

Overview

Vaccines in historic evolution and perspective: a narrative of vaccine discoveries

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Abstract

The sciences of vaccinology and of immunology were created just two centuries ago by Jenner's scientific studies of prevention of smallpox through inoculation with cowpox virus. This rudimentary beginning was expanded greatly by the giants of late 19th and early twentieth centuries biomedical sciences. The period from 1930 to 1950 was a transitional era with the introduction of chick embryos and minced tissues for propagating viruses and Rickettsiae *in vitro* for vaccines. Modern era vaccinology began about 1950 as a continuum following notable advances made during the 1940s and World War II. Its pursuit has been based largely on breakthroughs in cell culture, bacterial polysaccharide chemistry, molecular biology and immunology, which have yielded many live and killed viral and bacterial vaccines plus the recombinant-expressed hepatitis B vaccine.

The present paper was presented as a lecture given¹ on August 30, 1999 and recounts, by invitation, more than five-and-half decades of vaccine research from the venue of personal experience and attainment by the author. The paper is intentionally brief and truncated with focus only on highlights and limited referencing. Detailed recounting and referencing are given elsewhere in text references [Hilleman MR. Six decades of vaccine development — a personal history. *Nat. Med.* 1998;4 (Vaccine Suppl.): 507–14] and [Hilleman MR. Personal historical chronicle of six decades of basic and applied research in virology, immunology and vaccinology. *Immunol. Rev.* (in press)]. This narration will have achieved its purpose if it provides a background of understanding and guidelines that will assist others who seek to engage in creation of new vaccines. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Early history of vaccines

Vaccinology is a complex multidisciplinary science that is partly rational and partly empirical. It engages basics and breakthroughs in achieving practical and licensable products. Understanding and comprehending the specifics of vaccinology may be facilitated by review of its total history [1–4].

Fig. 1 provides a diagrammatic outline of the history of vaccines. Progress in the evolution of whole or sub-unit, live, killed or recombinant viral or bacterial vaccines can be divided into eras. The diagram depicts the

progress from the start of the scientific era followed by enlightenment derived from rational empiricism and transition to the modern era. The modern era has been the most productive of all, providing many new vaccines and technologies that have led to the contemporary period and to what the future may bring. Progress, for any particular time period, is rate limited by the kind and amount of financial support it receives, as shown on the left vertical columns of the diagram.

In the beliefs of ancient peoples (Table 1), diseases were inflicted on mankind by intangible and capricious deities as punishment for ill-defined transgressions. Fear of destruction by disease became an effective tool used by rulers, politicians and their shamans to instill terrors, which would prove useful in controlling human behavior in the long climb from early tribal to

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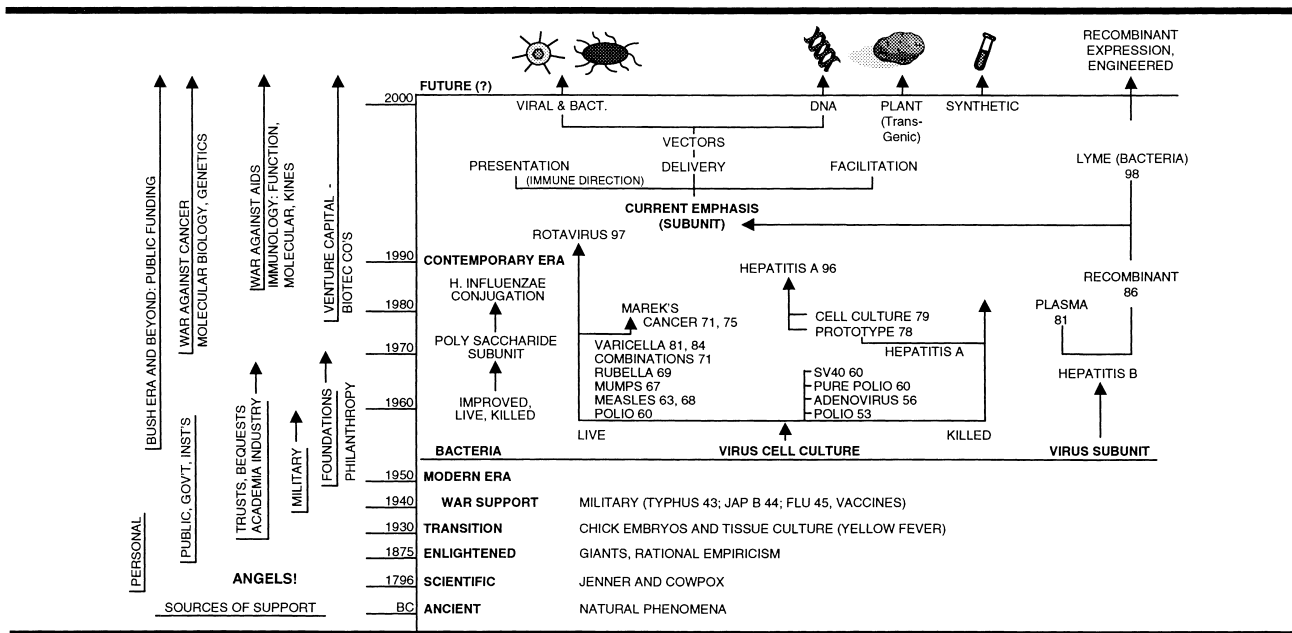


