Innovative Vaccines in China

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5.1 Japanese Encephalitis Live Attenuated Vaccine

- The first vaccine in China prequalified by the WHO

Yongxin Yu is an academician at the Chinese Academy of Engineering and a highly respected person when it comes to Japanese encephalitis (JE) live attenuated vaccine (Fig. 5.1). After the JE vaccine he developed was approved and marketed in 1989, hundreds of million doses have been used, demonstrating the safety and effectiveness of this vaccine under large-scale use. The effectiveness of this vaccine is over 95%, as recognized by the WHO and other international organizations.

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Fig. 5.1 Academician Yongxin Yu

Academician Yu is one of China's most famous virologists and experts in biological products; he has received an outstanding contribution award by improving China's medical health. He is also a renowned scientist in China and in the world with his great contributions. His science is rigorous and realistic; pays attention to the accuracy, reliability, and integrity of the experimental data; and is loved by scientific and technological personnel.

5.1.1 The Severe Burden of JE

In 2014, Xu Bai, a Xinhua Net reporter, interviewed Yongxin Yu, who was then 85 years old, and still wore a white coat. Bai said, "he still cannot forget the epidemic of JE in the middle of the last century in China. At that time JE epidemics were very serious. According to Yu, the fatality rate was as high as 30% among children and usually followed by serious sequelae, including development of paralysis from brain damage in 10% of the individuals. There were many JE

patients in hospitals. In children's hospitals, the wards and the aisles were full of pediatric JE patients."

JE, also called Japanese encephalitis, is an acute infectious disease transmitted by mosquitoes with a high mortality and morbidity rate among its victims. JE is caused by the JE virus and is one of the most serious threats to the population, especially to children. With an acute onset, the disease presents with varying severity, ranging from latent infection to mild and to severe encephalitis. Severe cases always involve invasion of the central nervous system with high fever, convulsions, coma, spastic paralysis, and even death. Obvious complications nearly always occur in survivors, including persistent confusion and paralysis. Epidemics of JE occurred in the 1960s and early 1970s across China. There were different intensities of epidemics in China (including Hong Kong, Macao, and Taiwan) except for Qinghai, Tibet, and Xinjiang where JE is rare.

About 30,000–50,000 cases are reported globally every year with 15,000 deaths annually [1]. The actual number of patients is far higher than the reported figures due to lack of diagnostic capacity and reliable data. JE is highly endemic in Southeast Asia and the Western Pacific Region and is endemic in the coastal areas of Russia, Japan, North Korea, South Korea, Vietnam, Laos, Cambodia, Burma, Thailand, India, Indonesia, Sri Lanka, the Philippines, Malaysia, Nepal, Pakistan, and other places [2]. In the most recent 25 years, the spread of JE has increased in some countries, while the endemic range has increased globally, with the disease extending to regions of Asia that were not affected previously and even to northern Australia [3, 4].

5.1.2 The Development of JE Attenuated Vaccine

There is no effective treatment for JE; therefore prevention and control of JE are critically important work of disease prevention and control institutions in epidemic areas. It is not currently feasible to control infected mosquitoes because they multiply in the natural environment. Given that 70% of JE cases are children, most experts advocate universal immunization for children in endemic areas to prevent JE. Many years of experience from China and other countries has shown that JE vaccination is the most economic and effective way to control JE [5].

There are two types of JE vaccines – inactivated vaccine and live attenuated vaccine. Historically, Japanese scholars developed a mouse brain-based, purified inactivated JE vaccine, which became widely used in epidemic areas. In China, an inactivated JE vaccine produced in primary hamster kidney (PHK) cells was widely used. Japan's mouse brain inactivated JE vaccine caused a few serious and even fatal allergic reactions due to residual brain tissue in the vaccine – events that were reported in Japan and South Korea. As with China's PHK JE vaccine, the primary schedule for Japan's mouse brain vaccine included two or three doses – burdensome to deliver and therefore difficult to complete the full immunization schedule to obtain adequate immunity.

Not long after he began to work, Yongxin Yu received a task: improve the JE vaccine. “There were lots of quality problems in the domestic JE vaccine,” he recalled. Hemin Li, director of a Department in the Institute of Control Biological Products, asked Yongxin Yu whether a better vaccine could be developed. “I didn’t know how difficult it would be,” Yu said lightly. He had no idea that his promise to develop an improved JE vaccine would occupy his entire life.

His first task was to find a suitable JE virus strain for producing a virus vaccine. It was not until 1967 that Yu’s team identified a potential vaccine strain that could be used as a live, attenuated vaccine – a strain that was demonstrated to be safe, stable, and effective in an animal model. However, experts did not reach agreement about testing the vaccine candidate in humans, so Yu and five colleagues made a bold decision: to vaccinate themselves. “It is impossible to say not to worry, but according to our own experimental data and study results, there should be no major problems,” he said. After 2 weeks of observation, they were free from infection with the vaccine virus. Yu became more confident. They decided to vaccinate their own children. “My daughter was six years old, and the kindergarten-age children of 5 other colleagues participated in this experiment. Everything went well; we were very happy and felt that we had achieved an initial success,” he said. However, their work was far from over, as more subjects were needed to prove the safety of the vaccine candidate.

In 1988, after more than 20 years of hard work, an expert group led by Yu in the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) finished the development of a strain for a live attenuated vaccine, SA14-14-2. This JE live attenuated vaccine (JEV-L) was produced successfully by Chengdu Institute of Biological Products. The vaccine, which had been independently developed in China with independent intellectual property rights, was included by the World Health Organization in its list of prequalified vaccines. The vaccine won first prize for Technological Progress in the Ministry of Health (MoH) in 1989 and won the National Science and Technology Progress award in 1990. The vaccine is now indicated for children above 8 months old and for adults who move from non-endemic areas to endemic areas. Vaccination has significantly reduced the incidence and fatality rate of JE in endemic areas, effectively protecting hundreds of millions of children.

