The Challenge: Biotechnology Transfer to Public Health. Examples from Arbovirology

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Introduction

Infectious diseases remain the preeminent health problems in the developing world, and immunization the most effective approach to controlling them. Recombinant DNA technology has provided a plethora of new approaches for the development of vaccines. The priorities for development of new vaccines and delivery methods have been redefined by the Children's Vaccine Initiative. Among the diseases targeted for vaccine improvement or development are three arbovirus infections, dengue, Japanese encephalitis, and yellow fever. The burden of disease caused by these mosquito-borne viruses, the current status of classical vaccines, and progress toward development of genetically-engineered vaccines are reviewed. Since 1984, the World Health Organization has promulgated research leading toward the development of molecular approaches to vaccines against these flaviviruses. Considerable headway has been made in the understanding of genome variation, the identification of protective epitopes, and in cloning and expression of relevant proteins in vaccinia and baculovirus systems. In addition, the development of full-length complementary DNA clones which yield infectious RNA transcripts is being pursued as a means of producing stable, attenuated and chimeric flavivirus vaccines. However, progress in molecular biology reveals a widening gap between our ability to devise new products and our ability to manufacture them at low cost and utilize them for the prevention of disease in the developing world. The high costs of research and development may preclude the use of new vaccines in the Third World; economic and political solutions needed to overcome this obstacle must keep pace with technical innovation. Moreover, biotechnology provides a countervailing force to the funding of research and the pursuit of careers in field biology, epidemiology, hygiene,

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and other traditional disciplines which are required to define the changing determinants of disease ecology, delineate targets for prevention, formulate public health policy, and evaluate the results of prevention programs. Yellow fever provides an example of a disease which is poorly controlled despite the availability of an effective vaccine.

The advent of molecular biology has revolutionized traditional approaches to the diagnosis, treatment, and prevention of diseases affecting plants and animals. Tracing the major accomplishments of a century of progress in the struggle against infectious diseases illustrates how biotechnology has provided fundamentally novel strategies for their control. The explosion of knowledge in molecular biology is now being applied to these problems by many groups engaged in research, including the pharmaceutical industry. This revolution has been accompanied by a predicament, however. A widening gap is revealed between our ability to devise new products and our ability to utilize them for the prevention and control of disease, especially in the developing world. Narrowing the gap between discovery and production of new technologies and their practical deployment will be one of the cardinal challenges of public health in the 21st Century.

Biotechnology had its origins during most of our lifetimes. I was a toddler of 3 years when Avery, MacLeod, and McCarty at the Rockefeller Institute identified DNA as the genetic material of prokaryotic cells. I was 12 years old, and just uncovering the mysteries of my first chemistry set, when Watson, Crick, Franklin, and Wilkins described the three-dimensional structure of DNA. I had reached medical school when Arber, Meselson and others developed site-specific restriction endonucleases and Nirenberg and his colleagues elucidated the genetic code for the synthesis of proteins. I was a young medical officer at the Centers for Disease Control (CDC), when Temin and Baltimore described reverse transcriptase and Boyer developed DNA cloning techniques. My hair was just beginning to gray in the mid-1970's, when Milstein and Kohler fused antibody-producing lymphocytes with myeloma cells to produce monoclonal antibodies and Sanger and Gilbert developed DNA sequencing techniques. The pace of technology then really exploded as I finally admitted I might be reaching middle age. In 1980, the U.S. Supreme Court ruled that microorganisms are patentable, opening the floodgates of research using recombinant DNA in the biotechnology industry. Within two years, the first diagnostic kit using monoclonal antibodies and the first genetically engineered pharmaceutical product (human insulin) were licensed by the Food and Drug Administration (FDA). In 1982, the first recombinant human vaccine – hepatitis B surface antigen expressed in eukaryotic cells – was described and, shortly thereafter, successfully tested in clinical trials. Also in 1982, Paoletti initiated the exploitation of vaccinia virus as a live vector of foreign genes, now being applied to a wide array of viruses, bacteria, and protozoa. By 1985, this technology was considered one of the most promising expression systems for recombinant vaccines. Vaccinia recombinants are now in field use against fox rabies, vaccinia-vectored vaccines against hepatitis B

and human immunodeficiency virus (HIV) have been tested in humans, and trials of a rinderpest recombinant vaccine are planned in Africa. In 1985, Saiki and colleagues described an enzymatic process for amplifying specific DNA sequences in vitro – the polymerase chain reaction – a technique which has revolutionized approaches to gene mapping, sequencing, and the development of sensitive diagnostic assays. In 1988, the first patent was issued for a genetically engineered (transgenic) animal. Work is now underway to exploit this technology to the control of infectious diseases, including the generation of disease-resistant animals and incompetent vectors. Within the next few years, technological approaches which are anticipated to produce major breakthroughs include the development of many new vaccines; wide application of therapeutic human monoclonal antibodies and immunological tissue targeting of drugs; new adjuvants, immunostimulators and delivery systems for drugs and vaccines; computerized modeling for drug and vaccine design; and molecularly targeted antiviral drugs, to mention only a few. The challenge for public health will be how to harness these sophisticated approaches to confront disease, especially in the developing world where infectious diseases will be preeminent for the foreseeable future. The challenge requires that economic and political solutions follow the scientific breakthroughs.

One index of progress in the application of biotechnology to medicine is the annual survey of products in development conducted by the Pharmaceutical Manufacturers Association (PMA). Since 1986, when these surveys were initiated, the number of biotechnology and genetic engineering patents issued has increased nearly 60%. More than half of the products are medicines or vaccines. As of March, 1990, there were 11 biotechnology products licensed for general use, 32 in final stages of FDA approval, and 72 undergoing clinical trials (Table 1). Among these are 3 licensed and 14 experimental vaccines, all but one against infectious diseases, including hepatitis B, *Hemophilus influenzae* type b, HIV, herpes viruses, and malaria (Table 2). However, this relatively short list does not accurately reflect the rapidly expanding worldwide scope of efforts within university and government laboratories on vaccine development, much of it directed at new approaches through biotechnology. In fact, the proliferation of new live vectors and candidate vaccines often makes it difficult to select the most advantageous approach for clinical development.

The most important health problems of the developing world continue to be the infectious diseases, and immunization is the most effective and cost-efficient means of protecting populations against them. However, despite an array of new vaccines in various stages of development, only a handful are in practical use in the developing world, and these old vaccines are not the children of biotechnology. Only six vaccines are currently in routine use for childhood immunization (Table 3), and vaccine coverage is still less than hoped for (1). It is estimated that without an accelerated rate of coverage, 30 million children will die of vaccine-preventable diseases in the 1990s (2). The toll from diseases against which vaccines are not yet deployed is even more staggering: 20 million deaths from malaria in this de-

Table 1. Biotechnology Products Approved or in Clinical Development as of March, 1990 (Source: Pharmaceutical Manufacturers Association)

Product type	Indication	No. products: clinical trials	No. products: licensed
Colony Stimulating Factors	Cancer, AIDS	7	
Superoxide dismutases	Reperfusion injury	2	
Erythropoietin	Anemias	2	1
Factor VIII	Hemophilia	2	
Growth Factors	Wound healing	7	
Growth Hormone	Growth failure	4	2
Interferons	Cancer, infectious diseases	8	2
Interleukins	Cancer, AIDS	10	
Monoclonal antibodies	Cancer, transplantation, infectious diseases	37	1
CD4's	AIDS	4	
Tumor Necrosis Factor	Cancer	3	
Vaccines	Infectious diseases, cancer	14	3
Other		4	2
Total		104	11

cade, 40 million from diarrheal diseases, and 40 million from acute respiratory disease. Nevertheless, there is optimism that the global vaccination programs can be strengthened and that new vaccines against these infectious diseases can be brought into use.

