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.

Prophylaxis: the foundation for our future progress

PREVENTION IN CONTEXT

The most persuasive evidence for the distinction between humans and the other animate organisms with which we share this planet is surely that we can consciously generate notions of what is in store for us and on that basis we can act in the most beneficial manner. We can accentuate, enhance or encourage what we think is in that future, if we believe that this will be to our advantage, or conversely, we can take measures to prevent outcomes that we judge will be to our detriment. It is the central theme of this editorial that human society will fail to progress unless it takes the necessary preventive measures against the many potential disasters that face us.

We could obliterate virtually all life on this planet were we to unleash the destructive potential of the nuclear weapons we have in store. Our profligate use of non-renewable resources can only lead to shortages, conflict and the misery of the unfortunate. The uncontrolled growth of population will exacerbate the rate of depletion of our dwindling inheritance, resulting in the inevitable decrease in the value we place on human life, with a consequent erosion of those moral systems based on the survival of our genetic and cultural information capital. Some nations already spend between 6% (UK) and 14% (USA) of gross domestic product on taking care of diseased citizens and we should recognize that in the future the amount spent must rise. It is thus clear that the prevention of disease can release resources that can provide a base for society to continue to progress and develop. In addition to human-based disaster scenarios, there are threats from the other organisms of our biosphere. To achieve the present rate of evolution, living organisms have had to walk the tightrope of associating intimately with the carriers and transporters of genetically valuable materials. On the one hand, such carriers could be the bearers of useful and valuable capabilities or properties while, on the other hand, they could effect their own propagation at the expense of the life or well-being of the host.

Having foreseen such disasters, we have to implement the prophylactic measures that are appropriate for the task. It is beyond the remit of an editor of VACCINE to suggest measures to prevent the nuclear holocaust, the depletion of Earth's concentrated reserves and the world's population problem. It would also be inappropriate to comment on the prevention of disease through the control of diet and behaviour. Rather, this author must focus his attention on the organisms that currently cause diseases in humans and their animals (and plants!), and he must show that by succeeding in preventing these infectious diseases, we contribute to the establishment of the foundations for our future progress.

In the past, society was ravaged by plagues. Humans, domesticated animals and agriculturally important plants succumbed to diseases whose origins were obscure. The control and elimination of bubonic plague, smallpox, rinderpest, foot-and-mouth disease, anthrax, polio, measles, leprosy, tuberculosis, whooping cough, syphilis, rabies, yellow fever and others have lessened man's dread of the untimely death of his loved ones and the despoliation of the animals (and plants) on which his livelihood depended. With increasing confidence that his life's work would not be in vain, the struggle for improvement and progress becomes worthwhile; it is this positive, proactive attitude that is the foundation for the advancement of our society and, indeed, our continuing evolution both personally and socially. Incidentally there are increases in material wealth and free time not dedicated to personal life-supporting activities. These provide further opportunities to invest in society. One such investment, amply rewarded in the past, is in measures to decrease the effects of those infectious diseases that have not yet been conquered. While such diseases, including AIDS, are not in the category of the plagues of yesteryear, their further control and extinction would contribute significantly to our future progress. It is in this context that I now consider the future development and use of vaccines.

VACCINE EVOLUTION

The starting point for the development of modern vaccines can be found in carefully twisted pledglets dipped in pustular fluids over fires doped with incense and mumbled incantations to deities revered and dreaded. The pustular fluids elicited by the smallpox virus may be held to have begun the process of seeking immunity by the inoculation of a disease-associated material under conditions that afford the greatest chance of a successful immunization. Such materials are far from being either defined or pure (see F. Brown, in this issue), so it is hardly surprising that they were fraught with problems, not the least of which was the killing of the inoculee by smallpox. Jenner's use of animal pox postular materials (in addition to his use of cowpox pustular fluid from the palm of Sarah Nelmes in 1796, he used swinepox in 1789 to inoculate his son Edward, who subsequently resisted challenges by inoculated variolus matter in 1791 and 1792[1]) led to the human-to-human transmission of pustular materials. (Cowpox pustular material was not always readily available.) During this process syphilis was transmitted and the recombination of pox viruses probably occurred. This lack of definition of the immunogenic material is clear from our present ignorance of the origin of the vaccinia virus used to protect against smallpox and which provides the basis for some of the deliberately recombined vaccines of the modern era.