Since the approval in 1989, JEV-L has been proven to be safe and effective through several large-scale clinical trials, serological studies, and field observational studies. This vaccine is now the most widely used JE vaccine in the world.

Yu’s JE vaccine has a very good safety profile. Through several clinical studies organized by Chinese and foreign experts, JEV-L has been demonstrated to be safe and effective, with no serious adverse reactions related to the vaccine. Compared with inactivated JE vaccine, local and systemic reactions are significantly fewer [6, 7]. In 2006 JEV-L was used widely in India for the first time, with more than 9 million children vaccinated and with the adverse reaction closely monitored by the India MoH. The WHO also conducted safety assessments during this mass vaccination effort and showed that the vaccine had a good safety profile [8].

Clinical studies showed that JEV-L was immunogenic, not only in a Chinese population but also in other Asian populations. A study in South Korea showed that the neutralizing antibody conversion rate was as high as 96% after a single dose of JEV-L [9] and that average neutralizing antibody GMT was significantly higher than among those who previously received JE mouse brain inactivated vaccine, suggesting that JEV-L could induce memory response [10]. A study conducted in Thailand showed that the seroconversion rate for developing neutralizing antibodies was 95% after a single dose of JEV-L and 100% after two doses [11]. Under the support of the Rockefeller Foundation, a joint clinical study by West China School of Medicine, Sichuan University, and the University of Pennsylvania was conducted in Chengdu, Sichuan Province. The study showed that the effectiveness of two doses of the vaccine was 97.5% (95% CI, 86–99.6%) [12]. According to clinical observations in Nepal in July 1999, the effectiveness of one dose of JEV-L was 99.3% (95% CI, 94.9–100%) [13].

Compared with the inactivated vaccine, the JEV-L is easier to administer, requiring only a single primary dose. A study of concomitant administration of JEV-L and measles vaccine in the Philippines showed that JEV-L can be co-administered with measles vaccine with no influence on the immunogenicity and safety of either vaccine, facilitating the introduction of JEV-L into national immunization programs [14].

5.1.3 Prequalification of JE Vaccine by WHO

In May 2013, Chengdu Institute of Biological Products Ltd. (Chengdu Institute), a subsidiary of China National Biotec Group Company Limited (CNBG), was inspected on-site by the WHO for prequalification of JEV-L. On October 9th, JEV-L was officially prequalified by WHO – an event that was widely reported in the media. This is the first time that a Chinese vaccine was prequalified by WHO, obtaining the first "ticket" for a Chinese vaccine to reach the international market. Prequalification means that JEV-L is qualified for procurement by the United Nations (UN), which contracts for vaccines for more than 120 developing and middle-income countries.

"This is a welcome development both in the fight to protect children in developing countries from JE and in the future availability of vaccines more generally, as China is now producing vaccines up to WHO standards," says WHO Director General Dr. Margaret Chan. "There is a huge potential for vaccine manufacturing in China and we hope to see more and more Chinese vaccines become WHO prequalified. The whole world will benefit."

Co-chair of the Bill and Melinda Gates Foundation (BMGF), Bill Gates, said that "vaccines can save lives, and can protect children's lives. China joins the global vaccine supply chain, and can provide high quality and inexpensive vaccines for many developing countries. This is a great boon for the millions of Asian children whose lives and health suffer from the threat of Japanese encephalitis."

The WHO representative in China Dr. Bernhard Schwartländer said, "JE vaccine is a start. The gate is opened, and more Chinese vaccines will go to the world."

5.1.3.1 The Road to Prequalification: China's JEV-L

Chengdu Institute's JEV-L prequalification effort started from its own internationalization.

After the successful development of JEV-L in 1989, it aroused the attention and interest of experts in the United States, South Korea, Japan, and the WHO. In 1992, Dr. Scott B. Halstead, deputy director of the Health Sciences Department in Rockefeller Foundation, learned of the development and production of JEV-L in Chengdu Institute by Yongxin Yu and showed great interest to make a site visit to the production facilities and meet the personnel involved with JEV-L. In order to enhance international confidence in the vaccine, experts from the United States and the West China School of Medicine, Sichuan University, vaccinated children aged 1 and 6 years old in Chengdu in 1995 and showed a seroconversion rate of 97.5%. The research also pointed out that "if the new vaccine, which is safe, reliable and affordable, is promoted widely, millions of Asian children will be protected from death or lifelong disability every year." Mark Steinhoff, from the School of Public Health in Johns Hopkins University, wrote: "health institutions and vaccine manufactures in Asia and the world should adopt best practices to produce and distribute this inexpensive vaccine that effectively prevents Japanese encephalitis." At about the same time, the Rockefeller Foundation funded experts from the United States and Chengdu Institute to design a modern production plant for JEV-L that would be consistent with current good manufacturing practices (cGMP). Due to limits on the level of economic development, the GMP production facility was not used.

The Lancet published a scientific article on the study conducted jointly by Chinese and foreign experts that showed satisfactory safety and effectiveness of JEV-L [13]. Once again, the safety and effectiveness had been demonstrated in clinical research, and this augmented the scientific and business rationale to enter the international market and to attract attention from investors.

The publication in *The Lancet* attracted attention and interest by Korean experts. Since 1996, the Chengdu Institute has cooperated with South Korea's Glovax company, striving to internationalize JEV-L. The joint preclinical and clinical research by China and South Korea once again demonstrated the safety and effectiveness of JEV-L. The seed viruses, PHK sera, PHK cell line, and vaccine samples were tested by the British authorities' laboratory (Q-One Biotech Ltd.) to determine whether there could be virus contamination. They searched for 10 mouse viruses, 18 viruses of rat mouse, and 2 bovine viruses, finding no contaminants and meeting international standards. Additionally, no retrovirus was detected. The safety of the vaccine had been demonstrated again.