Since 1984, the development of new vaccines and improvement of existing technologies have been actively promoted and funded by two programs administered by WHO and UNDP. In the *Program for Vaccine Development*, five targets are being addressed by individual Steering Committees under the overall guidance of a Scientific Advisory Group of Experts (SAGE). The targets include two arbovirus diseases – dengue and Japanese encephalitis; as well as hepatitis A and poliomyelitis; tuberculosis; acute viral respiratory infections; and encapsulated bacteria (3). The program attempts to coordinate research efforts world-wide, promote information-sharing, focus research on the most promising technologies, and provide limited direct funding of new research, with the hope that the results will attract other resources. The underlying principle guiding the efforts of SAGE and the individual Steering Committees is the commitment to more fully characterize disease agents at the molecular level, delineate the antigens responsible for induction of immunity, elucidate the basis for protective host im-

Table 2. Biotechnology Vaccines, Approved and in Clinical Trials, as of March, 1990[1]

Indication	In clinical trial	Approved
Hepatitis B	4	2
AIDS	3	
Herpes 2	2	
Malaria	4	
Hemophilus B		1
Melanoma	1	

[1] Source: Pharmaceutical Manufacturers Association

Table 3. Vaccines Included in the WHO Expanded Program of Immunization, 1991

1. BCG
2. Diphtheria
3. Pertussis
4. Tetanus
5. Poliomyelitis
6. Measles

munity, and develop and evaluate vaccine candidates. New approaches for the use of infectious clone technology and live vectors of foreign genes seem particularly promising as efficient and cost-effective means of inducing long-lasting immunity (4).

The second WHO *Program on Transdisease Vaccinology* is addressing the important issues of adjuvants and novel approaches to antigen presentation and delivery, including the use of live vectors, microencapsulated antigens, and oral administration of vaccines classically delivered by syringe and needle. One of the most important obstacles to the use of vaccines in developing countries is the requirement for multiple inoculations and repeated interactions between the population and the health care delivery system. Controlled-release technologies are being explored which show promise of multivalent immunization with a single inoculation.

In 1986, the Institute of Medicine sponsored a study on *New Vaccine Development, Establishing Priorities* (5) and identified 29 vaccine candidates for development against 19 diseases not currently incorporated in the WHO Expanded Programme of Immunization (Table 4). The new vaccines included 1) improved existing products, generally derived by changing from tissue-based manufacture to cell cultures; 2) new vaccines produced

Table 4. Diseases of Importance in Developing Countries Targeted for Vaccine Development, Institute of Medicine, 1986

Disease/Pathogen	Improve existing	New, classical	Biotechnology
Yellow fever	+		
Dengue			+
Japanese encephalitis	+		
E. coli (ETEC)		+	+
Hemophilus B			+
Hepatitis A		+	+
Hepatitis B			+
Leprosy		+	
Neisseria meningitidis			+
Parainfluenza			+
Malaria			+
Rabies		+	+
Respiratory syncytial virus		+	+
Rotavirus		+	
Salmonella typhi			+
Shigella			+
Streptococcus A			+
Vibrio cholerae		+	+

by classical approaches; and 3) new vaccines produced by biotechnology. Vaccines for 14 of the 19 target diseases affecting developing countries would be the result of genetic engineering or other biotechnological approaches. Three of the target diseases, for which significant health benefits would accrue by implementing vaccination programs, were arthropod-borne viruses – yellow fever, dengue, and Japanese encephalitis.

In 1990, WHO, UNICEF, UNDP, and the Rockefeller Foundation announced its new global *Children's Vaccine Initiative* (CVI), which has as its goal the development and implementation of new, multivalent vaccines for the protection of the world's children against the major killer diseases. Many of the vaccines required are yet to be developed or licensed (e.g., against toxin-producing *E. coli*, hepatitis A, respiratory syncytial virus, groups A and B streptococci, rotavirus, dengue, malaria, typhoid, parainfluenza, and pneumococcal meningitis). In addition to the vaccines themselves, new strategies for delivery are envisioned, including microencapsulated antigens for mucosal and parenteral presentation. The tech-

nological objectives thus include: 1) immunization with the minimal number of injections; 2) immunization at the earliest point in life, possibly at birth; 3) high thermostability; 4) provision of lifelong immunity; and 5) cost within reach of programs for universal immunization. In the United States, the CVI is coordinated by the National Vaccine Program of the Department of Health and Human Services and implemented in part by DHHS agencies, the Centers for Disease Control, National Institutes of Health, and Food and Drug Administration. Priorities for new funding for the National Vaccine Program have been defined by these agencies, and the target diseases are similar, in general, to those already identified by WHO and the IOM report.

To illustrate and extend some of the aspects of these initiatives, I have selected several examples from arbovirology which serve as specific paradigms to explore the general theme of vaccines from biotechnology and the implementation of vaccination strategies in the developing world. The examples selected – dengue, Japanese encephalitis, and yellow fever, – represent diseases which have also been targeted by WHO, the IOM, or the CVI for new or improved vaccine development. All three diseases are caused by members of the family Flaviviridae. Significant progress has been made in recent years on the molecular biology of this group of viruses and on approaches toward production of recombinant vaccines.

Dengue and Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS)

Dengue is widely credited as the most important vector-borne viral infection of humans, because of its worldwide distribution, high morbidity and cost associated with epidemics, the great difficulty in successful application of mosquito control as an alternate strategy to vaccination, and the occurrence of severe and potentially lethal disease – DHF/DSS – in a subset of those infected. The current global situation is characterized by an expanding geographic distribution of the disease, by an increasing incidence of DHF/DSS in Southeast Asia, and by the emergence of DHF/DSS in previously unaffected regions, principally the Americas and Pacific island nations. This changing pattern is attributable to expanding urbanization and the attendant increased distribution and population density of the vector, *Aedes aegypti*; to the reinfestation of South and Central America by *Ae. aegypti*; to the senescence of effective vector control programs; and to increasing air travel with introduction of dengue virus strains and serotypes to new and receptive areas. Figure 1 shows the present distribution of dengue and areas receptive to dengue by virtue of being infested with *Ae. aegypti*. Over 2 billion people reside in these areas, and millions are affected by dengue fever annually (6). Morbidity estimates are available only for the severe form, DHF/DSS. These data indicate an increasing incidence, with over 250,000 cases reported annually in recent years, principally in Southeast Asia (Table 5). However, within the last decade, epidemic DHF/DSS

Table 5. Global Incidence of Dengue Hemorrhagic Fever[1]

Time period	No. years	Cases	Mean cases/year
1956–1980	25	715,283	29,803
1981–1985	5	687,522	137,504
1986–1990[2]	5	1,338,461	267,692
Totals		2,741,266	78,322

[1] Gubler DJ (1991) Virus Info Exch Newsl 8: 2–3
[2] 1989–90 data provisional

appeared in a number of countries for the first time, notably Sri Lanka, and several countries in the New World – Cuba, Venezuela, and Brazil. The epidemiological pattern of DHF/DSS in the Americas in the 1980s resembles the emergence of this disease in Southeast Asia in the early 1960s and predicts an increasing incidence of severe disease in this hemisphere in the future.

