a successful campaign.

‘The miracle of vaccination’

The Guest Lecture, given at the Royal Society, was delivered by Professor Donald Henderson (Department of Health and Human Services, Public Health Service, Washington, DC), who headed the World Health Organisation Smallpox Eradication Program in the 1970s. His lecture ‘The miracle of vaccination’ was attended by HRH The Princess Royal, who has been actively involved in efforts to vaccinate children throughout her work for the Save the Children Fund. Professor Henderson hoped to persuade the audience that vaccination had indeed earned a place in the ‘Pantheon of Medical Miracles’, from which it traditionally has been excluded. To make his case, he pointed to smallpox eradication.

Smallpox had been a globally devastating disease; Jenner’s vaccine provided an essential first step in its containment. Numerous problems, from cost to loss of potency in hot climates, constrained the vaccine’s extension. In part inspired by the Lister Laboratory’s development of a freeze-drying method that allowed the vaccine to be transported in the tropics, the WHO, in 1966, made a commitment to eradicate smallpox from the world in 10 years. (The audience chuckled when Professor Henderson told how the figure of ‘10 years’ had been decided. Smallpox eradication was being discussed when the US government devised its 10-year space plan. The WHO committee decided that if a man could be sent to the moon in 10 years, smallpox could certainly be eradicated on earth in the same amount of time.)

The Smallpox Commission decided to concentrate on breaking the chain of transmission: they would vaccinate 80% of the population, focusing their efforts on areas of disease outbreak. The method was one of surveillance and containment. He then recounted the means by which this plan was adapted to situations in various countries, emphasising that the process also created networks for future vaccination efforts throughout the world. Henderson illustrated the Commission’s progress with annual maps of smallpox distribution. The final case of smallpox occurred in 1977. The Commission had attained its goal within a small bureaucratic structure for $100 million—and had done so in only a year over its 10-year target.

In the successful campaign against smallpox, members of the Commission increasingly became aware of the number of children who died annually from preventable diseases. After smallpox was eradicated, the Commission turned its attention to these diseases, which include tuberculosis (TB), tetanus and polio in its expanded programme of immunisation. Governments, having seen the efficacy of efforts to eradicate smallpox, were even more accommodating towards the new vaccine programme. Moreover, the public health network established for smallpox, remained in place. UNICEF, Rotary International, the Save the Children Fund and others have helped to distribute vaccines that now save three million people annually.

Henderson painted an optimistic future for vaccination. History has shown that problems of vaccine research, manufacture and distribution—with all its complexities—can be overcome. Still, viruses loom as threats to civilisation. Governments that devote large proportions of their budgets to defence must be persuaded of the miracle of vaccination so as to ensure the continued war against disease.

Conclusion

The day’s historical meetings not only celebrated Jenner’s legacy, they also underscored the complexities underlying vaccine development and distribution. Economics, political priorities and cultural perceptions of disease mesh with scientific concerns to make the story of vaccination a study of modern civilisation. In this context, history is particularly relevant to policy. Indeed, the lessons of history, as Dr Dobson pointed out, were concisely stated by Ronald Ross in 1910: ‘The history of malaria contains a great lesson for humanity: we should all be more scientific in our habits of thought, and more practical in our habits of government’.

The evening concluded with a soirée and viewing of the Royal Society’s vaccine exhibition, attended by the Princess Royal.

Immune response session

The immune response session was held at the Royal College of Physicians, London, on 15 May. The papers presented were concerned with the basic science of the immune response and how such knowledge could be harnessed in the design of new vaccines. The morning session was chaired by Professor Peter Lachman (University of Cambridge) and the afternoon session by Professor George Griffin (Head of the Division of Infectious Diseases, St George’s Hospital Medical School).