On October 13, 1998, the Conference for Vaccination Control of JE was jointly held by WHO and CVI in Thailand. The participants agreed that Chengdu's JEV-L was stable and that its virulence was shown to be attenuated by tests in sensitive animals (such as rhesus monkey and mouse). There had been more than 100 million people vaccinated with JEV-L, demonstrating that the vaccine was not only safe but

also effective. JEV-L was a new safe and effective vaccine against JE, requiring fewer injections, and was relatively inexpensive.

In early 2000, WHO held an expert committee meeting on JE and dengue fever, during which it was clarified that JEV-L would be the direction for development of JE vaccine, with its fewer doses and lower cost. WHO adopted TRS-980 based on Chengdu's "production and specifications for freeze-dried live attenuated JE vaccine." The adoption of this document paved the way for China's JEV-L to enter the international market and improved reputation of vaccines throughout the world.

According to the requirements of the WHO, as one of the most important raw materials for vaccine production, the hamsters used for cell lines should be specific pathogen-free (SPF). To this end, the Chengdu Institute established the first SPF hamster in China. The SPF hamster was verified by NICPBP and the UK Q-One laboratory. The establishment of SPF hamster raised the quality of experimental animals to a higher level.

However, prequalification by WHO was the necessary route for JEV-L to enter the international market and for widespread use by countries around the world.

The Program on Appropriate Technology in Health (American) (PATH) established a JE Project Team at the end of 2003. The team was funded by BMGF to promote the use of safe, effective, and inexpensive JE vaccine in Asian endemic countries and to control and reduce the burden of Japanese encephalitis. To achieve these objectives, the PATH JE Project Team searched for the most suitable JE vaccine in the world. PATH organized two evaluations in August 2004 and February 2005 by well-known, international experts in GMP and vaccine to assess in detail clinical studies and Chengdu JEV-L manufacturing facilities. After receiving the evaluation report on vaccine safety and effectiveness, PATH compared JE vaccines in the global market and in the developmental stage and ultimately selected Chengdu Institute as its partner. JEV-L (SA14-14-2) was selected as the target vaccine. Starting at that point, Chengdu Institute's JEV-L was on the road to prequalification.

5.1.3.2 The Process of Prequalification for China's Vaccine

According to the WHO vaccine prequalification procedure, a complex process must be completed for prequalification of vaccine:

- First, the country in which vaccine is produced must have a National Regulatory Authority for Vaccines that has been verified by the WHO to be functional – this is a prerequisite for the manufacturer to submit a prequalification application for a vaccine.
- Second, manufacturers must submit a vaccine product summary file (PSF) to WHO, and then WHO organizes experts to evaluate the PSF and clinical data about the vaccine.
- Third, after the PSF document is reviewed, manufacturers submit vaccine samples for laboratory testing by a WHO-designated independent laboratory.
- Fourth, WHO sends an on-site inspection team to evaluate the production of the vaccine, its quality, and cGMP compliance.

- Fifth, if the on-site inspection meets the relevant regulatory requirements of WHO, the inspection report and the manufacturer's reply will be submitted to a WHO Special Committee for a decision whether the vaccine can be prequalified.

Chengdu Institute began international cooperation in the mid-1990s to conduct assessments of the safety of JEV-L, to optimize the production processes, and to facilitate international market registration. Before prequalification, JEV-L had been registered in many countries, including South Korea, Thailand, India, Nepal, Sri Lanka, Kampuchea, and Laos, and these national registrations facilitated the prequalification process. To introduce the vaccine into the UN Procurement Catalog and make the vaccine available to more children globally, in 2006, Chengdu Institute began a cooperative agreement with PATH that was funded by BMGF and conducted with the guidance of China Food and Drug Administration (CFDA), initiating the process of the WHO prequalification for JEV-L vaccine.

Chengdu Institute decided to establish a quality management system in accordance with WHO GMP, the Pharmaceutical Inspection Co-operation Scheme (PIC/s) GMP, and the European Union GMP at the beginning of the program, before Chengdu Institute was about what a huge undertaking this would be. For example, Chengdu Institute originally planned to only build a JEV-L production plant and an SPF hamster plant but later found these were far from adequate. At that time, Chengdu Institute analyzed all plants involved with JEV-L production and identified gaps between current practices and the WHO GMP standard. Finally, new warehouses for raw materials, hazardous goods, and finished products were established, and the SPF experimental animal breeding plant, animal experiment room, medium building, QA/QC building, and central control building were renovated. All plants and facilities had to have complete validation data.

In addition to the "hardware," Chengdu Institute improved the management system in accordance with ISO guidelines, WHO GMP, TRS910 (WHO live attenuated JE vaccine production and quality control guidelines), and other regulatory documents, including the file management system, a verification management system, a measurement management system, a maintenance system, a raw material and supplier management system, compliance management, prevention and correction, modification control, risk management, trend analysis, product quality review, environmental monitoring, and deviation management.

In March 2011, CFDA passed NRA evaluation by WHO. In November 2012 National Institutes for Food and Drug Control (NIFDC) became a contract Inspection Laboratory for prequalification, and in January 2013 WHO designated the Institute for Biological Product Control (IBPC) of NIFDC as a WHO Reference and Evaluation Collaborating Center (WHO CC) for biological products. These achievements provided technical support for JEV-L prequalification.

Chengdu Institute raised funding to invest in JEV-L-related hardware facilities and management systems, with ten plants constructed or renovated; domestic and international clinical research conducted; a comprehensive quality management system (QMS) completed; staff trained; operations and management of QMS

Fig. 5.2 Japanese encephalitis live attenuated vaccine

enhanced; more than 10,000 new standard operating procedures and records drafted, issued, and implemented; and more than 300,000 person-time of training completed. After an almost 7-year effort, JEV-L was prequalified and production capacity expanded.