Fig. 1. Rise of vaccinology — BC to 2000.

civilized existence. Much of what was known to early civilizations about contagion, insect transmission, and sanitation was lost to Europe with the fall of Western Greco–Roman civilization following 400 AD and the onset of the Dark Ages. It was not to be revived in full until the nineteenth century. However, some, who were the forerunners of modern science, did discover microbial life forms, the relationships of environment to disease, and the fact that there was no second occurrence following certain clinically definable ill-

nesses. Such heretical concepts revealed that man himself, rather than devils and demons, were the source of pestilence, and that solutions to problems might exist outside an appeal to the supernatural.

The ancient Chinese practice of preventing severe natural smallpox by inoculating pus from smallpox patients was introduced into Europe in the early eighteenth century. This procedure was known to Edward Jenner as was the fact that milkmaids were protected against smallpox by prior infection with cowpox. Lay persons, such as farmer Benjamin Justy, inoculated his family with the cowpox pus to prevent smallpox, well before the time of Jenner.

It was with such background of knowledge that the English practitioner, Edward Jenner [5], conducted the first scientific investigations of smallpox prevention by human experimentation in 1796. These clinical studies proved that preinoculation of cowpox virus did prevent smallpox on challenge with virulent virus. From this beginning, the sciences of both vaccinology and immunology were born.

During the nineteenth century, cowpox vaccination became a worldwide practice, especially in Europe and North America. But the principles learned from Jenner's seminal findings lay fallow for more than a century-and-a-half during which time no new vaccine had appeared. The field was sorely in need of proofs that would reject the theory of spontaneous generation and that would establish the germ theory of disease.

Table 1
Vaccinology — in the beginning

Capricious gods and devils cause disease.
Fear of disease — powerful tool for rulers, shamans and politicians.
Early observations. Transmissibility of disease, sanitation, resistance to repeat infection, codified religious taboos promoting health.
Early knowledge diminished by fall of Rome and Dark Ages.
Superstition prevails.
Reawakening in the late 1700s.
Jenner investigates phenomenon of absence of smallpox occurrence in milkmaids infected with cowpox.
Jennerian prophylaxis.
Beginning science of vaccines and immunology.
Eclipse (period of maturation, smallpox vaccine improvement) of vaccinology to 1875 awaiting Pasteur's seminal findings.
Spontaneous generation of life does not occur.
Fermentation by microbes.
Establishment of germ theory of disease.

Table 2

Vaccinology — 1875 through World War I and 1930

Great awakening 1875 — discovery and rational empiricism. Focus on bacteria and antibodies (rabies virus exception).

Giants were

Louis Pasteur — immunoprophylaxis, attenuation.

Robert Koch — methodology, etiology, hypersensitivity, postulates.

Emil von Behring — antibodies and immunotherapy.

Paul Ehrlich — specific receptor—ligand binding, specific chemotherapy, antibody quantification.

By 1929:

Humoral immunologic phenomena described

Immunotherapy dominates the field

Credible and useful vaccines

Smallpox and rabies

Killed and/or attenuated typhoid, shigella, cholera

Plague, diphtheria, tetanus, pertussis, and tuberculosis

Both of them were accomplished, in great measure, by the French chemist, Louis Pasteur [1–4].

The fourth quarter of the nineteenth century (Table 2) was a period of great awakening in which the meaningful science of vaccinology was born. It extended for more than four decades, into World War I. The giants [1–4] of the early period were Pasteur, Koch, von Behring and Ehrlich. It was a period in which central focus was on bacteria, on medical application, and on empirical immunologic discoveries relating mainly to antibodies.

Having noted attenuation of fowl plague bacteria by laboratory cultivation, Pasteur also observed that they induced resistance to subsequent challenge with virulent bacteria. Further studies gave rise to his development of credibly useful vaccines against anthrax, cholera and virus-caused rabies. It has been suggested that Louis Pasteur's sudden and remarkable burst of

insight into vaccines might have been aided substantially by his unacknowledged acquaintance with the pioneering concepts of Auzias-Turenne [6], which were published several years earlier.

Robert Koch, in Berlin, was the master of pure culture technology and was heralded for his discovery of both the cholera and the tubercle bacilli. Koch's postulates gave rigid definition to establishing specific etiology in disease, and his discovery of clinical hypersensitivity ranked with Metchnikoff's discovery of phagocytic cells in relation to innate immunity.

Emil von Behring, the first recipient of the Nobel Prize, utilized Roux's and Yersin's discoveries of the soluble toxins of diphtheria and tetanus bacilli, that could be detoxified for purpose of immunization, and established the field of passive immunotherapy. This was to dominate therapeutic medicine against infectious diseases for decades to come.