Such lack of definition did not apply to the monocultures of bacteria used for the anthrax and fowl cholera vaccines of Pasteur in the 1880s, although the infected and dried spinal cord vaccines used for rabies were crude tissue homogenates. Vaccines protective against the bacterial diseases of diphtheria, tetanus, tuberculosis, bubonic plague and whooping cough followed. Each vaccine began as a monoculture but was far from being defined in terms of knowledge of the

0264-410X/92/140971-06

molecules and their quantities present in the vaccine (see papers by Walker and Cherry, in this issue). This mode of vaccine preparation was also used for the viral vaccines protective against yellow fever, mumps, measles, rubella, Newcastle's, foot-and-mouth and Marek's. In each case the monocultured seed material was amplified in a culture system and either stabilized or potentiated by mixtures of incompletely defined materials that resulted in preparations that could cause problems for the recipients of the vaccines[2]. From the accidents and upsets experienced by the vaccine producers during the first seven decades of this century, a body of knowledge and practice emerged that kept the deleterious effects of vaccination within tolerable limits. Yet, all the while, the memories of the disasters caused by ill-defined or poorly controlled vaccine preparations cast a cloud over the further development of prophylactic materials; was the cost really worth the benefit?

This situation, augmented by the thalidomide calamity, has led to the strengthening of regulatory and licensing agencies in most countries. Has this resulted in vaccines of greater definition and purity? This does not seem to be the case for the varicella vaccine described by Takahashi in this issue: sonicated cell culture fluid is the principal material used in the vaccine. By contrast, the most recently developed virus vaccine for humans, hepatitis A, is based on a purified cell culture fluid of infected and inactivated MRC-5 cells which is aluminium hydroxide adjuvanted and 2-phenoxy-ethanol preserved[3]. The purification process involved sterile filtration, ultrafiltration and concentration by column chromatography. These processes decreased the amount of bovine albumin (a component of the cell culture medium), to <1 ng ml^{-1}. Similar care was taken to ensure that the inactivation process using formaldehyde was more effective than that used for the well established inactivated polio vaccines. Although the recently introduced serum vaccine protective against hepatitis B has been largely superseded by genetically engineered equivalents, the introduction of a purified and much inactivated material[4] was permitted in the absence of an alternative vaccine that was yet more advantageous. Thus, purification and definition of the immunogens that have recently been licensed have increased to the point where it is difficult to make further progress. What, then, have the genetic engineers and peptide chemists to offer that can improve on the traditional ways of producing immunogens?

CHEMISYNTHESIZED AND GENETICALLY ENGINEERED VACCINES

In this anniversary issue of VACCINE there are two papers (Brown, and Arnon *et al.*) that look to chemisynthesized immunogens for the vaccines of the future. These authors argue with cogency that such vaccines can be produced in unlimited quantities by processes that are inherently inexpensive and yield products that are defined, pure and stable under the widely variable conditions of temperatures (ambients of up to 40°C) against which conventional vaccines have to be protected. But can they be made as effective as the conventional immunogens? There are three inherent difficulties for such vaccines. First, they are, perforce, based on linear epitopes. This limits their ability to mimic the conformation-dependent epitopes commonly found as immunogenic epitopes. Secondly, they cannot be glycosylated in chemisynthetic systems; although there is an epitope that is an effective immunogen and can be produced by the genetic engineering of yeast cells (hepatitis B surface antigen), it does not have the native glycosidic side chains: this could be an exceptional case. Thirdly, the chemisynthesized peptides which are not extensively modified are generally poorly immunogenic. By the time they are made into effective immunogens by being coupled to adjuvants or co-expressed by attachment to hepatitis B virus core antigen proteins or polymerized and coupled to T-cell epitopes, the resulting immunogens have lost some of their putative advantages such as low cost, purity and high stability. This has not, however, prevented the development of a possibly valuable vaccine protective against malaria by the use of polymerized synthesized antigens with a molecular weight of 150 kDa[5].