Prequalification of JEV-L (Fig. 5.2) was not only a ticket to the international vaccine market for Chengdu Institute but also was a means to build a new platform for internationalization of vaccines. The international standards of the new quality management system make Chengdu Institute and China National Biotec Group Company Limited (CNBG) leaders among the Chinese vaccine manufacturers, providing a distinct advantage for future domestic and international competition. With such an advanced platform, one can anticipate that more vaccines from CNBG and from other Chinese companies will be prequalified! Chinese vaccines are entering the world market to benefit the world population!

5.2 Hepatitis A Vaccine

5.2.1 Hepatitis A: A Highly Endemic Disease in China

Viral hepatitis is caused by five different viruses, A, B, C, D, and E, with hepatitis A ranking the first in terms of incidence. Hepatitis A is a usually self-limited disease with serious public health impact that is caused by the hepatitis A virus (HAV), which is transmitted by the fecal-oral route. HAV may survive in the natural world outside of the body for months. Dr. Fuqiang Cui, an expert in the National Immunization Program of China CDC, said that a seroepidemiological survey of viral hepatitis in 1992 in China showed that the average prevalence of antibodies against hepatitis A was 80.9% in the Chinese population with an annual incidence as high as 60 per 100,000 in the early 1990s. Thus, China was a highly endemic country.

Hepatitis A is prevalent throughout the world. According to the WHO, the global incidence of acute hepatitis A increased from 177 million in 1990 to 212 million in 2005 and deaths due to hepatitis A increased from 30,283 in 1990 to 35,245 in 2005. The majority of the increase was among children 2–14 years of age and adults over 30 years of age [15].

Most human infections with HAV are subclinical or asymptomatic, with only a small proportion of human infections manifesting symptoms. Generally, patients fully recover, and do not develop chronic hepatitis or become carriers; death is a rare outcome of HAV infection. However, in patients with chronic liver diseases for whom the threshold of new diseases is decreased – especially for viral superinfection – hepatitis A is the leading cause of exacerbations and death. During a hepatitis A pandemic in 1988 in Shanghai, the fatality rate among 27,346 people who were HBsAg-positive was 5.6 times [16] higher compared with HAV infection alone. HAV infection may cause heavy economic loss in developed and developing countries. For example, the annual average economic cost due to treatment and productivity loss from hepatitis A in the most recent 10 years was $200 million in the United States [17].

5.2.2 Development of Hepatitis A Vaccine

The best way to prevent hepatitis A is hepatitis A vaccination, and the main way to prevent hepatitis A for those at risk of infection or travelling to endemic areas on short notice is intravenous immunoglobulin. With the successful development of vaccines, hepatitis A vaccine has become the preferred means of prevention of hepatitis A, except in special groups and special circumstances (elderly people, immunocompromised patients, patients with chronic liver disease, or people who travel to high incidence areas with less than 2 weeks of advanced notice).

Before 2008, hepatitis A vaccination strategies varied by province according to local epidemiology and economic development. From 1992 to 2007, 156 million doses of hepatitis A vaccine were used, with most use among children and students. In 2007 hepatitis A vaccine was introduced into the National Immunization Program, and after that, vaccine coverage among age-eligible children increased to >90%.

With development of the economy, improvements in health, and widespread use of hepatitis A vaccine, the incidence of hepatitis A showed a declining trend in the past 10 years in China. Introduction of hepatitis A vaccine into NIP in 2007 reduced the incidence of hepatitis A among young children.

There are two kinds of hepatitis A vaccine: inactivated hepatitis A vaccine (HepA-I) and live attenuated hepatitis A vaccine (HepA-L). Because hepatitis A virus has only a single serotype, the vaccines made from any HAV in the world are suitable for the entire world. Breakthrough progress was made in China and abroad by the development of hepatitis A vaccine. In foreign countries, GlaxoSmithKline Co. Ltd. and Merck Co. Ltd. successfully developed HepA-I at the end of the 1980s and early 1990s. Chinese experts began to conduct related research, focusing on the development of HepA-L. China preferentially developed HepA-L for several reasons. First, humans develop immunity following vaccination through two mechanisms, humoral and cellular immunity. Inactivated vaccines only induce humoral immunity, but live attenuated vaccine can induce both humoral and cellular immunity, similar to natural infection. Second, live attenuated vaccine is relatively inexpensive to produce, which was suitable for China's economic condition. The key

was to select and culture a suitable virus strain for live attenuated vaccine. Serious problems could follow if the virulence of the virus is not well controlled. The selected vaccine strain should maintain a certain amount of activity, but not cause disease. Chinese researchers finally developed two vaccine strains – H2 and L-A-1 – through unremitting efforts of screening and cultivation of the virus strains to identify candidates suitable for production of live attenuated vaccines.

H2 was developed by Chinese Academy of Sciences academician, Jiangsen Mao, and his research team after 10 years of hard research. Mao went to Zhejiang Province to investigate viral diseases in 1978 and found that hepatitis A ranked first, from which farmers suffer seriously. That year saw a hepatitis A pandemic in rural with up to 41% of people suffering from jaundice hepatitis in Yuan Pu Cun on the outskirts of Hangzhou. He saw one family of five in which all had been infected with hepatitis A virus. Mao and his colleague Nianliang Chen conducted a house to house survey of hepatitis patients. His work of stool collection is unforgettable: every morning, the first thing he did was to go to rural farmers and hospitals to collect stool specimens from jaundiced hepatitis patients; each pack of stools was put in a plastic bag and brought back to the lab, with a hope of isolating HAV. He collected enough stool samples to fill two large refrigerators. Finally, in 1982, he isolated HAV from stool samples from a 12-year-old boy living in the outskirts of Hangzhou. The virus was initially cultured in a primary monkey kidney cell line (NMK) for 20 passages (15 passages at 35 °C followed by 5 passages at 32 °C), followed by 5 passages in human embryo lung diploid cell KMB-17 at 32 °C to attenuate the HAV. In 1988, a live attenuated hepatitis A vaccine strain was successfully developed. The strain is now owned by Zhejiang Pukang Biotechnology Co. Ltd.