The approach to dengue immunization is greatly complicated by the occurrence of 4 serotypes, which do not provide cross-protective immunity. Instead, heterologous immunity appears to underlie the pathogenesis of DHF/DSS, presumably by non-neutralizing antibody-mediated enhancement of dengue virus replication in Fc-receptor-bearing monocyte-macrophages. In addition, cross-reactive cytotoxic T cells target infected cells during secondary infection, with release of precursors of the coagulation cascade and vasoactive mediators of shock. For these reasons, primary immunization with a dengue vaccine must meet certain unique criteria: 1) simultaneous induction of immunity to all 4 dengue serotypes; and 2) induction of long-lasting immunity which does not wane from levels which are protective to levels which enhance virus replication.

The preparation of inactivated, whole virion dengue vaccines is generally accepted as impractical because of low virus yields in cell culture. Attempts to produce live, attenuated vaccines in mouse brain were begun in the 1940s by Sabin and Schlesinger, but the modern era began in the 1970s with efforts by the Walter Reed Army Institute of Research to produce vaccines in cell culture substrates acceptable by current regulatory standards. The first of these vaccines to be tested in humans was a temperature-sensitive, small plaque variant of dengue virus type 2 (7). During the 1980s, candidate vaccines with similar characteristics against all 4 serotypes were tested in humans. The results, summarized in Table 6, were disappointing. Either preimmunization with yellow fever 17D was required (dengue-2, Pr159/S1), or the vaccine demonstrated overattenuation (dengue-4, H241 and Carib 341750), or it caused unmodified illness (dengue-1, 45AZ5 and dengue 3, CH53489). In vitro and in vivo laboratory markers of attenuation were found to be unreliable predictors of clinical response. Therefore,

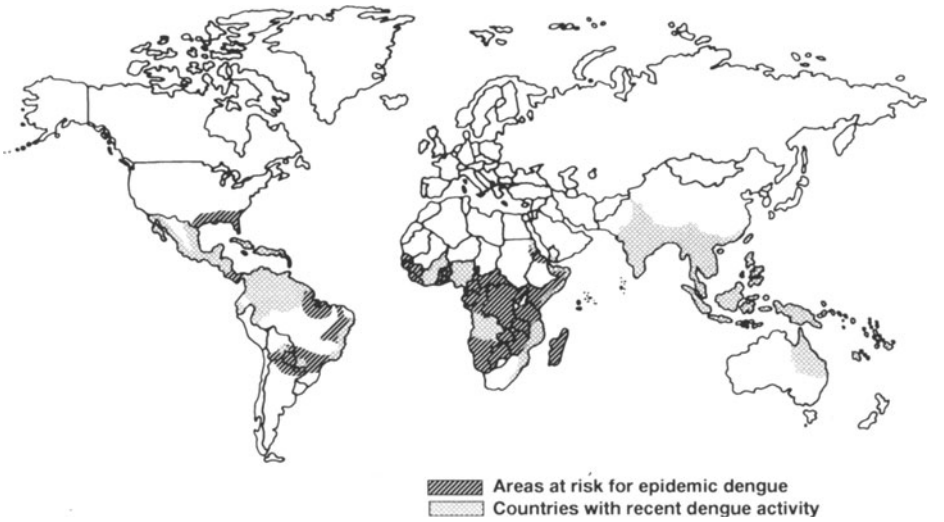

Fig. 1. World distribution of dengue and of areas at risk of epidemics by virtue of infestation by the principal vector, *Aedes aegypti.* Modified from Gubler (6), with permission

present efforts at finding candidate vaccines are directed at testing decreasing cell culture passage levels of selected virus strains in small groups of human volunteers.

A parallel effort at development of live vaccines has been undertaken in Thailand by Bhamarapravati and colleagues, with support provided by WHO. Candidate vaccines against all 4 dengue have been tested in flavivirus-naïve and JE-immune humans alone and in various simultaneous combinations, including a trivalent (dengue 1, 2, 4) vaccine (8, 9). The results have been encouraging and indicate 90–100% immunogenicity, apparently without significant side-effects. A number of problems remain to be overcome, however. The dengue type 1 candidate requires a high dose for adequate immunization, creating a problem for cost-effective production. It is still uncertain what the effect of preexisting dengue immunity, either acquired by active infection or by passive maternal transfer, will have on immunogenicity and reactogenicity of the live vaccine.

Since 1984, the WHO Steering Committee on Dengue and Japanese Encephalitis has sought alternative approaches to immunization through biotechnology. The response of the scientific community has been magnificent, and progress in flavivirus molecular biology is so extensive as to defy concise summarization. Only relevant highlights can be presented here [see Brandt (10) for a partial review]. Efforts have been directed at 1) cloning and sequencing representative strains of each dengue serotype and definition of genetic variation among geographic dengue virus strains; 2) defining the protective epitopes of dengue viruses, including both B-cell and helper T-cell activities; 3) expression of relevant subunit proteins in

Table 6. Status of Clinical Trials of Live, Attenuated Dengue Vaccines, Walter Reed Army Institute of Research, 1991[1]

Serotype	Strain	No. vaccinated by YF immune status	Seroconversion rate (%)	Clinical response	Conclusion
DEN-1	45AZ5	2 (I)[2]	100	Dengue fever	Not attenuated
DEN-2	PR159/S1	70 (I)	90	mild symptoms	Requires YF immunity
		28 (N)	62	mild symptoms	
DEN-3	CH53489	2 (I)	100	Dengue fever	Not attenuated
DEN-4	H241	5 (I)	40	mild symptoms	Over-attenuated
	Carib 341750	8 (N)	65	minimal symptoms	Further tests at lower passage

[1] Eckels K (1990) pers. comm.
[2] *I* previously immunized with yellow fever 17D; *N* not immunized

various expression vectors; 4) cloning genes for protective epitopes into recombinant live vectors; and 5) generation of dengue virus infectious clones.

Flaviviruses contain a single-strand positive-sense RNA genome about 11 kilobases long (11, 12). There is a single long open reading frame of about 10 kilobases which encodes 3 structural and 7 nonstructural proteins (Fig. 2). The structural proteins, encoded at the 5'-terminus, include a capsid protein (C) associated with the RNA genome; a glycosylated prM protein which is proteolytically cleaved to form a small membrane (M) subunit of the virion envelope; and a major glycosylated envelope (E) protein. The E glycoprotein comprises the surface projections of the whole virion and is involved in major biological functions such as attachment to cells and induction of humoral and cellular immunity. The nonstructural proteins have important enzymatic functions in post-translational proteolytic cleavage and RNA transcription.

