PERSONAL REFLECTIONS ON TWENTIETH CENTURY VACCINOLOGY

Maurice R Hilleman

Merck Institute for Vaccinology, West Point, PA, USA

Abstract. The science of vaccinology was created in the late 18th and 19th centuries by "giants" of the time including Jenner, Pasteur, Koch, von Behring, Ehrlich and Lister. Relatively little technologic advance was made in the period leading to World War II except for yellow fever and influenza vaccines. Support for war efforts fueled developments which led to the modern era of vaccines of 1950 onwards. The author's career in vaccinology, which began in 1944, was recognized in the 2002 Prince Mahidol Award given in Bangkok, Thailand. This review presents the substance of the Award lecture delivered on January 29, 2003.

INTRODUCTION

Among the many pursuits of mankind, none can be more rewarding than that of preventing diseases by procedures which derive from the practice of vaccinology. Vaccines, together with sanitation and nutrition, have served as principal tools employed in Public Health to increase the health and lifespan of human beings. Public Health was a dedication of Prince Mahidol of Thailand whose memory is celebrated each year with the Prince Mahidol Awards in Medicine and Public Health. We are reminded in this that clinical medicine treats the individual, but public health treats the community to the benefit of all who reside therein.

Present endeavors in the fields of vaccinology, and the pursuits in basic science which make them possible, are enabled by the rich heritage of discoveries made by the great pioneers of the 18th and 19th century Europe, particularly by Jenner, Pasteur, Koch, von Behring, Ehrlich, and Lister, who laid down many of the basic principles and technologies we still follow today. It has been the responsibility of 20th century vaccine research both to serve that period and to provide a legacy for the 21st century.

EARLY BACKGROUND

My own interests in science and biology derived from my birth and rearing on a farm in the state of Montana on the harsh Western Frontier where working with plants, animals, soil, mechanics, and electricity provided a crucible for learning, and a practical introduction to the workings of the biological and physical sciences. Having majored in science in high school, I went on to receive a baccalaureate degree in microbiology and chemistry from Montana State University in 1941 and a doctorate from the University of Chicago in 1944 in Medical Microbiology with specialization in virology. Knowledge of viruses was mostly a black box of disparate and anecdotal data in the early 1940s, without even a textbook. My professor Francis Gordon and I did, however, pioneer the first formal course in virology in the USA, including both lecture and laboratory components.

INTRODUCTION TO INDUSTRY

Having exhausted what university tenure had to offer, I joined the Squibb Pharmaceutical Laboratories in New Jersey in 1944 to gain practical and managerial experiences. I became the Head of the Virus Laboratories, directing and conducting research, development, manufacturing, and quality control. Among our many labors, we pioneered and produced a Japanese B encephalitis vaccine to protect military personnel in the Pacific Theater of World War II, and we collaborated with Wendell Stanley in commercial development of the Sharples centrifuge-purified influenza vaccine for military and civilian use.

Correspondence: Maurice R Hilleman, Marck Institute for Vaccinology, West Poit, PA 19118, USA.

BASIC RESEARCH AT WALTER REED

By 1948, I was convinced that my further career should be focused on basic research but with need to take promising possibilities to practical realities. I joined the Walter Reed Army Institute of Research in Washington, DC, in 1948 as chief of respiratory diseases research, with authority to explore them all but with special responsibility to create a knowledge base for influenza viruses, and to avert the next pandemic of the disease. My first investigations were aimed at the study of antigenic heterogeneity of the virus and to explain in full why the 1947-48 influenza epidemic was so severe and why the vaccine failed to protect. In comparing the antigenic composition of previous and current virus isolates, I discovered progressive antigenic change, with time, which is now called antigenic drift, and is caused by point mutations needed by the virus to evade the continuing evolvement of complementary herd immunity. The 1947-48 epidemic was caused by virus of very marked antigenic drift.

In 1957, when the World Health Organization and our military surveillance systems failed to detect, I noted a report in the press of a large and severe outbreak of respiratory disease with high fever in Hong Kong. This had all the potents of an influenza pandemic and our findings in strain analyses of specimens which we obtained from Japan allowed me to alert the national and international communities to a pending pandemic which would begin in the US with the opening of school in September. It did occur as predicted and was named the Asian influenza pandemic of 1957. With such early alert, we were able to engage the manufacturers early enough to produce 40 million doses of vaccine by year's end, averting many cases of influenza in the population.

In 1951, I conducted a large study of influenza at Fort Leonard Wood MO. to collect throat samples from patients with the intent to determine whether there was antigenic change in the virus on egg adaptation. Inadvertent shift in etiology in the overall epidemic left me with specimens from a different disease that was of unknown etiology and which gave an opportunity to explore. With availability of a trachea from a newly deceased soldier, we prepared explant cultures of respiratory epithelium, which showed transmissible cytopathic change on inoculation of throat samples from patients at Leonard Wood. This was the discovery of the adenoviruses, with which we could soon write a new chapter on epidemic respiratory diseases. In the contemporary time period, Dr Robert Huebner at the National Institutes of Health found similar agents in cell cultures of tonsils and adenoids of children who were latently infected but without evident disease. Viral adaptation to monkey kidney cells allowed us to develop a killed adenovirus vaccine that we showed to be near 100% effective in preventing adenovirus disease in two epidemics in the military. A killed vaccine for civilian use was licensed in 1958.