The L-A-1 strain was developed by Professor Mengdong Hu and her team in Shanghai CDC from HAV that was isolated in December 1980 from a 2-year-old boy with hepatitis A in Harbin, Heilongjiang Province. Shanghai CDC and Changchun Institute of Biological Products cooperated in the development of the vaccine strain, which went through 7 passages in human diploid lung cells SL7, followed by 4 passages in human embryo lung diploid cell line 2BS at 32 °C and 7–14 passages at 35 °C. The final strain was obtained after a successful marmoset monkey infection and challenge trial. The strain is now owned by Changchun Biological Products Ltd. Co. (formerly Changchun Institute of Biological Products).

The two manufacturers carried out the necessary research on the strains, including stability testing and nucleotide sequencing after passages, entered the strains into the three-tier seed banks, and obtained national approval for their use.

The H2 strain of Zhejiang Academy of Medical Sciences (now Zhejiang Pukang Biotechnology Ltd. Co.) is stable after passages in the human diploid cell line KMB17 and is in large-scale production. A liquid presentation of live attenuated hepatitis A vaccine made from the H2 strain obtained licensure in 1992. Zhejiang Academy of Medical Sciences assessed the duration of immunity and showed that seropositive rates were 98.6% and 81.3% at 2 months and 15 years after vaccination with the H2 strain, HepA-L, and concluded that the vaccine has a good immunogenicity and persistence. However, there is a serious weakness for the liquid

attenuated hepatitis A vaccine, as it must be transported in low-temperature conditions (under −20 °C), and the shelf life is very short – only 5 months when stored at 2–8 °C. This disadvantage is not favorable for large-scale use and leads to waste and inefficiency. Therefore, researchers aimed to develop a freeze-dried formulation that would make the vaccine more useful in China and globally. Following unremitting efforts, in 2000, a freeze-dried live attenuated hepatitis A vaccine obtained licensure and market authorization. The new-generation vaccine has been demonstrated to be safe and immunogenic through more than 10 years of use. In 2005, the vaccine received an import drug license and market authorization from the India Drug Administration and was exported to India in January 2006.

The L-A-1 strain is owned by Changchun Institute of Biological Products, is stable during passage, and can be produced in large scale in 2BS cells. In 1996, L-A-1 HepA-L received a formal production license from the national regulatory authority. The vaccine had been demonstrated to be safe in preclinical trials in monkeys, which showed no elevation of alanine aminotransferase (ALT). A large-scale epidemiological study with more than 500,000 participants showed that most subjects responded well to vaccination, with no serious adverse events being observed. The study showed that the immune response persisted 4 years after immunization, with a protection rate of 96.8% [18]. After 3 years of follow-up, the vaccine protection rate was 96.85%, with a disease incidence of only 1.99/100,000 [19]. Because the shelf life for liquid vaccine is short and liquid vaccine is less stable, researchers successfully developed a freeze-dried formulation and obtained a national patent (Patent No.: ZL 98120633.6)/international patent (South Korea) (10-0702086) and the second award of National Technology Invention in 2001 (the first award being vacant). The freeze-dried vaccine (trade name: Haiweike) received new drug certificate and production approval in 2000. Vaccine stability tests showed that vaccine virus titers remain unchanged for 18 months at 2–8 °C and for 28 months at −20 °C. Decreases in virus titer were less than 0.5 log when stored at 2–8 °C for 22 months and −20 °C for 30 months. The serum antibody conversion rate reached 98% by 1 month after vaccination and 100% [20] by 2 months. The vaccine has now been in use for 15 years, with nearly 50 million doses used, and its safety and effectiveness has been demonstrated.

China's independently developed HepA-I vaccine has also showed good results. In 1984, NICPBP and Tangshan Yi An Biotechnology isolated virus from the stools of a patient during a hepatitis A outbreak in rural Hebei Province. The extracted virus-containing liquid was inoculated into 2BS cells for three passages. With continued passage in 2BS cells, the replication period of the virus was gradually shortened. After ten passages, the replication was peaking at 14 days and was showing high ELISA titers with increasing HAV yields. At that time, the vaccine candidate virus was established with a name of TZ84 in a virus seed bank. By the end of 1999, Beijing Sinovac Biotech Ltd. (former Tangshan Yi An Biotechnology company) successfully produced HepA-I vaccine from this strain, retaining independent intellectual property rights and receiving new drug certificate and production approval from the regulators. The vaccine was made using human diploid cell and was marketed with the brand name of Healife.

NICPBP and the Biological Research Branch of the Institute of Chinese Medical Science isolated hepatitis A virus from stools from a Nantong patient during the 1988 Shanghai hepatitis A epidemic. The virus was cultured for 6 passages in KMB-17 cell lines, followed by an additional 20 passages. During these passages, the viral replication cycle was shortened from 35 days to 12–16 days and was shown to yield high antigen and infectious particle titers. The Biological Research Branch of the Institute Chinese Medical Science established a virus seed bank for this vaccine candidate with the strain name of Lu 8. HepA-I vaccine produced using this strain was made in a human diploid cell line by the Biological Research Branch; the vaccine obtained new drug certificate and production approval in 2003 and is still marketed today in China under that trade name of Weisairuian.