The most far-reaching discoveries of that era, however, were those of Paul Ehrlich who found specific affinities of dyes and other chemicals for cell components. Based on the principles of selectivity, he developed the world's first synthetic pharmaceutical drug, that of compound 606, or Salvarsan, for treating syphilis. Ehrlich's development of methods for specific quantification of antibodies made von Behring's passive immunity a practical reality. His concepts for specific complementarity of perceived cellular side chains with chemicals and with other proteins gave birth to what was later called specific receptor-ligand binding. This concept dominates our understanding today of immunologic specificity, of cellular chemistry and of specific therapeutics by drugs.

Many other workers followed with seminal investigations [see [1–4], and by the end of World War I in 1919, most of the kinds of humoral immunologic

Table 3

Vaccinology — transition 1930–1948, including early studies on influenza and adenovirus agents and vaccines at Walter Reed 1948–1957

1931	<i>Goodpasture</i> — virus propagation on membranes of embryonated hens' eggs.
1935	<i>Theiler</i> — safe and effective yellow fever vaccine attenuated by passage in minced chick embryo cultures.
<i>Squibb Virus Laboratories</i>	
Early 1940s	<i>Cox's</i> formalin-inactivated embryonated hen's egg (yolk sac) typhus vaccine for European invasion.
1944	Formalin inactivated mouse brain Japanese B encephalitis vaccine for Far East invasion, based on earlier Japanese and Russian studies and a Sabin report.
1945	<i>Wendell Stanley's</i> sharpless-purified chick embryo allantoic fluid-derived influenza virus vaccine. A paradigm for purified virus vaccines.
	Process and manufacturing developments at E.R. Squibb & Sons Labs. Military and civilian.
<i>Walter Reed Army Institute of Research (WRAIR)</i>	
1948–58	Discovery of progressive antigenic change (drift) and major change (shift) in influenza viruses by prospective and retrospective viral and seroepidemiologic studies.
	Detection of 1957 Pandemic; Vaccine development, 1957.
1953–57	Discovery of Adenoviruses (Epidemic — WRAIR; Latent — NIH) — 1953.
	Killed virus vaccine developed and proved effective(98%) — 1956. Commercial — 1958.

phenomena had been described. Live and killed vaccines of useable quality had been evolved. In addition to those already mentioned, they included vaccines against typhoid fever, Shigellosis, tuberculosis, plague, diphtheria and tetanus. Pertussis vaccine was not to be developed until 1926. All these vaccines continue to be researched and improved to the present time.

During this early period, resources for research (Fig. 1) were severely restricted unless serving an important military need or an ability to obtain public or private subscription, based on public attention and acclaim. It is important to emphasize again that, for all time, the volume and speed of research accomplishment has been consistent with the amount of available support.

2. Vaccines in transition from 1930–1950 (including 1950–1957)

The two decades between 1930 and 1950 (Table 3), which covered World War II, were a time of transition for what was to become a new era of vaccines. The large breakthrough of the period [1–4] was Goodpasture's demonstration in 1931 of viral growth in embryonated hens' eggs. From this came Theiler's safe and effective minced chick tissue vaccine 17D against yellow fever that found enormous application in tropical countries.

2.1. Early research at the E.R. Squibb & Sons Research Laboratories

At the Squibb Virus Laboratories [1,2], growth of typhus *Rickettsiae* in the yolk sacs of embryonated hens' eggs, according to the Cox method, rapidly led to mass production of licensed typhus vaccines that were seminal to the health of military personnel during World War II.

We at the Squibb Laboratories developed Wendell Stanley's influenza vaccine, which was purified by continuous flow centrifugation, and which became a paradigm for purified viral vaccines. In addition, using a report by Sabin [7], my colleagues and I were able to evolve and, rapidly, to develop a crude formalin-killed mouse brain-derived Japanese B encephalitis vaccine for commercial manufacture that was used in 1944 and 1945 to protect the troops in the Pacific offensive of World War II.

2.2. Research at the Walter Reed Army Institute of Research

Having joined the Walter Reed Army Research Laboratories in Washington, DC in 1948, my first assignment was to devise means for detecting and preventing the then "next pandemic" of influenza. In the

HONG KONG BATTLING INFLUENZA EPIDEMIC

Special to The New York Times

HONG KONG, April 16—Thousands of cases of influenza have been reported here during the last few days in the "worst epidemic outbreak in years" according to health authorities.

Because this colony does not require reports to be made on virus infections, an accurate estimate of the number of victims was not available. The vernacular press estimated there were about 250,000 residents receiving treatment. The population of the colony is about 2,500,000.

The influx of an estimated total of 700,000 refugees from Communist China has created a constant danger because of overcrowded conditions. Fires and epidemics are the worst fears of the Government authorities.

Throughout each day, thousands of sick persons have stood in long lines awaiting treatment in clinics. Many women carried glassy-eyed children tied to their backs.

Fig. 2. Reconstructed from NY Times, Wednesday 17 April 1957.

course of prospective and retrospective virologic and seroepidemiologic studies, I discovered [8–10] that there were both progressive and abrupt changes, with time, in the antigenic specificity of influenza virus that is now called drift and shift [11].