BACK TO RESEARCH IN INDUSTRY

In 1957, after 10 years at Walter Reed, which included a Sabbatical in 1951, as Visiting Investigator at the Rockefeller Institute, I accepted an invitation to join the Merck Research Labs in West Point, PA, to head a new Department of Virus and Cell Biology to be formed. This new laboratory was the brainchild of the famed Vannevar Bush, who had become chairman of the company and who believed that one day viruses would be of great scientific importance.

A new pattern for industrial organization was evolved for the Department in which basic research would serve as the foundation. I was given full responsibility, with authority and with accountability, to plan and direct all basic research and development, clinical investigations, engineering scale-up, and government relations, taking products, when developed, to manufacture and licensure with continued responsibility to maintain the products on the market. This centralized operation, while an overwhelming task, did provide a smoothly operating machine in which planning and decisions were quick to evolve and implement, and to pioneer new vaccines. Clinical working alliances were made with Dr Joseph Stokes in the Department of Pediatrics, and Children's Hospital, at the University of Pennsylvania, and with Dr Victor Villarejos at the Louisiana State Medical Research Unit in San Jose, Costa Rica. Above all, the central focus through-

out was on basic research with progression to targeted developmental research on what appeared promising.

MODERN ERA VACCINOLOGY

The beginning of the modern era of vaccinology arguably may be fixed as 1950 with Enders' breakthrough in tissue culture technology for growing polioviruses. Our first project was to develop a purified and precisely standardized killed polio vaccine which would correct the deficiencies of the Salk polio vaccine. This vaccine was licensed in 1960 under the name of PURIVAX.

A major problem with poliovirus vaccine production lays in the presence, in *Macacus* monkey kidney cell cultures, of more than 40 different endogenous contaminating viruses. Seeking remedy, we grew our viruses in *Cercopithecus* monkey kidney cell cultures which proved to be free of contaminating viruses. In using these cultures, we discovered hitherto undetectable viruses which grew and were detectable in *Cercopithecus* cells. One such agent was the SV40, monkey polyomavirus, which we also found to produce cancers, when inoculated into newborn hamsters. These findings, which provided means to remove contaminants, were important in making live polio vaccine possible, in simplifying killed poliovaccine production, in providing an early model both to study and understand virus-caused cancer, and to elucidate the immunology of cancer. SV40 virus became a principal model for studies of DNA virus-caused cancer, and a tool for basic studies in molecular biology.

PEDIATRIC LIVE VIRUS VACCINES

In 1957, it was perceived that live attenuated vaccines might be developed to protect against measles, mumps, rubella and varicella in children. These were only dreams at the time. Developing these vaccines faced common hurdles which involved etiologic discovery, and propagation and attenuation to achieve acceptable clinical reactogenicity while retaining adequate potency. Most important, was the lack of animal models and the need to conduct all basic attenuation, immunogenicity, protective efficacy, and proof of safety studies in thousands of susceptible child volunteers, with informed consent.

Individual problems with measles vaccine involved elimination of indigenous chicken leukemia virus contamination, attenuation and achievement of further attenuation – first by coadministration of immune globulin, and later by further attenuation of virus itself.

Mumps vaccine required finding a wild virus free of neurovirulence from which an appropriately attenuated vaccine virus could be developed.

Rubella vaccine relied on our finding of means to grow and attenuate the virus in duck cell culture, and in proving safety on contact of vaccinated children with pregnant susceptible women.

Varicella vaccine proved difficult to attenuate, while retaining potency, and the OKA strain was substituted for our virus in the vaccine.

Acceptable monovalent, bivalent, and trivalent vaccines were developed and licensed between 1963 and 1971.

HEPATITIS B

The breakthrough discovery of hepatitis B virus surface antigen in the bloods of hepatitis carriers by Blumberg opened the door to a vaccine. We found vaccine development to be feasible by applying a 4-step purification and inactivation process of carrier plasma which involved antigen purification and concentration by *zonal centrifugal separation*, followed by *digestion with pepsin, urea dissociation* and *re-association*, and *treatment with formaldehyde*. The pepsin, urea, and formaldehyde steps, individually, were able to destroy all detectable life forms and collectively constituted a process to develop a vaccine which was safe for man. Following demonstration of safety and protective efficacy in chimpanzees, of safety and potency for man, and determination of a vaccine regimen, two controlled clinical studies were carried out which showed essentially 100% efficacy. This plasma-derived vaccine was the world's first viral subunit vaccine and the first licensed vaccine against any human cancer.

The need for a more reliable source for hepatitis antigen led us, in 1975, to commission the preparation of an expression vector to produce surface antigen in yeast culture. The process was optimized in our laboratories and a vaccine of equal potency and safety was prepared when the recombinant antigen was substituted for the plasma-derived antigen in the preparation. Clinical performance was the same and the vaccine was licensed in 1986. It represented the world's first recombinant-produced vaccine.