5.2.3 Strict Quality Control of Hepatitis A Vaccine Production in China

Currently, there are four manufacturers producing freeze-dried HepA-L; all use similar production technology but use different vaccine strains, cell lines, freeze-dry processes, stabilizers, and vaccine specifications. Two manufacturers produce HepA-I, with vaccine strains and cell lines developed in China; aluminum hydroxide is used as an adjuvant, and the production technology is similar to the international vaccine production technology. Milestones of hepatitis A vaccine development and production in China are presented in Table 5.1.

Freeze-dried HepA-L uses an attenuated virus strain that is inoculated into human diploid cells for incubation, followed by harvesting, extraction, stabilizing, and lyophilizing to a final formulation of loose material that is white or cream colored. After reconstitution, the vaccine is a clear liquid. For HepA-I with an alum adjuvant, the virus is inoculated into human diploid cells, followed by culture, harvest, purification, inactivation, and adsorption onto aluminum. This product is a white liquid suspension and can be divided into layers but is easily mixed by shaking. The product, consisting mainly of inactivated HAV, may contain preservatives and other materials, including aluminum hydroxide, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, and sterile water for injection.

The manufacturing process for freeze-dried HepA-L is relatively simple compared to HepA-I. A key difference between the two manufacturing processes is after harvesting the virus. HepA-I must go through multiple purifications, a formaldehyde inactivation, and adjuvant adsorption. HepA-I, therefore, has greater purity compared with HepA-L. The freeze-dried HepA-L is processed with chloroform and then stabilized and lyophilized. HepA-I requires more preparation processing than does freeze-dried HepA-L.

Strict quality control in the production of hepatitis A vaccine is implemented in China:

(a) Virus seeds for production must be ensured to have had appropriate attenuation while retaining adequate immunogenicity.

Table 5.1 Hepatitis A vaccines in China

Vaccine type	Manufacturer	Brand name	Vaccine strain	Cell medium	Components	Specification	Antigen or virus content	Preservative
Freeze-dried live attenuated vaccine	Zhejiang Pukang Biotechnology Ltd. Co.	No	H2	KMB17	Live virus+ stabilizer	After reconstitution 0.5 ml per dose	Live hepatitis A virus no less than 6.50 lg CCID50/dose	No
	Institute of Medical Biology, Chinese Academy of Medical Science	Weisairuiji	H2	KMB17		After reconstitution 1.0 ml per dose		
	Changchun Institute of Biological Products	Haiweike	L-A-1	2BS		After reconstitution 1.0 ml per dose		
	Changchun changsheng Institute of Biological Products	Wanxin	L-A-1	2BS		After reconstitution 1.0 ml per dose		
Inactivated hepatitis A vaccine	Beijing Sinovac Biotech Ltd.	Healife	TZ84	2BS	HAVAG + aluminum adjuvant	Pediatric: 0.5 ml/dose	250 U	No
						Adult: 1.0 ml/dose	500 U	
	Institute of Medical Biology, Chinese Academy of Medical Science	Weisairuian	Lu 8	KMB17	HAVAG + aluminum adjuvant	Pediatric: 0.5 ml/dose	320 EU	2-Phenoxyethanol
						Adult: 1.0 ml/dose	640 EU	

Sources, histories, and biological characteristics of virus seeds must be clearly documented, and the relevant documents should be complete and approved by CFDA. Virus seeds are managed based on three batches, including the original seeds, the master seeds, and the working seeds. The genetic characteristics of viruses used in live attenuated vaccines must be consistent with the original seed and/or the master seed. Virus seeds used for production should be identified completely according to regulatory requirements, including identification testing, virus titer assays, serialization, *Mycoplasma* and exogenous virus test, and immunogenicity testing. Virus seeds are stored at −60 °C at specific sites in which other viruses cannot be stored. Because only primates can be used as testing animal models, the safety and immunogenicity of the master virus seeds have to be evaluated in monkeys.

In the *Chinese Pharmacopoeia* (2010 edition), passages for vaccine viruses are clearly defined, and the range of multiplicity of infection (MOI) in inoculation and culture is clearly defined to ensure stability and consistency during the production process. In the *Chinese Pharmacopoeia* (2015 edition), it is required that the background information of the whole genetic sequence for master virus seeds should be established and master seed of live attenuated vaccine should be sequenced completely.

(b) Establishment of cell line bank and conduction of comprehensive testing.

Cell lines are essential raw materials for virus vaccine production, and the quality of the cell line directly affects the quality and yields of the vaccine, including the safety profile [21]. The current hepatitis A vaccines on the market are all made from human diploid cell (HDC) which ensures cell line safety. In the production process, the sources of cell lines should be very clear and must correspond to the appropriate cell bank. The HDC age is estimated according to reproduction times, which double the cell population in one generation. The age of the cells used for vaccine production is limited to the first two thirds of the cell life expectancy. Quality control for the cell line is concerned primarily with the potential presence of exogenous factors and the biological characteristics of the cells [22]. HDCs must be identified comprehensively according to the current pharmacopoeia, including identification, serialization, *Mycoplasma* test, exogenous factor test, specific virus test, chromosome test, and cell tumorigenicity.

(c) Strict standards for final product testing.

In the *Chinese Pharmacopoeia* 2010 edition, the HepA-L final product must be tested for the following items: identification, appearance, water, chloroform residues, virus titration, thermal stability test, bovine serum albumin residues, antibiotic residues, sterility, abnormal toxicity test, and bacterial endotoxin test. In addition to the above items, the *Chinese Pharmacopoeia* 2015 edition requires pH and concentration of osmotic pressure testing.

The *Chinese Pharmacopoeia* 2010 version required that final product of HepA-I must be tested for the following items: identification, appearance, volume, pH, aluminum contents, free formaldehyde contents, chloroform residues, 2-phenoxyethanol contents and relative potency test in vitro, antibiotic residues, sterility, bacterial endotoxin, and abnormal toxicity. In addition to the above items, the 2015 edition of *Chinese Pharmacopoeia* included concentration of osmotic pressure testing.