The genetic engineers, by contrast, have had more success. Vaccines protective against hepatitis B, Aujeszky's, rabies and piglet diarrhoea are in use while there are many others at present in the wings awaiting the obligatory licence, [diphtheria, pertussis (see Rappuoli in this issue), cholera (see Manning in this issue), shigella, salmonella and other enteric pathogens (see a forthcoming issue of VACCINE containing the papers given at the Cambridge meeting in April 1992), malaria[6]]. Are the genetically engineered vaccines better defined or more pure than their conventional counterparts? Many of them, for example, the vaccinia recombinant vaccines, are cell culture fluids or sonicates thereof. Others based on the enteric bacteria are presented as live organisms and therefore cannot be defined, while a smaller remaining group are built around bacterial toxins that have been specifically engineered so as to remove the pathogenic components, for example, diphtheria and, in some cases, pertussis. It would only be in the latter cases that more defined molecules have emerged as prophylactics.

There are of course some novel approaches that do not yet appear to have led to putative vaccines. For example, the construction of chimaeric vaccines based on polio type I vaccines should result in the development of safe type III vaccines (or vaccines that do not revert to pathogenic virus on administration)[7]. The techniques that have resulted in the polio chimaeras can be used to achieve similar constructs containing foot-and-mouth disease virus immunogenic epitopes or sections of the V3 loop of the gp120 glycoprotein from HIV. A second approach, particularly suitable for those viruses that exhibit an icosohedral capsid, is founded on the use of 'empties', complete icosohedral virus capsids differing from the native virus in lacking the nucleic acid. This has been assayed for foot-and-mouth disease (Brown), could be useful for polio and may be a product of agriculture via the cow pea plant and cow pea mosaic virus chimaeras[8].

Neither genetic engineering nor chemisynthetic approaches to vaccines are inherently conducive to the production of defined and pure materials for vaccination. It would appear that the principal thrust behind the use of these techniques is the creation or innovation of materials capable of initiating an immunogenic response that would be more protective than anything previously tried. As a secondary consideration

the ability to produce such materials with as large a margin of safety as possible, as would be the case were the vaccines both defined and pure, would also improve the product because the cost factor would be commensurately decreased.

This outline is but a thumb-nail sketch of a fraction of the activities that are in progress. Licensed products are slowly beginning to emerge from the maw of the regulatory agencies. The effects of the vaccines that have recently been granted licences have yet to be felt. To this we must add the effects to be achieved by the vaccines not yet licensed. The assured outcome has to be a decrease in infectious disease with a corresponding burgeoning of progressive activity. However, there is still no reason to adopt a complacent attitude as no account is taken of the latest and most perplexing of the infectious diseases, that caused by the human immunodeficiency virus, HIV.

A POST-MODERN VIEW OF VACCINES

We can use the opportunity of VACCINE's tenth anniversary to identify a new era of vaccine research and development. There are four features that typify this new age. First, it follows and extends in a novel way the revolutionary progress that has resulted from our newly found abilities in genetic engineering and associated techniques and knowledge; hence the concept of the 'post-modern'. Secondly, there is an increasing need to recognize that most of the single organism-caused disease syndromes have been dealt with, so we have now to rise to the challenge of the multiple organism disease syndromes such as those that arise in respiratory, gastro-intestinal and mastitic diseases. Thirdly, we wish to achieve a prophylactic effect in a non-infectious disease such as the elimination of a cancer before it becomes an incurable disease (immunotherapeutics?), or the prevention of a pregnancy. Fourthly, although Pasteur demonstrated in 1885 that it was possible to prevent the emergence of the rabies disease in patients who had been infected, we are still struggling to achieve equivalent success in diseases such as those caused by the herpes simplex virus (HSV) or HIV. The post-modern era therefore calls upon us to create and exploit a new armoury of skills and approaches to improve our condition.