Antigen presentation

Professor Alain Townsend (Institute of Molecular Medicine, Oxford) gave an overview of cellular
processing and presentation of viral antigens via the class I major histocompatibility complex (MHC). He showed how an understanding of the biochemical steps involved in the system might facilitate the rational design of vaccines. Two major systems have evolved to allow the immune system to detect intracellular or extracellular foreign antigen. The class I system is concerned with processing intracellular proteins, predominantly viral antigens but possibly also antigens resulting from malignant transformation of cells. Antigen present in the cytosol is rapidly degraded by proteases such as the ubiquitin dependent, multi-subunit protein, proteosome. The evidence that proteosome is the major source of viral antigen is strong but not complete. Elegant experiments in which cytoplasmic proteins are linked to ubiquitin demonstrate increased antigen expression via the class I pathway. However, preventing protein ubiquination by amino acid substitutions does not abolish protein presentation. Data from mutant cell lines that lack two subunit constituents of proteosome, and experiments with proteosome inhibitors, failed to confirm or refute the protein’s unique importance. It is also possible that other proteases, including enzymes within the cytosol and endoplasmic reticulum (ER), play an important role.

The importance of the transport associated proteins (TAP) in the processing of antigen also remains unclear. Cell lines with established defects in TAP have reduced expression of class I molecules. The MHC molecules in these cells are also unstable, suggesting a role for peptide in stabilising the complex. The occurrence of class I molecules associating with TAP in the ER membrane is of uncertain significance. Interestingly, human subjects who lack TAP are relatively well. Generation, binding and export of MHC/peptide complexes to the cell surface needs to be rapid for cells to survive viral infection. Generation rates are extremely rapid and binding affinities are in the order of 10^7 litres/mole, which is similar in magnitude to antibody/antigen interactions, whereas export rates are anywhere between four and six hours. It is not clear how four class I receptors in an outbred population are able to bind such an array of peptides with sufficient affinity. However, once bound, providing they possess appropriate properties, peptides are buried within the groove of the MHC molecule. In terms of vaccine design Professor Townsend felt the most efficient way of generating a cytotoxic response to a vaccine would be via a non-replicating viral vector or with DNA.

Professor Donald Mason (MRC Cellular Immunology Unit, Oxford) discussed an experimental system developed to study the relationship between the neuroendocrine and the immune systems. Using the Lewis rat model of experimental allergic encephalomyelitis (EAE), he and his group have utilised modern immunological techniques to study the evolution of this disease. In this model, subcutaneous injection of myelin basic protein (MBP) in complete Freund’s adjuvant (cfa) leads to the development of distal paralysis over the ensuing ten days, with complete recovery over the subsequent week. Studies using adrenalectomised rats confirmed the immunosuppressive role of corticosterone in these rats. Indeed, adrenalectomised rats given cfa alone die from massive release of pro-corticosterone including interleukin-1 (IL-1), IL-12 and tumour necrosis factor alpha (TNF-α). In order to prevent this, rats were fed corticosterone pellets at the time of immunisation. Without further corticosterone supplementation all rats developed disease and failed to recover, whereas when given steroid supplementation all recovered. Attempts to explain these effects by culturing cells ex vivo with antigen with or without steroid suggest that disease is prevented by the development of antigen specific T helper 2 (Th2) cells and that disease develops as a result of a Th1 response to MBP.

Professor David Raith (Department of Pathology and Microbiology, Bristol) also made use of an EAE model to study the possible modulation of autoimmune disease by reinstating self-tolerance by immunisation with synthetic peptides. Extensive work on the mouse EAE model has defined the dominant T cell epitopes of both the MBP and proteolipid protein (PLP). One T cell epitope is located at the N-terminal of MBP. Interestingly, studies with transgenic mice that express a T cell receptor (TCR) capable of recognising this epitope suggest that it is a poor antigen. Analysis of the epitope binding affinity to MHC suggests that its poor antigenic qualities relate to low affinity binding to class II MHC. Substituting lysine at position 04 with tyrosine leads to enhanced binding affinity and greatly enhanced T cell activation. This suggests that potentially autoreactive T cell clones escape negative selection in the thymus because of their weak affinity interactions with MHC molecules. This theory is borne out by injection of the high affinity analogue into the developing thymus of transgenic animals. Immunohistochemistry and FACS analysis of thymic subpopulations demonstrate that the analogue has a profound effect on the CD4+ CD8+ population, considerably reducing their number, whereas injection of the wild-type peptide has no effect. These cells are eliminated by apoptosis. As yet there is no evidence that this mechanism applies to humans.