Fig. 5.3 Live attenuated hepatitis A vaccine

5.2.4 Prospects

China independently developed HepA-L (Fig. 5.3) and HepA-I vaccines, which have been marketed for many years and have made great contributions to the control of hepatitis A endemics. With rapid progress in biotechnology, many researchers are focused on the development of new types of hepatitis A vaccine – for example, a combination vaccine or a genetically engineered vaccine. At least one of these innovative vaccines has been marketed – a hepatitis A and B combination vaccine.

5.3 Inactivated Enterovirus Type 71 Vaccine

5.3.1 Enterovirus Type 71 (EV71) and Hand, Foot, and Mouth Disease

In March 2008, Fuyang People's Hospital, Anhui Province, China, admitted and treated numerous pediatric patients with hand, foot, and mouth disease (HFMD); many were critically sick, and some died. The epidemic caused widespread concern by the public and the media [23].

HFMD is a common infectious disease caused by several human enteroviruses. Most patients present with mild symptoms – fever and rash and herpes-like lesions on the hands, feet, or mouth [24]. A small number of patients develop aseptic meningitis, encephalitis, acute flaccid paralysis, neurogenic pulmonary edema, and myocarditis, with a rapid progression that can lead to death [25–27]. Viruses causing HFMDs include EV71, coxsackievirus group A type 16 (CV-A16), and ECHO virus. Of these, EV71 is, arguably, the most important HFMD pathogen [28].

From 2008 to 2015, a total of about 13.8 million HFMD cases were reported in China, with an average annual incidence of 147/100,000. Among these, approximately 130,000 cases were severe and more than 3300 patients died. HFMD represents a serious threat to the health of children in China [28]. According to Yu Hongjie's study of the epidemiology of HFMD in China from 2008 to 2012, published in January 2014 in *The Lancet*, EV71 accounted for 41% of all laboratory-confirmed cases, 81% of severe cases, and 93% of deaths [29].

During the past 20 years, HFMD was widely prevalent in the Asia Pacific region, including in Malaysia, Japan, Singapore, Vietnam, China (Mainland, Hong Kong and Taiwan), South Korea, and Cambodia [30–33]. The largest outbreak of HFMD in recorded history happened in 2000 in Singapore, in which 3790 cases were reported, with 73% being caused by EV71 [33]. In 2012, at least 54 Cambodian children died of EV71 infection [34]. EV71-related HFMD has long been prevalent in many countries and regions in Asia and is considered an important public health problem.

5.3.2 EV71 Vaccine: Research and Development

To reduce the incidence and mortality of HFMD, the World Health Organization and disease control departments in several countries have adopted measures such as surveillance, disinfection, isolation, and education to identify and control HFMD epidemics. EV71 virus spreads through direct contact; virtually all children are susceptible, and after infection, most are asymptomatic. Given these characteristics, it is difficult to prevent and control HFMD with hygiene, alone [28].

To prevent and control HFMD epidemic caused by EV71 infection, many countries or regions have begun to develop EV71 vaccines, including inactivated vaccines, live attenuated vaccines, subunit vaccines, DNA vaccines, peptide vaccines, and recombinant virus-like particle (VLP) vaccines [28].

Research on whole-virus inactivated vaccine has been the fastest, as there are five manufacturers or institutes whose EV71 vaccine has entered into clinical trials. A vaccine developed in Singapore completed its Phase I trial in Taiwan and then entered a Phase II trial. The Institute of Medical Biology, China Academy of Medical Sciences (Kunming Institute), Beijing Sinovac Biotech Co. Ltd. (Beijing Sinovac), Biological Technology Research Co. Ltd., and Wuhan Institute of Biological Products (CNBG) completed Phase III trials in 2013. These three EV71 vaccines by Chinese manufacturers received new drug certificate in 2015 and 2016 from CFDA [28].

The Chinese government gave strong support to development and clinical research on EV71 vaccine, through the National Major New Drug Development in the "11th Five-Year Plan," the "12th Five-Year Plan," and other projects.

EV71 vaccine from Beijing Sinovac has obtained several patents in China, including "HFMD vaccine and its preparation and application" and "EV71 virus neutralization epitope assay kit and preparation method." The next section will introduce research and development of EV71 vaccine by Beijing Sinovac.

5.3.2.1 Selection of Virus Seed

There is only one serotype of EV71. From 1999 to 2008, all EV71 strains isolated from mainland China were C4 subtype, providing a basis for selection of candidate virus genotypes for EV71 vaccine [35–38]. Through several years of cooperation with China CDC, Beijing Sinovac isolated 20 strains of EV71 that varied by patient symptoms, genotype, and region of isolation. To identify EV71 strains for vaccine development, candidate viruses were screened for the ability to generate high titers after culture, ability to induce immunity with cross-protection, and protection against death in animals. Genetic stability of selected strains was demonstrated by comparison of whole genome sequences with the original virus.

5.3.2.2 Evaluation of Protection in Animals

The traditional mouse model was not suitable for evaluation of EV71 vaccine. Beijing Sinovac created a new animal model through collaboration with the Laboratory of Infectious Diseases and Immunology Research, Australia University of Sydney. Two doses of vaccine were administered parentally to female rats. After conception, the suckling mouse obtains maternal EV71 antibody. Challenged by a lethal dose of EV71 (mouse-adapted strain) on suckling rats, morbidity and mortality were able to be monitored to evaluate protection by EV71 antibody induced by the candidate vaccine.