Studies employing monoclonal antibodies, active immunization with purified or expressed proteins, and synthetic peptides have demonstrated protective epitopes on at least 3 glycoproteins, including two structural proteins [the intracellular precursor to the membrane protein (prM) and the envelope (E) glycoprotein] and a large glycosylated nonstructural protein (NS1). However, the individual amino acid sequences constituting protective epitopes have not yet been completely defined. Protection of animals by antibodies to the E glycoprotein is clearly correlated with virus neutralization, but antibodies to prM and NS1 protect without directly neutralizing virus. The protection afforded by antibodies to NS1 appears to be mediated by complement-dependent lysis of infected cells. Since NS1 is not a struc-

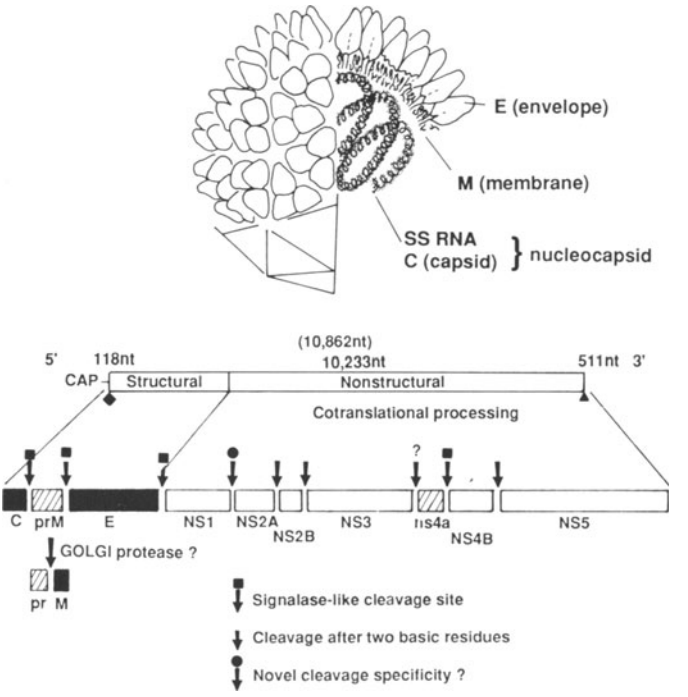

Fig. 2. Structure of the flavivirus virion, showing the organization of the genome, coding assignments of the structural and nonstructural polypeptides, and enzymatic cleavage sites for posttranslational processing. The data shown were first described by Rice et al. (11)

tural component of the virus, it is an attractive target for vaccine development. Antibodies to this protein would not bind to virions or engage the immune enhancement phenomenon. However, a body of evidence is accumulating that effective subunit vaccines derived from expression vectors should probably include at least 4 proteins, prM, E, NS1, and NS2a, both because this provides multiple antigens relevant to protection and because of the complex interdependency of individual proteins in their functional expression. The production of authentic NS1 appears to depend on the presence of the downstream (NS2a) sequences, and, similarly, functional assembly and conformation of the E protein appears to depend on prM and possibly on NS1(13, 14). Development of successful recombinant flavivirus vaccines thus depends on native presentation of viral proteins, and prokaryotic expression systems or synthetic peptides may have limited usefulness. Moreover, it appears from studies with synthetic peptides that both B and T-cell responses are strongly dependent on MHC haplotype. Thus, incorporation of multiple epitopes subserving protection in a recombinant vaccine may be important to avoid variation in immune responses of an outbred (human) population.

Considerable effort has been made to investigate vaccinia and baculovirus expression of dengue proteins as approaches to vaccine development (13–15). Recombinant vaccinia virus has received the most attention, because of the potential advantages of a live vector in the induction of both humoral and cellular immunity. The molecular strategy includes construction of plasmids for homologous recombination of flavivirus gene sequences and a vaccinia promoter into the nonessential thymidine kinase gene locus of vaccinia virus. Recombinant constructs containing genes for individual or multiple flavivirus proteins have been investigated, with variable success in immunizing and protecting laboratory animals (Table 7). In early experiments, neither binding nor neutralizing antibodies to the dengue type 4 E glycoprotein were detected in animals receiving vaccinia or baculovirus expressing this protein, probably because the proteins were synthesized intracellularly and were not secreted in an extracellular form. Recently, Mason et al. (16) demonstrated that a vaccinia construct of Japanese encephalitis (JE) virus containing genes for prM through NS2a secreted particles into the supernatant fluid with characteristics of the slowly sedimenting hemagglutinin (SHA) produced by wild-type virions. The SHA particles represent empty envelopes with the M and E proteins in a conformationally native, immunogenic configuration. It appears that deletion of the capsid (C) protein but retention of NS1 were essential to secretion of SHA particles. Mice immunized with this vaccinia construct produced high titers of neutralizing antibodies and were solidly protected against a high dose of JE virus.

Table 7. Immunization of Mice and Monkeys with Recombinant Baculovirus-Infected Cell Lysates and Vaccinia Recombinants Expressing Dengue Proteins[a]

Expression system	Dengue genes	Antibody response, mice	Survival, mice (%)	Protection, monkeys (%)
Baculovirus	C-M-E-NS1-NS2a	NS1	100	33
	RSVG-E[b]		100	33
	Control		0	0
Vaccinia	C-M-E-NS1-NS2a	NS1	97	0
	C-M-E		100	
	E		100 (ill)	
	RSVG-E		100 (ill)	
	NS1-NS2a	NS1	100	
	NS1-15%NS2a	weak NS1	67	
	Control		16	

[a] From Bray et al (1989) J Virol 63: 2853; Zhang et al (1988) J Virol 62: 3027

[b] Dengue animo-terminal signal sequence replaced with respiratory syncytial virus glycoprotein sequence

It is still uncertain whether vaccinia or baculovirus will provide a suitable approach to dengue immunization. Live vaccinia vectors which secrete particulate antigen may offer the advantage of long-lasting immunity, but this remains to be tested, and at present, the durability of immunization cannot be predicted. The potential disadvantages of vaccinia, including neurologic and dermatologic accidents and unchecked replication in immunologically-deficient hosts, are well-known and not specific to dengue vaccines. The possibility that replication of the vector might be restricted by preexisting immunity to expressed dengue antigens deserves investigation. Baculovirus expression offers the possibility of efficient production of large quantities of expressed protein, but methods for purification from infected cells or for inducing secretion of dengue particulate antigens will have to be worked out. Preliminary studies suggest that deletion of the carboxy-terminus of the E glycoprotein gene produces a truncated protein missing the anchor sequence and that this form is secreted from infected cells (17, 18), providing another approach to improved expression of flavivirus proteins.

A most promising advance, which follows on similar work with polio and yellow fever viruses, is the successful cloning of full-length complementary DNA (cDNA) of dengue type 4 by C-J. Lai and his associates at NIH (19). RNA prepared by in vitro transcription was used to transfect cells, with recovery of infectious progeny virus identical to the parental virus. This accomplishment sets the stage for the preparation of well-characterized dengue vaccines using infectious clone technology. The use of cDNA avoids the high rate of mutation associated with RNA virus passage and permits discrete engineering of the dengue viral genome. The cDNA clone can be manipulated by site-directed mutagenesis or by deletion of coding regions, with the objective of inducing an attenuated phenotype or even a vaccine lacking determinants engaged in immune enhancement. It also provides a cassette system for insertion of foreign genes, including those of heterologous dengue serotypes, to make chimeric vaccines inducing broad protection. Although this approach has advantages over the empirical development of live vaccines, it has the same inherent difficulties mentioned earlier: lack of markers of attenuation short of human testing and the uncertain safety and immunogenicity in persons with preexisting immunity.

It is reasonable to predict that the pressures exerted on health authorities in the Americas by the threat of DHF/DSS will lead to deployment of the live, attenuated dengue vaccines years in advance of successful development of a genetically-engineered product. If the live vaccines prove to be effective, it may be more difficult to pursue the expensive course of full development of a second-generation recombinant vaccine. Nevertheless, the long-range view must consider the need to incorporate dengue antigens in vaccines administered to infants with maternal antibodies, limiting the use of traditional live vaccines. The ultimate goal of a rationally-designed, multivalent, recombinant dengue vaccine will require a full exploration of the molecular determinants of dengue virulence, immune enhancement, and immunological protection.