To establish whether this regulatory system is operative in the peripheral lymphoid system, the effect of immunising with high affinity mutant peptide on the course of the disease was studied. The high affinity analogue caused disease if animals were injected with peptide and complete Freund’s adjuvant, but prevented or treated established disease if injected in saline up to one month before and five days after immunisation with MBP. Further experiments with high affinity epitope analogues of MBP and specific T cell clones demonstrated dramatic changes in cytokine
release, specifically in the amount of tumour TNF-α, in response to challenge with peptide. *In vitro* immunisation with soluble peptide analogue has been shown to be capable of bystander suppression, preventing and treating established disease resulting from exposure to an unrelated protein or peptide.

**Antitumour vaccine strategies**

Dr Mary Collins (Institute of Cancer Research, London) discussed methods of enhancing antitumour responses by genetic modification. The first evidence for the development of specific antitumour cytotoxic responses was from experiments involving transplantation of tumours in a mouse model. A major goal of these studies is to identify tumour-specific antigens that might be rarely expressed, or mutated cell proteins or viral antigens, which could be utilised to engender specific antitumour responses. She particularly concentrated on work with melanoma, as attempts at immunootherapy with this tumour have had measured success in the past and melanoma cell lines are relatively easy to culture *in vitro*. Various systems have been developed to engineer specific antitumour responses in animal models. They usually involve modification of the tumour cell line to express foreign antigen, enhance antigen presentation or express cytokines. Dr Collins discussed in some detail a trial that involves establishing *in vitro* cultures of tumour cells from patients with melanoma. These cells are transfected with a retroviral IL-2 expressing vector and maintained *in vitro*. Tumour cells are then returned to the host after irradiation, and pre- and post-vaccination antitumour cytotoxic T lymphocyte (CTL) responses are evaluated. The study is still in progress but preliminary results have been favourable.

**DNA vaccines**

Perhaps one of the most exciting recent developments in vaccine technology is the potential use of non-replicating DNA vectors as vaccines. Dr Margaret Liu (Merck and Co Inc, USA) discussed the rapid progress made in evaluating this new technology. The vectors make use of strong viral promoters, polyadenylation and termination sites. They were initially developed as part of a project to develop gene therapy and were first administered to animals as negative controls in a transfection experiment. The construct used included the beta galactosidase gene and unexpectedly, beta galactosidase was expressed in myofibrils and an anti-beta galactosidase response was generated. DNA based vaccines have the theoretical advantage of mimicking viral infection with presentation via class I and the generation of CTL responses but without the potential risks associated with live viral vector. Preliminary experiments, using the gene for influenza nucleoprotein, demonstrated cross strain protection with both humoral and cytotoxic responses. Responses were long lasting and DNA persists at the injection site for up to four months in an unintegrated, episomal form. Studies using the reporter gene luciferase demonstrated persistent expression two years after immunisation, although more immunogenic systems may lead to more rapid elimination through a CTL response against DNA infected cells.

Studies with primates have made it possible to compare licensed influenza vaccines with the DNA preparation. Results suggest persistence of antihemagglutinin antibody after immunisation with the DNA vaccine but not with either whole inactivated or split inactivated licensed vaccines. The DNA vaccine produced an excellent boosting response, with higher titre than with either of the licensed preparations. DNA vaccines incorporating plasmodial, bacterial and viral antigens have been investigated and show promise. *In vitro* analysis of cytokine production in response to rechallenge with antigen reveals production of Th1 cytokines, particularly gamma interferon.

A number of safety issues need to be addressed before DNA vaccines can be generally accepted and the question of efficacy in humans remains. However, the first study in human volunteers with the influenza nuclear protein construct is under way. Conceptually, DNA vaccines have a number of attractions, they are simple to produce, utilising fairly generic technology, and if results with humans are as successful as those obtained in animal models, DNA vaccines will be a very promising and exciting technology.