HEPATITIS A

Hepatitis A vaccine was pioneered in our laboratories, with the first isolation of true hepatitis A virus in marmoset monkeys in 1973. Virus that was purified from infected livers was used to develop tests to *define the virus*, the *clinical picture*, and the *epidemiology* of hepatitis A. Such purified virus was also used to create a prototype killed vaccine in 1978, which was highly effective in preventing infection in vaccinated marmosets following challenge with live virus. Our breakthrough cultivation of the virus in cell culture in 1979 opened the door to a killed vaccine for man, which copied the process of the prototype marmoset liver vaccine. It was licensed in 1996.

MAREKS NEURAL LYMPHOMATOSIS

Our research with SV40 virus had piqued our interest to develop the world's first licensed anticancer vaccine. We accomplished this using Burmester's live turkey herpes virus which was apathogenic in chickens but immunized against neural lymphomatosis, called Marek's disease, when given to newly hatched chicks. A whole infected cell Mareks vaccine was licensed in 1971 and a purified dried virus vaccine was licensed in 1975. The vaccine proved highly effective in preventing tumor, death, and declining egg production. It revolutionized the economics of the poultry industry.

MENINGOCOCCI, PNEUMOCOCCI, *HAEMOPHILUS INFLUENZAE*

We were asked in 1971 by the US military to develop a vaccine against meningococcal disease to provide a solution to the drug resistance problem which had arisen against meningococcal meningitis of military personnel. Polysaccharide antigens were extracted from meningococcal types A, C, Y and WI35 organisms and used to prepare vaccines which were safe and immunogenic. They were licensed as single and bivalent preparations between 1974 and 1977. The quadrivalent vaccine was licensed in 1982.

Having entered the bacterial polysaccharide arena, we developed 14 and 23 valent pneumococcal vaccines which were licensed in 1977 and 1983, respectively. Purified polysaccharide vaccine against *Haemophilus influenzae* was eagerly pursued by a number of pharmaceutical companies following discovery that coupling of their polysaccharides with proteins elicited T helper as well as humoral antibody immune responses. Our product was licensed in 1989.

VIRAL INFLUENZA

Toxicity due to impurities in killed influenza vaccine led us to develop a filter-purified and a zonal gradient-purified vaccine in 1969 and 1970, respectively. These refinements vastly reduced the reactogenicity of killed influenza vaccine.

INTERFERON AND INTERFERON INDUCERS

One of our earliest programs of research at Merck, lays with purification and characterization of interferon following Isaac's and Lindenmann's discovery of the substance in 1957.

Chicken allantoic fluid interferon induced by cultivation of influenza virus in embryonated hens' eggs, was *purified* 4,500-fold and *had an activity* of 42 ten thousandths microgram of protein *per unit* of activity. The purified interferon was shown to be a monomeric glycoprotein of 7,900 molecular weight (also multimeric). It was the first of the cell cytokines to be discovered and characterized, and serves to control viral infection in nature.

Because of species-specificity of interferon, chicken interferon had no application to man. We set about, therefore, to develop useable interferon

inducers for direct application in man by trying to find out how viruses accomplished induction. We found, in three different approaches, that double-stranded RNA is *foreign to cells* and provides the *danger signal for a cell to express* antiviral interferon. Interferon induction is now recognized as an early response effector substance elicited by the innate immune system in response to viral exposure. We found homopolymeric synthetic poly I:C to induce interferon on injection into man. It is of particular interest as a pattern recognition signal to the innate immune system.

BENEFITS FROM VACCINE APPLICATION

The accomplishments of the 20th century vaccine research have proved to be of worldwide significance. Its vaccines are now finding their way increasingly from the developed world into routine use in the developing nations. Before vaccine, eight million persons worldwide died of measles per year. The annual mortality rate is now less than one million. Combined MMR, where amply applied, has essentially eliminated clinical disease caused by these agents, and congenital rubella syndrome is now a rarity. Cost savings from use of MMR in the US alone, in 1992, came to 5.1 billion dollars. Hepatitis B vaccine, now given routinely to newborn babies in more than 100 nations, has drastically reduced the incidence of hepatitis B virus carriers with major reduction in cirrhosis and cancer of the liver in countries where the vaccine has been extensively applied.

HERITAGE

Many of the technical breakthroughs in the 20th century, though not well exploited, will be the heritage to the 21st century. These include the sciences of vectors, adjuvants, recombinant expression, and the fledgling discoveries in genetics, genomics, proteomics and computation science which will be importantly used. New live attenuated vaccines, and subunit bacterial vaccines will also be developed.

Vaccinology is a field in which dreams may be turned into realities. It is an activity which is heavily overshadowed by uncertainties, but can be conquered by persistent rational pursuits and by selective choices needed to surmount the hills and mountains in the quest. I would add finally that vaccine progress can best be made in close cooperation between government, academia, and industry. He or she who wishes to engage in vaccine research will be served by being well positioned into all three of these enterprises.

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