Then, on April 17, 1957, an article (Fig. 2) appeared in The New York Times, which provided a first alert to influenza in Hong Kong. Virus studies [12–14] allowed my colleagues and me to predict the occurrence of the Asian Influenza Pandemic of 1957 that would start in the Fall in the US with the resumption of school. It did occur on schedule. Collaborating with commercial manufacturers [14], it was possible to achieve production of 40 million doses of vaccine by Thanksgiving when the Pandemic peaked and rapidly declined thereafter.

An inadvertent shift in the etiology of a respiratory disease epidemic, which occurred during a field study of influenza in 1951, at Fort Leonard Wood, MO [15], left me with a huge collection of blood and throat samples from cases of noninfluenzal acute respiratory

Table 4

Modern era — bacterial capsular polysaccharide vaccines — 1950 continuing

Dominant vaccine research is polysaccharide subunit	
<i>Pneumococcus</i>	
By 1945	Antibody response is marker for type-specific immunogenicity and protective efficacy.
1946	Hexavalent vaccine licensed but displaced by antibacterials.
1964–1968	Effective chemotherapy may not prevent death (R. Austrian). Renewed vaccine research.
1977	14-valent vaccine licensed.
1984	23-valent vaccine licensed.
<i>Meningococcus</i>	
1963	Sulfonamide resistance creates military problem.
1969–1972	Walter Reed vaccine feasibility and protection shown.
1974–1982	Vaccine development and licensure: A 1974; C & A–C 1975; A–C–W135–Y 1982.
	Sporadic occurrence limits commercial interest.
<i>Haemophilus influenzae</i>	
1985	Polysaccharide vaccine licensed for older children.
<i>Reality</i>	
Mid-1970s	Polysaccharide vaccines are poorly immunizing in young children.
1987–1990	Discovery, conjugation and protein elicits protective response in new born animals.
1992	Licensure of diverse <i>H. influenzae</i> conjugate vaccines.
1998	Intensive work on all polysaccharide vaccines.
	New Subunit Lyme Disease Vaccine licensed to SmithKline Beecham.

illness. This large team study, with its tactical support, was very expensive and it was necessary for me to accomplish something of value before the deed was discovered. A newly deceased military recruit netted me a warm trachea from which my colleague, J. Werner, and I prepared explant cultures of tracheal lining that grew out ciliated epithelial cells. Inoculation of throat specimens from the Leonard Wood patients gave three isolates [15,16] (types 3, 4 and 7) of a new virus that was propagable in series. This was the discovery of the adenoviruses.

Discovery of the adenoviruses causing epidemic disease was made in my laboratory, while those causing persistent latent infection in tonsils and adenoids of children were made in Robert Huebner's [17] laboratory, both in 1952. Enders' [18] breakthrough propagation of poliovirus (see below) in cells of embryonic tissues in 1949 opened the way to cultivation of viruses in cells in culture. A killed monkey kidney cell-grown epidemic adenovirus vaccine [19,20] was developed in my laboratory and was proved to be 98% effective in a large controlled clinical study at Fort Dix, NJ, in 1956. This was just four years after discovery of the virus. Killed adenovirus vaccine was licensed for commercial distribution in 1958 for pediatric application.

Much of the support for research between 1930 and 1950 derived from military initiatives and from the rise of Foundations, which gave philanthropic donations and supported laboratories such as those of the Rockefeller Institute.

Between 1950 and 1985 (Fig. 1), many new vaccines were pioneered, developed, and put into clinical trials, a few with licensure delayed until the late 1980s and 1990s. But after 1985, there was rapid decrease in the pioneering and achievement of licensable new vaccines.

Modern era vaccines (Fig. 1) are divided into whole and subunit bacterial, viral recombinant subunit, and live and killed whole virus preparations using virus grown in cell culture. Most of the vaccines of this entire era were pioneered and first licensed in our laboratories [1,2], where resources and a uniquely appropriate organization with central authority favored successes.

3. Bacterial vaccines

Principal bacterial vaccines (Table 4) of the modern era focus on subunit capsular polysaccharide preparations, though much progress with attenuated whole bacterial vaccines has also been made.

Pneumococcal vaccines, containing but a few serotypes, were first licensed in 1946 [4] but were discontinued shortly thereafter because of the introduction of therapeutic sulfonamides and antibiotics. Though highly effective in eliminating bacterial infections, these drugs did not prevent death in some effectively treated patients. The field of pneumococcal vaccine research was kept alive by the persistent efforts of Dr. Robert Austrian [21]. We entered into pneumococcal vaccine research in the early 1970's and this resulted [22,23] in development of 14- and 23-valent vaccines that were licensed in 1977 and 1984.

Development of meningococcus vaccines was initiated by us at the request of the military, following the pioneering studies at Walter Reed [24] of prototype vaccines to circumvent the problem of sulfonamide resistance and resurgence of meningitis among recruits at army installations. Monovalent, bivalent and quadrivalent groups A, C, W135 and Y vaccines were devel-

Table 5

Modern era — viral vaccines — poliomyelitis 1950

National Foundation — supports establishment of basics. Antibody is marker for protection.

Killed vaccine. Macacus renal cell culture grown and formaldehyde inactivation with first order kinetics.

1955 *Licensure*

Problems

Incomplete poliovirus inactivation — caused paralysis. Process change and released.