In addition to the three approaches to molecular dengue vaccines described – vaccinia-vectoring, baculovirus-expression, and infectious clones – other laboratories are exploring alternative uses of biotechnology. These approaches include the use of synthetic peptides, chimeric molecules of hepatitis core protein and dengue sequences, anti-idiotypic antibodies, other recombinant live vectors, such as BCG and avian poxviruses. The embarrassment of riches afforded by molecular biology may complicate the search for the optimal vaccine, as it is often difficult to sort out the relative advantages and disadvantages of the multiple candidates. Another problem illustrated by the current effort on dengue vaccines, and molecular vaccine research in general, is the gap between laboratory research and clinical development. Vaccine research is being conducted in laboratories with little understanding of the process of vaccine development. There are relatively few institutions with expertise and infrastructure capable of the transition to clinical development of a product for human use, and they are not readily accessible to most laboratories engaged in research on molecular vaccines. Innovative solutions to this problem are required and may require fundamental changes in our national research institutions.

Japanese Encephalitis

Japanese encephalitis, another mosquito-borne flavivirus disease, causes an estimated 30,000 cases of central nervous system (CNS) infection annually in areas of Asia inhabited by 2.5 billion people (5). Surveillance and reporting in many areas are insensitive, however. Children under 15 years are principally affected; approximately 25% of the cases are fatal, and a high proportion – perhaps 50% – of the survivors have neuropsychiatric sequelae. In temperate regions of Asia, the disease often appears in intense summertime epidemics, with thousands of cases and attack rates of 10–100/100,000 population. In some areas, such as northern Thailand, the lifetime risk of acquiring JE infection approaches 100%. Fortunately, most infections are inapparent. Various estimates indicate that 1 encephalitis case occurs per 20 to 400 infections. It follows that the annual number of infections in Asia is between 600,000 and 12 million. In addition to the burden of human disease, JE causes encephalitis in horses and stillbirth and reduced fertility in swine. Since pigs are the principal amplifying viremic host in the epidemic transmission cycle, immunization of swine has a potential role in the protection of human populations.

Classical JE vaccines prepared from infected weanling mouse brain were first developed in the Soviet Union and in Japan in the 1940s. The crude vaccines were purified in Japan by precipitation steps and ultracentrifugation, and the purified product came into routine use in schoolchildren in 1966. The vaccine contains no detectable myelin basic protein and has not been associated with allergic encephalitis despite the administration of nearly 600 million doses. Similar vaccines are produced in Taiwan, South Korea, and to a limited extent in Vietnam and India. Safety and efficacy of the two-dose primary immunization schedule of the

mouse-brain vaccine was proven in a field trial conducted in Thailand in 1985 (20). Although widely used in several affluent Asian countries, with a decline in disease incidence attributable in part to vaccination, the mouse-brain vaccine has the disadvantages of high cost ($2.30/dose or $4.60 for the recommended two-dose primary series) and the requirement for repeated booster inoculations. As an alternative to the expensive tissue-based vaccine, the Chinese developed an inactivated primary hamster cell culture vaccine, widely used for immunization of children under 10 years of age. Annual production is given as 100 million doses. The cost of this product is unknown, but since yields are low (6.0–7.0 logs/ml), the cost per dose is probably relatively high. Most countries in Southeast Asia and the Indian subcontinent currently have no vaccination policy, and these populations remain susceptible to endemic and epidemic disease.

Because of the high cost of killed vaccines and the need for repeated inoculations to induce and maintain immunity, alternative vaccines are required. Several candidate live, attenuated JE vaccines have been produced in China and tested in humans. All are derived from the same parent, designated SA14, by sequential passages in a variety of hosts. Vaccines for human use are prepared in primary hamster kidney cells. One live vaccine, designated SA14-5-3, has been given to more than 5 million children, but was found to immunize fewer than 80%. A more promising vaccine, designated SA14-14-2, proved safe and highly immunogenic in human trials in China (21). At the Walter Reed Army Institute of Research, the SA14-14-2 vaccine was passed in an acceptable substrate (primary canine kidney), shown to retain all phenotypic markers of the original vaccine, and to be free from adventitious agents (22). Because of the obvious benefits of an inexpensive live vaccine for use in China and other developing countries, further development of SA14-14-2 is being pursued in China and the U.S. In fact, the CDC has recently identified this vaccine as a priority for new funding under the National Vaccine Program.

The live attenuated SA14-14-2 vaccine and its virulent parent have been genetically characterized in United States by Dennis Trent's group at CDC, Fort Collins. The vaccine and parent are separated by 121 passages, with biological cloning in a variety of host systems. The vaccine differed from parent virus by a total of 45 nucleotides resulting in 15 amino acid substitutions (Fig. 3), with the highest rate of change in the amino-terminal half of the E glycoprotein, including a change at position 107 in the highly conserved region which presumably subserves fusion during virus uncoating (23). While the specific attenuating mutations have not yet been defined, these studies provide a basis for the future genetic manipulation of JE infectious clones and selection of coding regions for producing chimeric viruses.

The WHO Programme on Vaccine Development has promulgated molecular approaches to new JE vaccines. The approaches used to date are essentially identical to those described for dengue and include vaccinia and baculovirus expression of single or multiple viral proteins. The complete nucleotide sequence of JE virus was described by Sumiyoshi

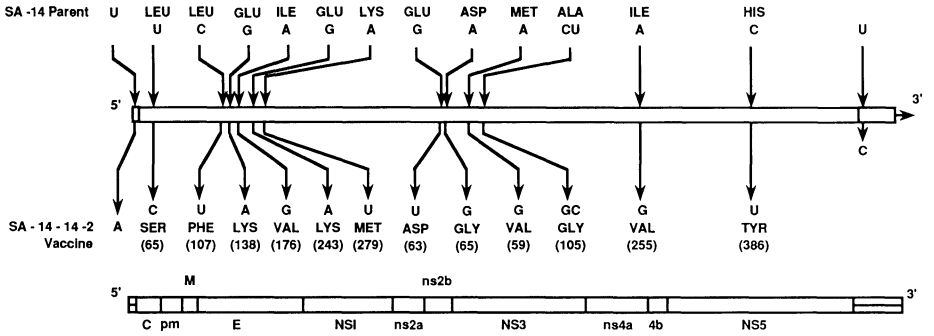

Fig. 3. Identification of the nucleotide and amino acid differences between the virulent parent (SA-14) and the live, attenuated Japanese encephalitis vaccine (SA14-14-2) derived therefrom; modified from Nitayaphan et al (23), with permission. Since reversion to virulence has not been demonstrated, it is likely than there is more than one change responsible for attenuation. Multiple changes in the amino-terminal half of the E glycoprotein are likely candidates because of the functional importance of this region

et al. in 1987 (24). Several groups have now successfully expressed the E glycoprotein in vaccinia and baculovirus systems, demonstrating neutralizing antibodies and protective immunity in mice. I have already mentioned the work of Mason, showing the highest degree of protection by a vaccinia recombinant having the prM-E-NS1-NS2a sequences and secreting SHA-like particles from infected cells (16). This vaccine candidate is being tested as a veterinary vaccine candidate in horses and pigs at USDA's Plum Island Animal Research Laboratory and is a promising candidate for a human vaccine.