**Mucosal vaccines**

Professor Myron Levine (Center for Vaccine Development, Baltimore, USA) discussed important theoretical and potential practical advantages of mucosal immunisation. The vast majority of pathogens are primary mucosal pathogens, or locally or systemically invasive following mucosal colonisation. Although mucosal immunologists had concentrated on production of local antibody, the potential for generation of both local and systemic cell-mediated responses makes mucosal immunisation an attractive goal. Practical considerations include ease of administration compared to parenteral routes and growing concern over possible detrimental interactions and greater risk of reactions to multi-component parenterally administered vaccines.

One of the most promising of the new orally administered vaccines is the live oral cholera vaccine, CVD103-HgR, a derivative of the classical Inaba serotype. The gene for the toxic, ADP ribosylating A subunit of cholera toxin has been deleted, leaving the B subunit intact. Placebo-controlled phase II studies have confirmed this to be an extremely well tolerated vaccine and highly immunogenic in children aged 7-17 months following a single dose. Experimental challenge data confirm protection in volunteers
challenged with either the classical or El Tor strains and either serotype. This vaccine will be the first recombinant live vaccine to be licensed. Professor Levine then discussed another attenuated live oral vaccine, the typhoid vaccine Ty21 A. This vaccine is not the most immunogenic vaccine in terms of seroconversion but engenders good protection rates in field trials. Other attenuated mutants developed include the double Aro CVD 908 Salmonella typhi vaccine. This has been extensively evaluated in terms of both efficacy and safety. It was associated with extremely high seroconversion rates, and vaccine excretion in stools of volunteers was transient (approximately three days). A potential concern was vaccinæmia, which developed in all volunteers receiving 10^9 organisms and in 50% of those receiving 10^7 organisms. This problem was solved by further genetic manipulation which had no effect on immunogenicity but prevented bacteraemia.

A major hurdle to evaluation of attenuated mutants has been the lack of a good animal model. This changed with the recognition that intranasal immunisation led to responses in mice to S typhi and derived mutants. Subsequent manipulations have allowed this model to assess the potential efficacy of CVD live salmonella strains to deliver heterologous antigens via the mucosal route. Promising results have been obtained with antigens derived from tetanus, diphtheria and pertussis, expressed in CVD103 derivatives, in terms of both production of neutralising antibody and protection on rechallenge with wild-type organism.

Although there have been tremendous advances, there are still considerable problems to overcome. The efficacy of the Ty21 vaccine varies tremendously depending on the formulation and the number of doses administered. A number of vaccines, including the oral Sabin polio vaccine, have looked highly promising when evaluated in industrialised countries, but their efficacy seems severely diminished when tested in developing countries. Such results have led investigators to consider the co-administration of cytokines with soluble antigens to drive the immune response or alternatively encode the genes for cytokines in live vaccines.

Immune responses to lentivirus

Professor Ian McConnell (Veterinary Pathology, Cambridge) discussed an animal model of lentivirus infection which provides a unique insight into pathogenesis and immunity in persistent viral infection. There is a whole group of lentiviruses that infects various animal hosts, including maedi–visna virus (MAV) which leads to a slowly progressive lymphoproliferative condition and is endemic in sheep flocks. Differing pathologies observed among the lentivirus group relate to varying cellular tropism. The advantage of studying MAV is that it specifically infects macrophages. The sheep model involves cannulation of the efferent lymphatic channel for sampling efferent lymph following direct inoculation of MAV into the skin of study animals. At certain intervals after infection, analysis of lymph node histology allows an assessment of the immune response in the node, the cellular phenotype of responding cells and estimates of viral DNA, RNA and antigen load.

A vigorous T and B cell response is observed shortly after infection, with evidence of CD4+ cells proliferating in response to viral antigen in vitro. Cytotoxic lymphocyte precursors can be recovered from the node and efferent lymph. Virus can be detected in the efferent lymph, all of it cell associated. The major problem is viral persistence in accessory cells and viral replication is tightly linked to the state of activation of these accessory cells. Professor McConnell is using this model to understand mechanisms of viral persistence in the face of an immune response and looking at ways of bringing about viral elimination.