Variable and less than acceptable potency.

Indigenous wild viruses in kidneys — assurance of inactivation.

1960: Hitherto undetectable monkey polyomavirus discovered, SV₄₀.

Resists total inactivation.

1962: SV₄₀ virus eliminated.

1960: Merck's Purivax licensed. Purified standardized potency vaccine. Discontinued for commercial reasons.

Live Vaccine. (nonneurotropic-attenuated).

1960 *Licensure*.

Problems

Retained or reversible neurovirulence.

SV₄₀ contamination — removed.

Live vaccine became paradigm for poliomyelitis prevention and worldwide eradication.

oped and evaluated by us [25] and were licensed between 1974 and 1982.

Polysaccharide vaccines, especially that of *Haemophilus influenzae b*, do not immunize young children. Discovery and presentation of an early paper in the late 1960's or early 1970's by an unrecalled hero, that conjugation of polysaccharide with protein elicits T cell help and immunizes infant animals, opened the door to development of the highly effective conjugate vaccines for young children by our own [26,27] and many biologics companies [see [1,2,4], which continue to the present.

Several highly effective conjugated *H. influenzae* vaccines have been licensed and are currently available. This same technology for conjugation is being applied at present to improve the immunizing capabilities of meningococcal and pneumococcal vaccines.

Recombinant subunit polypeptide Lyme disease vaccine is new and licensure was granted to SmithKline Beecham laboratories in 1998 [28].

4. Viral vaccines

Vaccines against poliomyelitis (Table 5) were created by programs that were funded and conducted under the auspices of the National Foundation for Infantile Paralysis. This foundation was an outgrowth and successor to the annual President Franklin Delano Roosevelt Birthday Ball for support of the Warm Springs Poliomyelitis Foundation — A Rehabilitation Center.

4.1. Inactivated poliovaccines

The enabling breakthrough for the vaccine came with Enders' poliovirus propagation [18] in cell cultures of nonneural tissue. Trivalent killed Salk polio-vaccine was prepared using virus grown in *Macacus* monkey renal cell cultures and was licensed in 1955. This vaccine was faced with three immediate problems that related to incompleteness of poliovirus inactivation, to highly variable immunizing potency, and to the discovery by us of a new indigenous contaminating *Macacus* monkey polyoma virus, SV₄₀ [29,30]. SV₄₀, prior to that time, was undetectable. We also found that the SV₄₀ virus was resistant (one in about 10,000 particles) to total inactivation by formaldehyde in the poliovirus vaccine.

Discovery of SV₄₀ virus [29,30] derived from our efforts to use kidneys for cell culture from *Macacus* monkeys that were not infected with the then ubiquitous presence of indigenous viruses. We identified and introduced the African *Cercopithecus* monkey to circumvent this problem. Renal cells from this species were found to be highly permissive to viral replication with cytopathogenic change, and allowed us to detect the presence of hitherto undetectable agents. In particular, use of these cells permitted our detection of SV₄₀, virus that we later found to be oncogenic [31] for baby hamsters.

There was major disruption in killed poliovaccine manufacture when a small amount of live SV₄₀ virus was found in the finished product [29,30]. The problem was rapidly solved, however, by substitution of *Cercopithecus* monkey kidney cells that were free of indigenous viruses. Efforts to overcome the highly variable potency of the killed vaccine led us to develop a purified poliomyelitis vaccine with precisely standardized potency (Purivax) [32]. This product was licensed in 1960 but was ultimately discontinued for commercial reasons.

Live oral-fed Sabin poliovaccine [33] was based on use of nonneurotropic poliovirus strains and was licensed in 1960. It also suffered the problem of SV₄₀ virus contamination [29–31], but, as for killed vaccine, the problem was easily solved by use of *Cercopithecus* monkey kidney cultures. The live vaccine retains, to this day, very low level neurovirulence [33] for man but rarely causes poliomyelitis in vaccinees or in contacts of vaccinees. In spite of this, live poliovaccine is the paradigm for poliomyelitis prevention and for worldwide poliovirus eradication.

4.2. Live vaccines for preventing pediatric diseases

The live attenuated pediatric vaccines, measles, mumps, rubella, varicella and their combinations were conceived by us [1,2] as future possibilities in 1957,

Table 6

Modern era — pediatric live virus vaccine — hurdles

Target: live attenuated vaccines: measles, mumps, rubella, varicella, combinations.

Common hurdles

Discover — grow virus in acceptable cell culture.
 No animal models, no markers of attenuation.
 All tests in children — decisions by judgment.
 Preparation of numerous passage level vaccines of commercial quality.
 Testing by judgment starting with likely most attenuated and working backward to acceptable balance in reactivity and immunogenicity.
 Retention of acceptance of research by scientific and regulatory committees (overcoming objections, e.g., varicella).
 Elimination — avoidance of viral contaminants.
 Huge and long-term vaccine preparation and testing.
 Protective efficacy in controlled studies.
 Safety validation 10–20,000 susceptibles.
 Safety for susceptible contacts.
 Retained protection — long term.
 Vaccine stable on storage and distribution.
 Combined vaccines — no increase in reactogenicity, and formulation adjustment to prevent interference.

even though they were only theoretical dreams at the time. The importance of the concept was, eventually, to provide a simple solution to a large segment of the pediatric viral disease problems.