A major obstacle to the success of global immunization is the requirement for multiple inoculations of inactivated antigens, such as DPT and polio. This problem may extend to many recombinant subunit and synthetic immunogens currently under investigation.The need to inoculate the same child multiple times during the first year of life greatly increases costs and represents the central impediment to complete immunization. One promising approach to overcome this obstacle is the use of controlled-release technology. Microspheres composed of biocompatible and biodegradable polymers of lactide and glycolide – the same material used to manufacture resorbable sutures – have been intensively studied as a way of long-term delivery of drugs and hormones and are now being explored for vaccine delivery (25). Antigen embedded in microspheres is released at a rate which can be controlled by manipulating the size of the microsphere and the ratio of lactide to glycolide in the copolymer. Increasing the proportion of lactide in the copolymer results in higher stability and prolonged time to release of the entrapped antigen. By using a mixture of copolymers in a single parenteral inoculation, it is possible achieve a "pulsed" release of antigen mimicking the effect of multiple

inoculations spaced weeks or even months apart. Such formulations are under active study as a means of delivering the 3-dose tetanus toxoid series in a single inoculum.

The inactivated JE vaccines currently in use must be administered to children in a series of at least three inoculations – a primary series of two inoculations and a booster dose at 12 months. Preliminary studies have been conducted on the development of a microcapsule system, which would achieve programmed release of JE vaccine with a single injection (Tice, T.R., unpublished data, 1985). In these experiments mice received a mixture of unencapsulated vaccine and microspheres programmed to release vaccine antigen at 2–3 weeks (microspheres polymer containing 50:50 ratio of lactide:glycolide) and 6–8 weeks after inoculation (65:35 ratio of lactide:glycolide). The results indicated that a single inoculation of the controlled-release formulation achieved immunization equivalent to three separate injections of equivalent doses of vaccine. If similar results can be achieved in humans, significant improvements in vaccine coverage could be achieved at reduced cost.

Yellow Fever

Official reporting of yellow fever belies the epidemiological importance of this disease. In South America, 100–300 cases of jungle yellow fever are reported annually. Since approximately 90% of the reported cases are fatal, surveillance detects only the most severe cases and underestimates the true incidence by at least fivefold.

However, the most pressing problem in South America is not the control of jungle yellow fever. In the late 1970's Brazil and Bolivia were reinvaded by the urban vector, *Ae. aegypti*. This reinfestation rapidly involved other countries of South America and was followed by explosive epidemics of dengue fever (Fig. 4). The reintroduction of *Ae. aegypti* greatly complicates the strategy for yellow fever vaccination in the continent. In Brazil alone, over 121 million people inhabit *Ae. aegypti*-infested coastal areas and are at risk of the introduction and spread of urban yellow fever for the first time in over 50 years. Because of the absence of the urban vector for many years, this large coastal population of Brazil has never been immunized. Similar considerations now apply to urban populations in coastal Peru and Ecuador. Public health authorities are now faced with a difficult decision. Should the immunological barrier to the urbanization of yellow fever be extended to the susceptible coastal populations? The logistical and economic problems in providing vaccine coverage to a population 8–10 times larger than that inhabiting the jungle areas are staggering.

In Africa, the annual incidence of yellow fever has been highly variable, reflecting periodic, spectacular epidemics. The incidence has increased dramatically since 1986, due principally to a period of sustained epidemic activity in Nigeria (Fig. 5), where 1000–3000 cases have been reported each year. However, the official reports represent only a small fraction of the true incidence, as shown repeatedly by direct epidemiological investigation

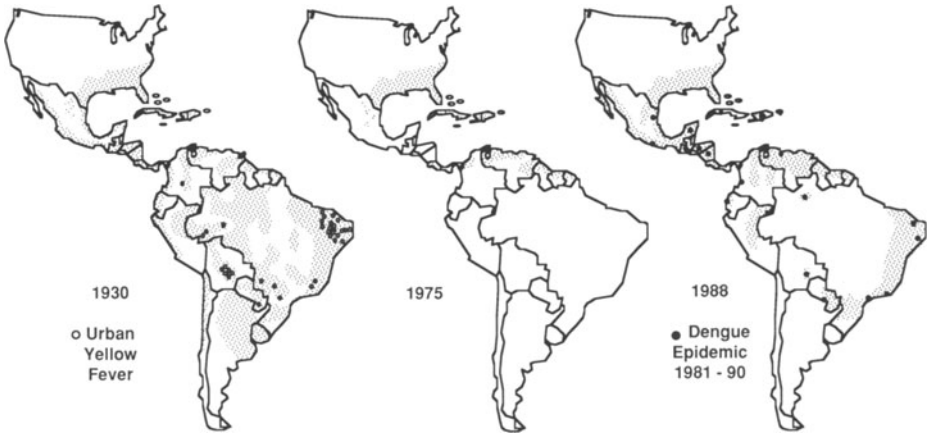

Fig. 4. Changes in the distribution of *Aedes aegypti* as a result of efforts between 1947 and 1974 to eradicate the mosquito and dissolution of vector control thereafter. Prior to the eradication effort, urban yellow fever epidemics were commonplace in South America. In the 1980s large outbreaks of *Ae. aegypti*-borne dengue fever have occurred, illustrating the renewed risk of urban yellow fever in the Americas. Reproduced from Monath TP (1991) Yellow fever: *Victor, Victoria.* Conqueror, Conquest? Am J Trop Med Hyg with permission

of epidemics. In 1986 and 1987, for example, only 2% of the cases and 3% of the deaths were officially recorded (26, 27).

Yellow fever in Africa comes to light only during major epidemics. In fact the disease burden of endemic disease is probably much greater than that attributable to epidemics. In Nigeria, for example, serosurvey data indicate an annual incidence of endemic disease with jaundice to be 1.1–2.4 per 1,000 and yellow fever death at 0.2–0.5 per 1,000 – approximately 20-fold lower than the incidence during epidemics and far below the threshold of detection by the existing passive surveillance system. Extrapolation to the total population at risk gives a nationwide annual total of 163,642 jaundice cases and 32,728 deaths due to endemic yellow fever (28). Many factors contribute to the lack of recognition of endemic yellow fever, including the difficulty in distinguishing the disease from other causes of jaundice; the widely-held belief that jaundice is best treated by traditional herbalists rather than western medicine; occurrence of the disease in remote, rural areas; and lack of death registries.

Because the disease goes unrecognized during the relatively long intervals between epidemics, many African countries have established a policy of emergency control rather than preventive immunization. This policy is flawed by the absence of surveillance, late recognition of epidemics, and logistical difficulty of mounting an effective mass immunization campaign in the face of an epidemic. The result has often been that only a small proportion of the affected population is immunized before the epidemic has run its natural course. The advent of the Expanded Program of Immunization in 1974, which provides an effective cold-chain for delivery, makes possible the inclusion of the thermolabile yellow fever vaccine in national