Mycobacterial vaccines

Professor Douglas Young (Department of Medical Microbiology, Imperial College of Medicine at St Mary's) gave an extensive overview of the current state of mycobacterial vaccine research. The tuberculosis epidemic in Europe peaked around the time of Edward Jenner, with an approximate mortality of 500 per 100,000 population. The incidence of tuberculosis started to decline well before the introduction of BCG in the early 1920s. Although BCG is the most widely used vaccine in the world, there is great interest and need for new improved vaccines. Molecular mycobacteriology has recently highlighted the genetic differences between BCG and its parent organism, Mycobacterium bovis. The characterisation of proteins present on some of the gene deletions described in BCG may identify important virulence genes. It is clear that for BCG to stimulate protective immunity it must be live, suggesting that there are either important differences in the manner antigens are presented or differences in the nature of antigens produced between live and dead organisms. There is the suggestion that early T cell responses to secreted antigens lead to protective responses whereas similar, late responses to cytoplasmic antigen might lead to pathology.

Many vaccine studies are proceeding and making use of new technology, including DNA libraries and whole genome screens. Some are showing great promise in a variety of animal models. The great problem is transferring these experimental data to field trials, which take a long time to conduct and require careful thought regarding the best measure of protection. However, despite these obstacles, a coordinated strategy towards vaccine development has been adopted by institutions such as the World Health Organization (WHO), National Institutes of Health.
Vaccinia

**Dr Geoffrey Smith** (MRC Cellular Immunology Unit, Oxford) returned to vaccinia virus and discussed a number of new findings with an old vaccine, advancing a strong case for continuing vaccinia based research despite the eradication of smallpox. Vaccinia has become a popular expression tool, and as it replicates within the cytoplasm, it is used to study DNA replication and transcription. It also provides an excellent model of viral pathogenesis. The first demonstration of an immune response to a heterologous antigen was to hepatitis B surface antigen (HBsAg) expressed in vaccinia. This experiment led the way in developing technology to modify and manipulate existing vaccines or microorganisms to develop protective responses to foreign antigen. However, if vaccinia were to be used as a recombinant vaccine, it would require rational attenuation and so attention turned to defining mechanisms of vaccinia pathogenesis.

Vaccinia is a prototype orthopox virus with an uncertain origin and natural host. Its genome is approximately 200 kb and includes genes encoding a variety of virulence factors facilitating immune evasion and pathogenesis. Factors include eukaryotic cell growth factors, proteins that prevent apoptosis, complement regulatory proteins, intracellular proteins that disrupt the class I MHC pathway and a number of cytokine receptors. Vaccinia encodes at least four cytokine receptors: interleukin (IL)-1β receptor, interferon (IFN) α/β receptor, γIFN and a TNF receptor. Dr Smith discussed the IL-1 receptor (B15R) and the IFN α/β receptor in some detail. The IL-1 receptor is responsible for controlling the febrile response in vaccinia infection; vaccinia strains most associated with complications, particularly encephalitis, eg Copenhagen, do not express this receptor. The majority of vaccinia strains also secrete a type 1 α-IFN receptor, which seems to be a major virulence factor; the least virulent vaccine strains such as Lister and Wyeth do not produce it. This receptor analogue is different from the other IFN receptors which are members of the type 2 cytokine family and this raises the possibility of an as yet undescribed eukaryotic equivalent.

In the final part of his talk, Dr Smith turned his attention to the possible origins of vaccinia virus. Theories include derivation from cowpox or variola by *in vitro* passage and subsequent mutation or alternatively through recombination between cowpox and variola. However, sequence analysis makes these theories unlikely. Numerous strains of vaccinia have been used and all are distinct from cowpox. The vaccine taken to the USA in 1876 is vaccinia and all the vaccine strains used in the 20th century are vaccinia. One of the most credible theories is that it represents a distinct form of orthopox virus that no longer infects its natural host, and could possibly be horsepox. Dr Smith ended his talk with a tribute to Jenner and an enthusiastic call to vaccinologists and immunologists to continue to develop new and innovative vaccines.