The research and development (Table 6) of the individual pediatric live virus vaccines were faced with numerous hurdles, usually common to all of them. One hurdle was to develop large numbers of different passage level vaccines, of commercial quality. These were tested clinically to find a level with acceptable toxicity and adequate potency, starting at highest passage level, lowest virulence, and working backwards. With no animal models and no markers of attenuation, all tests needed to be done in child volunteers. Detailed clinical observations and the exercise of judgment were the principal guidelines. Efficacy of each candidate vaccine was proved in two placebo-controlled protective efficacy trials. Safety validation of each vaccine was usually made in tests in 10 to 20,000 susceptible children and their susceptible contacts. Suitable vaccines needed to provide long-term protection. The combined vaccines required dose adjustments to retain the same favorable immune response as for the individual vaccines when given alone. Finally, all vaccines needed to be stable on storage and on distribution. Each of the live virus vaccines had individual problems and solutions (Table 7) that are discussed below.

4.3. Measles vaccine

Seminal to preparation of the measles vaccine [34,35] was the need to eliminate the ubiquitous avian leukemia virus contamination of the hens' eggs used to

Table 7

Modern era — pediatric live virus vaccines — notable problems and solutions

Measles — chick embryo cell culture.

Reduce reactions.

Coadministration of immune globulin.

Further attenuation (no globulin).

Eliminate avian leukemia virus from cultures — development of experimental leukemia-free flocks.

High-level potency and safety.

Mumps — chick embryo cell culture.

Jeryl Lynn strain lacks neurovirulence.

High-level potency — nonreactogenicity.

Rubella — discovery — propagation in duck cells.

Rapid and reliable attenuation.

Nontransmission to susceptible adult contacts.

Bivalent and trivalent formulations

Acceptable potency and reactogenicity.

Very successful clinical acceptance.

Flagship pediatric immunogens.

Varicella — all aspects — worked out with KMcC strain by 1981.

Virus refused to achieve acceptable balance of reactogenicity vs. immunogenicity on application of attenuation procedures.

OKA strain substituted.

provide the tissue needed for cell culture. This problem was solved through the development of leukemia-free chicken flocks [36]. Further, the original Enders' Edmonston B measles virus had excessive virulence for children that we were able initially to reduce by coadministration of measles immune globulin. The problem was better solved by our development of the further attenuated Moraten line [37] of measles virus that required no immune globulin. High-level potency and safety needed to be proved for the modified Moraten virus substrain as had been required for the original virus.

4.4. Mumps vaccine

The Jeryl Lynn mumps virus isolate that was recovered and attenuated in our laboratories provided a very suitable nonneurovirulent and highly immunogenic vaccine [38,39].

4.5. Rubella vaccine

Rubella vaccine development [40–44] was aided by our breakthrough discovery of propagation and of rapid and reliable attenuation of the virus in duck embryo cells in culture [40]. An important attribute was lack of communicability of the vaccine virus to susceptibles who were in contact with vaccinated persons.

4.6. Combined MMR vaccine

Combined bivalent and trivalent formulations of

Table 8
Modern era — Marek's chicken cancer vaccine

Marek's disease, a neural lymphoma or visceral lymphoma of chickens (range paralysis) is caused by a herpesvirus. Economic loss to poultry industry is large, through lowered productivity (eggs and meat) and condemnations at slaughter.

1970	Burmester discovers turkey herpesvirus antigenically related to chicken Marek's. Burmester virus causes no disease in chickens but protects against Marek's. After long and complicated research to establish safety and efficacy for chickens and safety for man in food consumption, two highly effective vaccines were developed in our laboratories.
1971	Frozen infected cell vaccine.
1975	Purified free virus vaccine, dried. This was the world's first licensed vaccine against any cancer. It revolutionized the economics of the poultry industry!

measles, mumps and rubella vaccines [45–48] were developed that were safe and effective in all respects. The trivalent vaccine, MMR, became the flagship for pediatric immunization and continues to the present with very significant cost savings [48].

4.7. *Varicella vaccine*

Our KMcC *varicella* vaccine [49], that was studied for more than 15 years, was used to pioneer and to develop all aspects of chickenpox vaccine preparation and protection, but for one aspect. It proved impossible to achieve acceptable potency for KMcC at an attenuation level, which also had acceptable reactogenicity. The Japanese Oka strain [50] was successfully substituted and brought to licensure. It is being readied for addition to the trivalent MMR vaccine.

5. Live vaccines against Marek's chicken cancer

Marek's disease (Table 8) is a neural and visceral lymphoma of chickens that causes huge economic losses to the poultry industry through lowered productivity and condemnations at slaughter.

Burmester and colleagues' turkey herpesvirus [51] was shown to protect against the antigenically related Marek's herpesvirus, without causing disease in chickens. We developed and licensed [52] infected frozen cell Marek's vaccine in 1971, and purified dried virus vaccine in 1975. These licenses were granted by the United States Department of Agriculture after long and complicated studies to prove protective efficacy and safety for chickens, and acceptability for human food consumption as well. This was the world's first licensed vaccine against any cancer and it revolutionized the economics of the poultry industry.