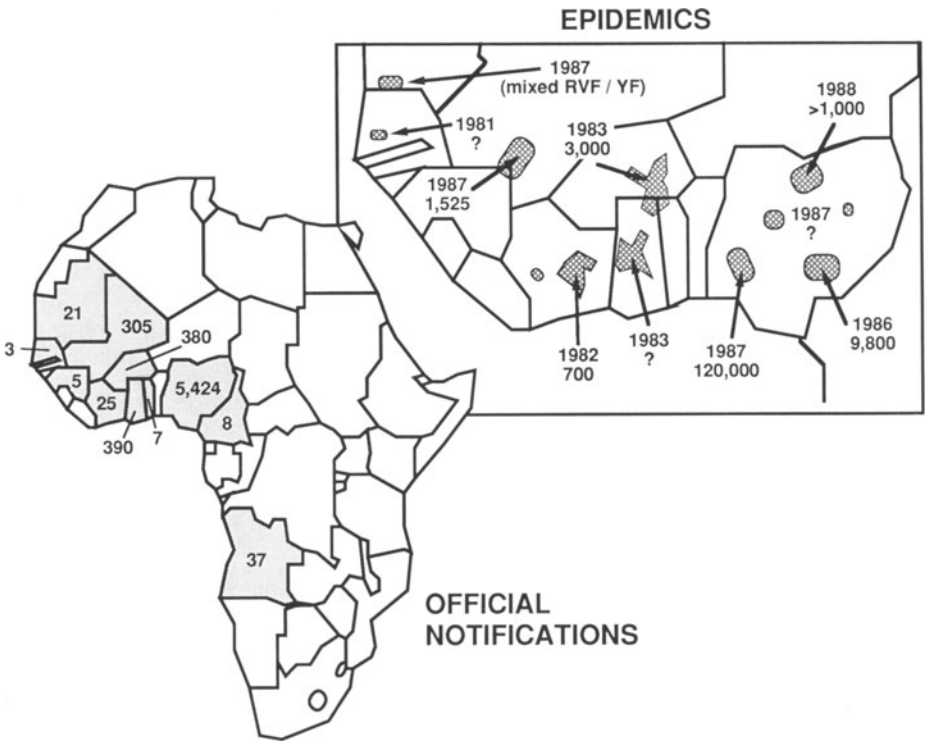

Fig. 5. Incidence of yellow fever reflected by official notification to the World Health Organization, and location and estimated morbidity associated with epidemics, Africa, 1980–1988. Reproduced from Monath TP (1991) Yellow fever: *Victor, Victoria.* Conqueror, Conquest? Am J Trop Med Hyg

programs of childhood immunization. Eight African countries have recently incorporated yellow fever vaccine in their EPI, and this year, Nigeria has been added to the list. Because of Nigeria's large size and population (currently 116 million, expected to reach 154 million by the end of the decade, and representing nearly 60% of the entire population in the yellow fever endemic zone in West Africa), the allocation of scarce resources to the inclusion of yellow fever vaccine in the EPI must be carefully considered. A recent analysis of the cost-effectiveness of yellow fever vaccination strategies in Nigeria indicates that routine vaccination of infants alone, as part of the EPI, would eliminate the risk of epidemics within 20–30 years (28). Endemic yellow fever morbidity would decline from 130 cases/100,000 population to 51 cases/100,000 over a time horizon of 35 years. The cost of the program per death prevented is $158 in the early phase of the program, and falls to $76 as cumulative immunity increases. Prevention of endemic disease would thus be cost-effective at ratios comparable to other diseases currently targeted by the EPI.

If it has been difficult to implement an effective policy for utilization of the present yellow fever vaccine, is it reasonable to suggest biotechno-

logical innovations? The current vaccine is produced in embryonated eggs by the same technology developed 53 years ago. There are currently production facilities in 11 countries, but nearly all of the vaccine available for use in developing countries is produced in Brazil and Senegal and sold at modest cost: US$ 0.18–0.20/dose. It is important to note that the cost of adding this vaccine in countries with an operating EPI should not be much more than the cost of the vaccine alone, since yellow fever and measles vaccines can be mixed and given in combination to children >9 months of age. The vaccine induces neutralizing antibodies in >90–95% of those vaccinated, and immunity is probably life-long. Over 200 million vaccinations have been given, and there have been only 18 recorded serious reactions, all in the form of postvaccinal encephalitis, with one death. Any new yellow fever vaccine would have to beat this remarkable record of achievement!

In 1981, the first of several meetings was held by WHO/PAHO to discuss the desirability and feasibility of modernizing yellow fever vaccine production. A number of problems with the present vaccine were considered.

1. The limited capacity for production and scale-up. Current stocks worldwide approximate 25 million doses, enough to vaccinate only the population of Sao Paulo, Brazil.
2. The instability of the vaccine to heat, even in its lyophilized state, increasing the logistical problems associated with vaccine delivery;
3. The contamination of many vaccines with avian leukosis virus, clearly an unacceptable aspect by modern standards, although no effects on human health have been noted.
4. The residual neurovirulence of the vaccine that limits its use in young infants. Monkeys inoculated intracerebrally develop inflammatory pathological changes in brain tissue, and clinical signs of severe encephalitis occurring in up to 10% are acceptable for vaccine to pass neurovirulence testing. Eighteen cases of encephalitis following inoculation of 17D vaccine have been reported, all but four cases in infants less than 4 months old. Since the danger to young infants was recognized in the 1950s and immunization practices were modified to exclude infants under 9 or 12 months from vaccination in the 1960s, denominator data are inadequate to assess fully the incidence of this complication. On the basis of the limited available data, the risk of postvaccinal encephalitis in fact may be significant for young infants, ranging from 0.06–0.97% (20).

A special concern is the age at which infants would receive 17D vaccine in the EPI. The current practice in most countries is to administer live measles vaccine to infants more than 9 months old, an age considered safe for use of 17D vaccine. This strategy has been questioned because increasing urbanization has lowered the age at which children are affected by epidemic measles, so that vaccination at an earlier age is desirable (29). The Edmonston-Zagreb vaccine may be effective as early as 4 months (30) and is currently being introduced in some programs for infants 6 months old. Because measles may be the last vaccine given to infants in the EPI sched-

ule, yellow fever vaccination must be given at the same window of opportunity to be economical. If encephalitis complicates administration of 17D to young infants, the annual immunization of millions of infants less than 6 months old in the EPI may uncover an unacceptably high incidence of untoward reactions.

5. The heterogeneity of the strains used for vaccine production and of the virion populations contained in these strains. At least two distinct substrains of 17D (17DD and 17D-204) are used by different vaccine manufacturers and can be distinguished by monoclonal antibodies and RNA fingerprinting (31). All existing vaccines in turn represent uncloned mixtures of virions with variable biological properties. Plaque variants within 17D vaccine have markedly different virulence for mice and ability to replicate in human macrophages (32). The heterogeneity of uncloned virus can produce dramatic changes in the dominant population under selective pressures, but these pressures are not predictable and are host-dependent.

There has been one fatal case of 17D vaccine encephalitis, a 3-year old girl in the United States in 1965. The brain isolate differed from the vaccine by RNA fingerprinting and lethality for adult mice by the intranasal route (Barrett ADT, Monath TP, Miller BR, unpublished data, 1988). This suggested that a virus variant, possibly one already contained in the heterogenous mixture of 17D vaccine, selectively invaded the central nervous system producing fatal encephalitis. This possibility was supported by other studies using monoclonal antibodies, that detected a variant within commercial 17D vaccine with antigenic and neurovirulence characteristics of wild-type, parental (Asibi) virus (33).

Previous considerations for improving the present yellow fever vaccines focussed on the development of 17D virus in cell culture rather than embryonated eggs and on the addition of stabilizers to improve resistance to heat. Although this approach would resolve some problems with the vaccine, others would remain, particularly those of vaccine heterogeneity, genetic stability and neurovirulence. However, molecular approaches are now being explored, which may resolve these issues. In 1985, Rice and colleagues reported the entire gene sequence of 17D vaccine (11) and, in 1987, of the parental Asibi strain (34). Of the 32 amino acid differences that evolved during 230 passages in the development of 17D vaccine, it is still uncertain which change or changes are responsible for loss of virulence. To address this question, Rice's group has constructed full-length cDNA templates of 17D and Asibi viruses, which yield infectious RNA transcripts (35). These infectious clones can be used to map individual nucleotide changes with respect to their phenotypic characteristics in animal models, including those responsible for neurotropism. This goal has considerable practical interest, since elimination of the remaining neurotropic properties of 17D vaccine might permit its use with Edmonston-Zagreb measles vaccine in young infants in the EPI. Moreover, the use of recombinant 17D viral DNA as the starting material for live vaccine production may provide a better approach than the present seed-lot system for stabilizing the yellow fever vaccine genome.