Multi-organism diseases

Although we may be infected with a single organism at any one time, the situation where a number of otherwise unrelated organisms can cause the same effect has to be dealt with in a manner different from that where the disease and the causative organism have a one-to-one relationship. For example, respiratory disease may be caused by the following viruses: rhinovirus, influenza virus, coronavirus, adenovirus, respiratory syncytial virus, parainfluenza 3, coxsackievirus and echovirus, and also by the following bacteria; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, *Bacteriodes* spp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus* spp. The renewed need to deal with the bacterial pathogens looms ever larger as the exchange of antibiotic resistance genes becomes more commmonplace.

The situation is made more complex by the 100 or so different types of rhinovirus, each of which results in a convalescent state that does not afford protection to another virus type. A new approach to this kind of situation may be obtained through the preparation of a monoclonal antibody to a rhinovirus that is capable of cross-neutralizing a wide range of such viruses. This antibody may then be used to identify the neutralization determinant on the virus, to provide passive protection as an inoculated antibody or to generate an active virus mimicking antigen by the generation of the anti-idiotypic antibody. Arvind Kumar and the author have shown that it is possible to obtain such cross-neutralizing monoclonal antibodies to a range of rhinoviruses that even neutralize some of the related polioviruses and coxsackieviruses (unpublished results). This approach, when used on those diseases where there are a large number of non-cross-protecting types of the individual infectious agent, for example influenza, gonorrhoea and AIDS, could provide a method for the development of appropriate immunoprophylactics.

There is a second novel component that is required in the multi-organism disease syndrome. For a vaccine to be useful in protecting humans or animals against infection it has to be able to counter the infectivity of a majority of the disease-causing agents both individually and collectively. The acceptance of such a vaccine will depend on the user being convinced that there is less than a one-in-three chance of the vaccinees contracting any of the individual diseases associated with the syndrome.

The third barrier to successful anti-syndrome vaccines is that it is likely that such vaccines will be formulated from many antigens. This has to be effected both economically and with sufficient technical expertise that the volume of the administered material does not exceed that which is acceptable. This may necessitate a re-examination of the technical procedures for the generation of immunogens, particularly those of viral origin.

The need for new vaccine production techniques. Well developed conventional cell culture systems for the generation of viruses result in the production of 1–20 $\mu g\ ml^{-1}$ of viral material. The viruses may be produced from cells held in suspension cultures of $\approx 10^6$ cells ml^{-1} or in surface-adherent cell cultures normally effected in roller bottles or on microcarriers but affording about the same cell concentrations as are found in the suspension culture counterparts. To achieve the concentrated antigens needed for the syndrome vaccines it would normally be necessary to use one or more of these procedures: ultrafiltration, precipitation, sedimentation associated with an adjuvant, chromatographic purification or ultracentrifugation. All these processes are effected at considerable expense and with a decreased overall yield of antigen. It may therefore be necessary to look to novel techniques to achieve the high antigen concentrations that are obligatory for such vaccines.

Two techniques that have been used for the production of monoclonal antibodies at concentrations of milligrams of antibody per millilitre of culture fluid (i.e. about 100–1000 times more concentrated than conventional virus vaccine processes), are based on either hollow fibre cartridges or on porous particles held in either packed or fluidized beds (see Ref. 9 for a review). The exploitation of such systems, with cell concentrations in excess of 10^8 cells ml^{-1}, would be an exciting way to

achieve the virus concentrations that can be formulated into the new polyvalent mixtures described above.

Immunotherapeutics. Therapeutics is the art or technique of ministering or treating, as applied to medicine or healing. There would clearly be a need for overt disease to evoke the ministering/treating activity. Hence there is an evident distinction from prophylaxis which is effected in the absence of any overt distress or disease. However, there is a sense in which 'therapeutic' action may be taken ahead of the eruption of disease, for example when a vaccine is administered to prevent the development of the rabies disease in a person who had been previously infected. A similar situation occurs in the case of herpes simplex infection when, after infection, repeated episodes of the disease can occur: the vaccine would be used to increase the time between recurrences. Again it would be appropriate to design a vaccine to prevent the onset of AIDS in patients who had been infected with HIV. In these cases the 'therapeutic' actions may be properly reclassified as prophylactic.