6. Discoveries and development of vaccines against hepatitis

Large-scale laboratory and field studies were initiated by our laboratories in the early 1960s with intent to discover viruses causing hepatitis A and B.

6.1. *Hepatitis A virus and vaccine (Table 9)*

In 1973, we published our earlier isolation of the CR326 strain of hepatitis A virus in marmosets [53,54]. The GB virus that had been isolated previously by Freidrich Deinhardt in marmosets has been found recently [55] to be a *Flavivirus* and not the virus of hepatitis A. Deinhardt's GB virus discovery is of special significance since it predates that of the discovery of hepatitis C *Flavivirus*. Studies to characterize, completely, hepatitis A virus and hepatitis A disease were carried out [56,57] in our laboratories making it possible to write [58] a new chapter on infectious hepatitis. A highly protective formaldehyde-killed virus vaccine was developed and reported by us in 1978 [59] in which we used virus that was purified from infected marmoset liver. In 1979, our laboratory [60] made the breakthrough discovery of cell culture propagation of hepatitis A virus that opened the door to preparation of a vaccine for use in man. Such killed virus vaccine [61], based on the 1978 marmoset liver prototype procedure [59], proved highly safe and protective in controlled field studies [62]. The vaccine was licensed in 1994 and is now used routinely in many parts of the world. We also pursued long-term development of live virus vaccines [63].

6.2. *Hepatitis B virus vaccines (Table 10)*

6.2.1. *Plasma-derived hepatitis B vaccine*

The discovery in 1965 by Blumberg and by Prince of the surface antigen of hepatitis B virus present in the

Table 9

Modern era — Hepatitis A virus vaccine

Early 1960s	Program initiated to discover viruses of hepatitis.
1973	Recovery of CR326 hepatitis A virus in marmoset monkeys. (Deinhardt's GB virus, originally believed to cause hepatitis A, was recently shown to be the earliest example of flavivirus, hepatitis, nonA–nonB (predating hepatitis C).
1978	Highly effective formalin-killed infected liver cell derived hepatitis A prototype vaccine shown safe and effective in marmoset and chimpanzee studies.
1979	Breakthrough cultivation in cells in culture opens door to vaccine for man.
1991	Cell culture-grown formalin-killed vaccine prepared using marmoset liver vaccine procedures.
1992	Safety and efficacy.
1994	Licensed.

blood of human carriers of the infection, opened the door to a hepatitis B vaccine. Starting with a near zero data base, probes were carried out in our laboratory beginning in 1968 to explore purification, yield, inactivation, safety and efficacy of a possible candidate hepatitis B vaccine using surface antigen purified from human carrier plasma [64–66]. The processes that were evolved were successful [67–69]. Hepatitis B virus does not propagate in vitro and tests for inactivation of surrogate viral agents in each of the multiple inactivation steps were used to assure safety from live hepatitis B virus and likely all possible microbial life forms that might be present in human blood. High level protective efficacy of the vaccine was proved, first in chimpanzee challenge studies and then in controlled clinical trials [70] in man in 1980. The vaccine was proved safe and highly protective, and was licensed for general use in 1981. This was thirteen years after our intensive vaccine investigations were first initiated.

6.3. Recombinant-expressed vaccine

Supplies of acceptable human carrier plasma were inadequate to meet market needs and a cooperative study was established in 1975 with Drs. Rutter and Hall of the Universities of California and Washington to develop a recombinant expression system for producing hepatitis B antigen. Recombinant expression was achieved in yeast [71] and cultivation and expression were optimized in our laboratories. The purified recombinant antigen was substituted for the antigen in the plasma-derived vaccine and was shown to yield a product, which performed the same as the plasma-derived vaccine [72,73]. The recombinant hepatitis B vaccine was licensed in 1986, just 11 years after the first recombination studies were initiated. The two hepatitis B vaccines represent the world's first viral subunit vaccine, the first licensed vaccine to prevent human cancer, and the first recombinant-expressed vaccine. This vaccine, with the urging of the World Health

Table 10

Modern era — Hepatitis B virus vaccines

Early 1960s	Search for hepatitis viruses.
<i>Plasma-derived vaccine</i>	
1965	Blumberg and Prince discover surface antigen of hepatitis B virus in blood of carriers.
1968	Probes initiated for purification, inactivation, safety and efficacy.
1980	Efficacy proved.
1981	Vaccine licensed (after 13 years of research).
<i>Recombinant yeast vaccine</i>	
1975	Initiated collaborative studies with Rutter and Hall to develop vector-expressed hepatitis B surface antigen.
1982	Expression system in recombinant yeast. Antigen extracted, purified and substituted for plasma-derived antigen.
1986	Recombinant vaccine licensed.
<i>Hepatitis vaccines represent</i>	
World's first subunit vaccine.	
World's first licensed vaccine against human cancer.	
World's first recombinant expressed vaccine.	