The availability of a full-length yellow fever infectious clone also provides the opportunity for inserting foreign genes containing protective epitopes on the E and NS1 proteins of other flaviviruses, such as dengue or JE, into the 17D backbone. At the WHO meeting in 1990, the Steering Committee recommended that, prior to the construction of chimeric infectious cDNA vaccines, proof of concept should be achieved by developing a new 17D yellow fever vaccine derived from the infectious clone. Accordingly, studies have been initiated as a collaborative effort between Rice's group, the Instituto Oswaldo Cruz in Rio de Janiero, and the U.S. Army Medical Research Institute of Infectious Diseases.

The new vaccine would be derived in a straightforward manner by transfection of an approved cell culture substrate and passage of the progeny virus to embryonated eggs to prepare a new primary seed. This, in turn, would be used to prepare secondary seed and vaccine lots using the standard manufacturing process in eggs. Quality controls, including monkey neurovirulence tests, would be performed as required by WHO standards, and the new vaccine would ultimately be tested in humans. A preliminary study of progeny virus from cell cultures transfected with RNA transcripts from the full length 17D cDNA demonstrated acceptable characteristics of attenuation in monkeys. The unique aspect of this work was the likelihood that the proof of concept – the development of a new vaccine derived from cDNA – could be accomplished with very limited resources, probably less than $1 million.

However, the ultimate development of an improved yellow fever vaccine presents a very different set of problems. The objectives would be much more complex and might include:

- High-yield production in an cell culture substrate in lieu of egg-derived tissue;
- Deletion or substitution of gene(s) responsible for neurovirulence and the extensive biological characterization of such a vaccine in nonhuman primates and ultimately in humans (including infants); and
- Construction of chimeric vaccines incorporating foreign epitopes conferring multivalent heterologous immunity – for example against dengue viruses – as well as homologous protection, and the biological and clinical evaluation of such vaccmes.

The resources required for the research and development for such a vaccine might be conservatively estimated at $5–10 million, an amount which would purchase 25–50 million doses – 1–2 year's requirements – of standard 17D vaccine for the entire world! There would be no commercial incentive to develop such a vaccine, and the vaccine development costs could not be passed back to the consumer, since that would defeat the purpose of its application in the developing world.

This example illustrates a problem common to other new vaccines anticipated for inclusion in the Children's Vaccine Initiative. Although the

technological basis for vaccine development is within grasp, the economics and ability to coordinate efforts among industry, the research community, foundations, national governments, and international agencies are much less clear. In general, vaccines are the neglected stepchildren of the pharmaceutical industry. The economic incentives of vaccine development are limited, and liability and patentability problems well-known (36). One possible solution is to redirect funding from international aid programs to vaccine development through contractual agreements with the biotechnology industry. This represents a departure from past policies, which have funnelled aid resources into needy programs in the developing world itself. Many countries may resist the notion that international aid programs should divert funds to private industry in the developed world, and strong political and nationalistic forces strongly favor the development of biotechnology in the developing countries themselves. This issue has yet to be fully aired, and I suspect it will be the focus of considerable debate.

If the technical problems of vaccine development are yielding to biotechnology, the obstacles to utilization of vaccines in general and of new vaccines in particular have their roots in politics and economics and are much less easily solved. It is clear that the development of a vaccine is not in itself an answer to a public health need. In the case of yellow fever, a tissue-based vaccine developed over 50 years ago meets nearly all the criteria for an ideal intervention but has not been effectively utilized. Vaccine development must be followed by a clear strategy for its application and implementation of a public health policy. As illustrated by the case of yellow fever, disease identification and definition is deficient throughout much of the area affected. Only a small fraction of the cases are recognized and reported. The true requirements for vaccine are not clear, and consequently, effective health policies are not formulated. Surveillance and disease definition were the essential first steps in developing the strategy for vaccine implementation and for convincing governments of the need for childhood immunization in the EPI (1, 29). Data from sentinel sites and other surveillance mechanisms formed the underpinning of the programs aimed at tetanus, poliomyelitis, and measles. Similar mechanisms have not been implemented for most of the diseases targeted for new vaccine development. The clinical diagnosis of diseases such as yellow fever, dengue, rotavirus, respiratory syncytial virus, typhoid, shigella, enterotoxic *E. coli* and others is inherently much more difficult than for tetanus, polio, and measles. Laboratory-based epidemiological studies are required to provide the empirical data to convince governments of the need for preventive immunization and are essential for monitoring the value of immunization strategies. However, little priority is currently given to such surveillance or field research efforts by national authorities or international funding agencies.

The biotechnological revolution has captured the imagination of scientists around the world. The ability to explore and understand biological systems at the most fundamental level is an extraordinarily powerful intellectual lure. Moreover, strong economic incentives have been created by an

expanding biotechnology industry. Today, molecular biology is the unitary focus of most undergraduate and graduate students in the biological sciences. Awards of government research grants are largely predicated upon innovative applications of molecular techniques, even in subject areas as far afield as epidemiology. In the developing world, students and young scientists are following the track of modern science, encouraged by their governments, which view this development as a matter of national pride, and by efforts of international agencies to strengthen scientific infrastructures in areas most afflicted by disease, famine, and other problems potentially addressable by biotechnology.

Despite its tremendous potential, it is also true that biotechnology provides a countervailing force to the pursuit of careers in field biology, epidemiology, hygiene, and other traditional disciplines. This is true in the developed world and also – perhaps with more serious consequences – in many developing countries. The accelerated pace and progress of biotechnology in the developed world is accompanied by an increasing flow of physicians and scientific talent out of the developing world – the well-known "brain drain". The gap is widening between developed and developing countries in the opportunities for education, monetary rewards, and personal scientific achievement, and it is exacerbated by the deteriorating economic situation and unchecked population growth in the developing world, particularly Africa (37).

The new opportunities for the prevention and control of infectious diseases that arise with technological breakthroughs in vaccines, diagnostic tests, and therapies, are outpacing the ability to evaluate and apply them in the areas most affected by disease. The targets for new approaches often remain ill-defined, by virtue of inadequate disease surveillance, absence of accessible laboratory diagnosis, and lack of reliable data on disease incidence – problems that can be addressed only by field and epidemiological studies. New viral hemorrhagic fevers and arbovirus diseases continue to emerge in tropical areas of the world, yet the laboratories, medical virologists and entomologists working in this area have fared poorly in the competition for resources with molecular biology and may be in danger of extinction. The first task in developing a health policy – that of identifying and defining the major health problems – is too often forgotten, and there are fewer trained people and resources available to undertake this task. The problems are not static. The emergence of new diseases demands effective surveillance and field investigation. Changing demographic patterns, urbanization, ecologic and climatic perturbations, and expanding human population groups with immunodeficiency disease make the problem of disease definition an ever-moving target. Thus, a parallel challenge to the transitioning of the new tools for disease prevention from biotechnology must be the strengthening of the public health infrastructure, including traditional disciplines such as medical microbiology and virology, epidemiology, and medical ecology.

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