However, in the case where it is intended to *prevent* a predicted non-infectious disease based on a sophisticated symptomatology, such as cancer, it is less clear whether we are dealing with a therapeutic situation or a prophylactic activity. In the early stages of cancer the patient is hardly 'not at ease' (diseased). Therefore the need for medical ministrations is not apparent. We can therefore consider a vaccinal approach to such cases. This in itself breaks new ground for vaccine manufacturers. While it is recognized that vaccines protective against infectious agents that can cause cancer have been successfully applied for many years, for example in the case of Marek's lymphoma cancer of chickens, and are in preparation to protect humans against the cancerogenic papilloma infections of the genitalia, such situations differ considerably from the cancers that arise from a breakdown in any one of several of the endogenous cell-proliferation control mechanisms. Yet it is possible that the non-specific stimulation of the immune system by adjuvants may prevent the development of diagnosed cancers (see Azuma, this issue). This also marks a break from the specificity of the traditional vaccine approach and therefore may be considered post-modern. As a further development of this approach, it may be possible to inoculate killer T cells of the $CD8^+$ lineage, which had been grown in cell culture and whose individuality had been either culturally or genetically expunged in the manner reported to proffer success in the protection of bone marrow transplant patients against infection by cytomegalovirus[10]. If such an approach succeeded, we can expect the production of specific and non-specific T cells to become important in vaccine manufacture.

Vaccines protective against pregnancy. By stretching a point, pregnancy may be regarded as an infectious disease or, more conventionally, as a state whose prevention would, on occasion, alleviate distress. Vaccines to protect against pregnancy can be based on the stimulation of antibodies that will react with human chorionic gonadotrophin, leuteinizing hormone receptor, luteinizing hormone beta-subunit, the fertilization antigen of sperm, luteinizing hormone-releasing hormone, follicle-stimulating hormone or finally, the sperm-specific lactate dehydrogenase (LDH-X)[11]. If we develop a post-modern vaccine against a human hormone or antigen specific to the generation or continuance of the pregnant state we may also achieve one of the most desirable objectives: the decrease in the rate of increase in the world's population. In this sense the prevention of pregnancy is *pari passu* with the efforts to limit the numerical growth of humans.

A corollary to the use of antibodies that react with the hormones controlling pregnancy is that it would become possible to use other antibodies directed against other hormones, cytokines or lymphokines. As every agonist seems to have an antagonist, the use of antibodies could possibly control all the reactions effected by the body from both the negative and positive standpoints. Could this be the preferred way forward as a rival to somatic genetic engineering to achieve the same effects? In such situations one might consider the antibody-inducing agent as a material that 'prevents' the *status quo ante*!

The HIV enigma

> 'How often have I said to you that when you have eliminated the impossible, whatever remains, however improbable, must be the truth.' (Sherlock Holmes to Dr. Watson in Conan Doyle's *The Sign of Four*, 1889).

It is unusual for there to be a clarion call to think in a radically new and post-modern way about a vaccine to protect against an infectious disease. Hilleman makes such a plea in his article in this issue. So at variance with the conventional approach has this new departure to be, that one or more specialized, probably international, institutes have to be set up from scratch. They must be sufficiently prestigious to attract and assemble a comprehensive spectrum of diverse talents into a juxtaposition from which will emerge the solutions to the problem. The pseudocompetitive individual investigator contracted to a project for a short time can but nibble at the issues that need to be addressed to come to prophylactic solutions suitable for those both uninfected and already infected with HIV.