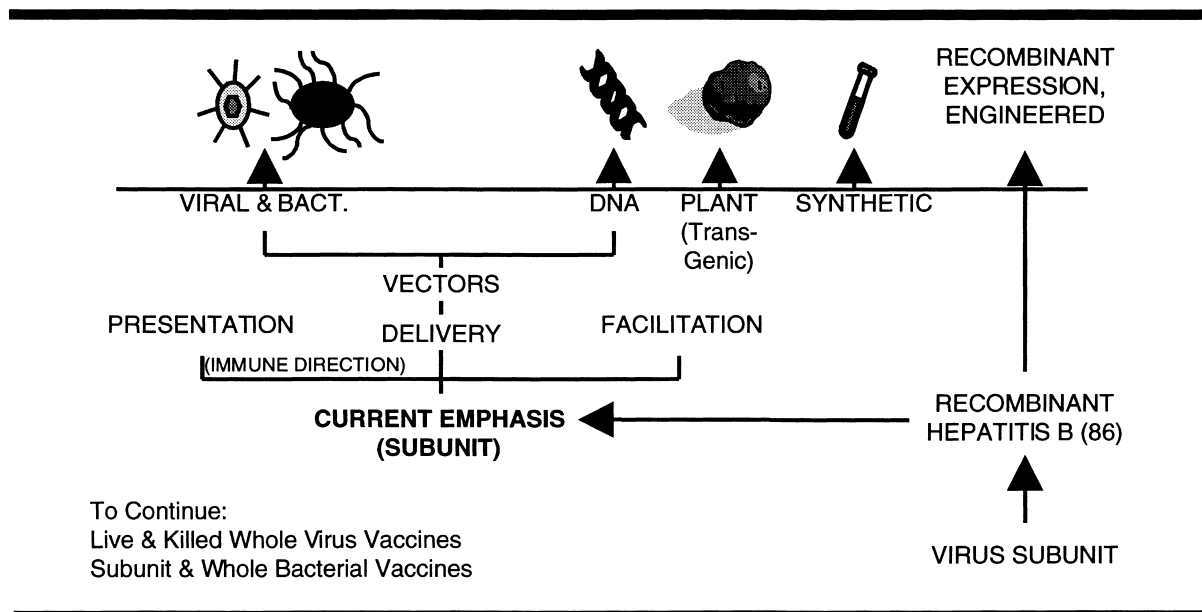


Fig. 3. Present and future vaccinology — building on past and recent discoveries.

Organization, is now being programmed for routine use to immunize all babies in more than 100 countries.

7. Contemporary era and future vaccines

Contemporary vaccinology (Fig. 3), at least for viral vaccines, is very complex and is dedicated largely to the subunit vaccine approach. Contemporary subunit vaccines are built on the same foundation and may be considered to be an extension of recombinant subunit hepatitis B technology, that was extensively pursued from the beginning in the attempt to develop a vaccine against AIDS. Save for the Lyme vaccine [28], no recombinant vaccine, other than that for hepatitis B, has been licensed to the present time. Whole live and killed virus and bacterial vaccines may also continue to be explored. Live Rotavirus vaccine, recently licensed to the Wyeth Lederle Laboratories, is such an example [74].

The contemporary era [75,76] is eagerly begging for new vaccines to control more than 20 diseases, especially tuberculosis, malaria, hepatitis C and AIDS. Pioneering new vaccine development, in the period since 1985, has been remarkably sterile and filled with “gonna’s and promises” but few successes.

The real question of the present is what will drive the future? Belated recognition of the importance of cell-mediated as well as humoral effector mechanisms in the immune response has initiated a whole new era of vaccine research that promises rewards greatly in excess of anything we have seen in the past. Seminal

to this new era are the remarkable advances made in defining and understanding immune function. The necessary achievement of both humoral and cell-mediated immunities has been clearly established, as revealed in numerous publications.

New vaccines rely on identification of appropriate antigens and epitopes. Importantly, they must rely on the “*what*” to present to the immune system as well as the “*how*” to present. To the current period, the cart has been mostly in front of the horse with exquisite advances in antigen presentation technology and a dearth of knowledge of *what* to present. The *what* to present will be a major problem for vaccines such as that against AIDS until more simple and more efficient methodologies for antigen and epitope discovery and identification have been achieved.

The *how* to present, as illustrated in Fig. 3, is already filled with new and exciting possibilities [75,76]. Central to it all will be molecular genetics with continued evolvement of eukaryotic cell expression following the breakthrough technology used for recombinant hepatitis B vaccine [72,73]. Added to this is the immense promise that live recombinant microbial and DNA vectors may give. Endogenous expression and presentation by antigens in transfected dendritic cells [77] give immense opportunity for development of vaccines to prevent infections as well as to treat persistent infections and cancer. Transgenic plant tissues [78] might provide a possible answer to the need for cheap and easily administered vaccines for the huge populations of the developing world. Improved synthetic chemistry, creating appropriate antigens and epitopes

in linear tandem composition [79], or in multiple arrays on scaffolds, may also find a significant role in the future.

The platforms of knowledge developed during the 20th century are ripe for exploitation and for anticipated successes early in the 21st century. It is not unreasonable to be optimistic and to expect that what must be, will be!

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