The concept of (a) dedicated institute(s) is not new. In 1924 a research establishment of the Foot-and-Mouth Disease Committee of the Ministry of Food and Agriculture was set up at Pirbright, Surrey, UK[12]. Similar institutes focused on the same objective (the implementation of an effective vaccine that would protect cloven-hooved animals against foot-and-mouth disease) were established in America (Plum Island), Denmark (Lindholm), the Netherlands (Lelystadt), Italy (Brescia) and many other countries (see contributors to Ref. 13). The value of the work of these institutes may be estimated from the present situation in America (free from foot-and-mouth disease since 1929 although threatened from Canada in 1952 and Mexico in 1953[14]), and in Europe where, having developed effective vaccines in 1947 (Frenkle) and 1963/4 (BHK cell culture)[15], vaccination to protect cattle against foot-and-mouth disease stopped in 1991/91 (A. Donaldson, personal communication). It is important when considering ventures of this kind to appreciate that once the goal of such an institute has been achieved there must be a recognized programme to redirect, retrain and redeploy the talented staff to areas that are similarly bedevilled. One can think immediately that the diseases of malaria, schistosomiasis and the re-emerging tuberculosis could

provide new material for such an operation once the HIV problem has been laid to rest.

Notwithstanding this call for novelty in engineering the prophylactic agents, there is still a need to develop our abilities further to test and evaluate putative products in an ethically acceptable manner. This matter is dealt with by Karzon in this issue. There is little doubt that the large-scale use of chimpanzees is not practicable and that monkey models may or may not be suitable subjects from which to learn what we need to know. Humans at risk may, in the final analysis, prove to be the most useful test system. The biggest problem is probably the time taken between the application of a material and the realization of the value of that material in either the prevention or the cure of the disease; this could be over 15 years! Feedback of this type is almost as much a curse as a blessing, but it is likely to be all we can get.

New routes to vaccines protective against HIV. In 1988 Langley and the author examined the prospects for a vaccine protective against AIDS[16] and concluded that '... an ideal prophylactic would have to initiate a reaction, probably in the macrophages, such that intracellular virus is destroyed. This implies that the vaccine we are seeking represents a departure from the conventional vaccinal materials in that the immune system as a whole has to remain quiescent while the macrophage and other cells that harbour virus are stimulated to purge themselves of their passengers.' Clearly, one way of achieving this is by the use of antisense nucleic acids. In September 1990, Wong presented a paper at the Cold Spring Harbor Symposium detailing the use of antisense nucleic acid for the prevention of outbreaks of HSV. It would seem sensible if this route were taken to prevent the emergence of the HIV during the development of AIDS, although one of the main problems with this approach is that not all of the infected cells become superinfected with the antisense material. Would it not be of interest to engineer an HIV-like particle that would contain at its surface the gp160 so that it would provoke those antibodies that would enable it to be taken up by the macrophages that harbour the wild-type HIV, thus delivering the anti-sense nucleic acid to the infected cells?

This approach should avoid the problems presented by opportunistic pathogens and should circumvent the complexities of the cells of the immune system, which may or may not be infected. It also obviates the need to consider the complex problems resulting from a change of the physiological function of the immune system cells that could lead to a change in the lymphokine profile; the latter can be held responsible for the apothosis of the T cells in the absence of the infection of those cells[17]. While it is appropriate to call for the discovery of the aetiology of the disease (but see Ref. 18) in order to 'rationally' design a vaccine, if this method of achieving a vaccine were to be successful it would probably be the first such rationally designed vaccine in the history of vaccination. Rather, we have to proceed in a reasonable way by trial and error using the empirical method of allowing the results of experiments to determine how we proceed, as opposed to theoretical notions derived from rationally derived models, the dogmatic acceptance of which can do more harm than good. It is clear to this author that, having discovered that the genome of the HIV integrates with the host-cell chromosome, the battlefield for the viability of the cell is the host-cell chromosome itself; that is where the antisense material can exert its greatest effect.

There is not a shortage of potential targets for a vaccine[19]. However, legislatures and regulatory agencies will ensure that there will be few opportunities for the effective testing of the alternatives. We can hope, therefore, that the establishment of an institute that would have on hand the full range of disciplines and procedures necessary would lead inexorably to progress such that the threat from this most enigmatic of viruses would be eliminated.

CONCLUSIONS

Our progress as a society is as much dependent on the prevention of disasters as it is on the extension and enhancement of that which we know to be advantageous. Vaccine prophylaxis has a key role to play in the prevention of these catastrophes that are caused by microbes on our planet. It can also be used to prevent the increase in world population and the diseases of the body that are susceptible to the control of the immune system. Our increasing ability to read the language that is used by the cells to control their activities will lead to further developments in the use of induced antibodies, though in such cases it will be difficult to adhere to the concept that the interference is of a preventive nature and the inducer of the effect is a vaccine. Nevertheless, by the controlled modulation of the components of the immune system and through them the other controlling systems in the body, we are set fair to achieve not only what we desire but also that which we have not yet had the courage to dream about.

R.E. Spier
School of Biological Sciences,
University of Surrey,
Guildford, Surrey, UK

REFERENCES

1 Baron, J. *The Life of Edward Jenner, Vol. 1.* Henry Colburn Publisher, London, 1838, pp. 103–137
2 Wilson, G.S. *The Hazards of Immunization.* Athlone Press, London, 1967, p. 323
3 Peetermans, J. Production, quality control and characterization of an inactivated hepatitis A vaccine. *Vaccine* **10** Supplement 1, 1992, pp. S99–S101
4 Prince, A.M. Hepatitis B Virus: active and passive immunization. In: *Vaccines and Immunotherapy* (Ed. Cryz, S.J. Jr) Pergamon, Oxford, 1991, pp. 256–270
5 Patarroyo, M.E., Amador, R., Clavijo, P., Moreno, A., Guzman, F., Romero, P. *et al.* A synthetic vaccine protects humans against challenge with asexual blood stages of *Plasmodium falciparum* malaria. *Nature* 1988, **332**, 158–161
6 Schorr, J., Knapp, B., Hundt, E., Kupper, H.A. and Aman, E. Surface expression of malarial antigens in *Salmonella typhimurium:* induction of serum antibody response upon oral vaccination of mice. *Vaccine* 1991, **9**, 675
7 Burke, K.L., Dunn, G., Ferguson, M., Minor, P.D. and Almond, J.W. Antigen chimaeras of poliovirus as potential new vaccines. *Nature* 1988, **332**, 81–82
8 Bowsley, P. and colleagues. (Work quoted.) *Financial Times* 25 June 1992
9 McCullough, K.C. and Spier, R.E. *Monoclonal Antibodies in Biotechnology: Theoretical and Practical Aspects.* Cambridge University Press, Cambridge, 1991, pp. 265–315
10 Riddell, S.R., Watanabe, K.S., Goodrich, J.M., Li, C.R., Agha, M.E. and Greenberg, P.D. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science* 1992, **257**, 238–241
11 Talwar, G.P. (Ed.) Contraception research for today and the nineties. Progress in birth control vaccines. In: *Progress in Vaccinology, Vol. 1* Springer Verlag, New York, 1988, p. 401
12 Burns, K.N. Diseases of farm animals. In: *Agricultural Research 1931–1981* (Ed. Cooke, G.W.) Agricultural Research Council, London, 1981, p. 270
13 *Dev. Biol. Stand.* 1977, **35**,

14 Bachrach, H.L. Foot-and-mouth disease: worldwide impact and control measures. In: *Viruses and Environment*, Academic Press, London, 1978. pp. 299–310

15 Spier, R.E. Production of veterinary vaccines. In: *Advances in Biotechnological Processes, Vol. 2* (Eds Mizrahi, A. and van Wezel, A.L.) Alan R. Liss, New York, 1983, pp. 34 61

16 Langley, D. and Spier, R.E. Is a vaccine against AIDS possible? *Vaccine* 1988, **6**, 3- 5

17 Meyaard, L., Otto, S.A., Jonker, R.R., Mijnster, M.J., Keet, R.P.M. and Miedema, F. Programmed death of T cells in HIV-1 infection. *Science* 1992, **257**, 217 219

18 Brown, P. How does HIV cause AIDS? *New Scientist* 18 July 1992, 31 35

19 Edington, S.M. Is an AIDS vaccine possible? *Biotechnology* 1992, **10**, 768- 771