To evaluate immunogenicity and effectiveness, Beijing Sinovac manufactured EV71 vaccine in different dose amounts and commissioned Peter McMinn, professor of Medicine of Infectious Diseases and Immunology, Medical School University of Sydney, to conduct challenge trials in mice. The results showed that after two doses of vaccine given to maternal mice, the morbidity and mortality of suckling mice were significantly lower than a control group, suggesting that EV71 vaccine can produce effective neutralizing antibodies to protect a sensitive animal from infection. EV71 vaccine was also demonstrated to provide cross-protection against a challenge by other gene subtype of EV71 [39].

5.3.2.3 Evaluation of Protection Efficacy in Large-Scale Clinical Trial

Beijing Sinovac began to develop EV71 vaccine in 2008 and completed a preclinical study in December 2009. After the clinical trial approval was obtained in December 2010, their EV71 vaccine became one of the first EV71 vaccines to enter a clinical trial in China. The clinical trial showed that EV71 inactivated vaccine was safe and effective, using a schedule of two doses – day 0 and day 28. The vaccine is indicated for prevention of HFMD, herpangina, encephalitis, and other diseases caused by EV71 infection. EV71 vaccination can effectively reduce hospitalization due to HFMD and severe HFMD. On December 30, 2015, Beijing Sinovac's EV71 vaccine (brand name, Evlife) was approved by CFDA.

The results of Phase I–III trials and three consecutive clinical trials showed that EV71 inactivated vaccine had good safety and immunogenicity. The overall adverse reaction rate of the experimental vaccine was 52.52%, but there was no significant difference in the incidence of adverse reactions between the vaccine group and the control group. In Phase III clinical trials, the subjects were followed for 14 months,

with no severe HFMD cases in the experimental vaccine group or EV71-associated severe disease or pathological immune response. Following completion of the schedule, efficacy was 94.6% against HFMD of any severity within 1-year period (95% CI, 86.6–97.8) and 100% against severe HFMD. In the 2nd year, EV71 vaccine showed an efficacy of 95.1% (95% CI, 63.6–99.3) against HFMD by EV71 infection. The immunogenicity against different genotypes indicated that the candidate vaccine induced cross-protection against different genotypes and subtypes [40]. Relevant results were published in international journals, including the *New England Journal of Medicine*, *Vaccine*, and *The Journal of Infectious Diseases* [41–43].

In addition, an immunological surrogate endpoint was assessed in the Phase III trial. The threshold of neutralizing antibody correlated with protection against diseases was 1:16 [43].

Three consecutive clinical trials showed that 95% CI of GMT log difference between three batches of vaccines was in the expected range (−0.176 to 0.176), suggesting that three consecutive batches of vaccine were consistent. Phase III clinical trial of four batches of 400 U vaccine showed that, 56 days after immunization, the coefficient of variation of GMT was 7.97%. These results showed that the vaccine was stable, with excellent consistency between the batches and production process [44].

5.3.2.4 Vaccine Evaluation Criteria and Standards

EV71 vaccine is a new vaccine developed in China, for which there is no global reference for development processes, no global standards, and no reference vaccine. The Center for Drug Evaluation (CDE), CFDA, and NIFDC developed standards for EV71 vaccine manufacturing and testing. In order to ensure safety, CFDA strengthened the criteria for purity of inactivated EV71 vaccine, requiring that purity should be no less than 95% by a HPLC detection method, a similar standard used for recombinant genetically engineered vaccines. Residual DNA and Vero cell host proteins are controlled according to the most stringent international standards.

In 2010, NIFDC worked with the manufacturer to jointly develop a standard vaccine for potency testing and to establish a standard assay for EV71 vaccine neutralizing antibody and antigen content (2010 National Biological Standard 0023; 0024). In addition, in order to meet the anticipated demand for EV71 vaccine in other countries or regions, NIFDC worked with the British National Institute for Drug Control to develop a WHO reference assay for EV71 neutralizing antibody. This was the first time that China undertook the development of a vaccine reference for WHO [45].

5.3.3 Prospect of EV71 Vaccine

EV71 vaccine is a Class 1 new biological product developed independently by China (Fig. 5.4). Registration of EV71 vaccine suggests that China's biological product industry is becoming advanced in the world. The EV71 vaccine and antibody reference developed by China provide a "reference" for the research and development of EV71 vaccine globally.

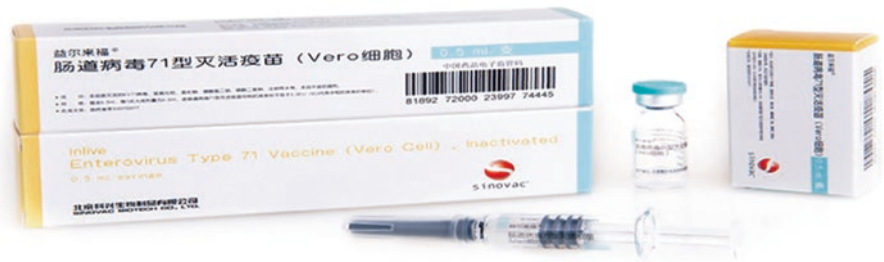

Fig. 5.4 Enterovirus type 71 vaccine

The successful development of EV71 vaccine will play an important role in the control of HFMD outbreak and epidemics related caused by EV71 globally. Peter C. McMinn, professor of the Institute of Infectious Diseases and Immunology, Medical School, University of Sydney, wrote a commentary in the *New England Journal of Medicine*: "… if these vaccines prove to be effective in preventing EV71-associated neurologic disease, an important tool for controlling, or even eradicating, EV71 infection in regions where it is endemic may have been developed. If its promise is realized, a priceless gift will have been given to the children of the Asia–Pacific region and to the rest of the world" [46].

5.4 Hepatitis E Vaccine

On March 5, 2015, a study was published in the *New England Journal of Medicine*, showing that the world's first hepatitis E vaccine (Fig. 5.5) developed by Professor Ningshao Xia from Life Sciences, Xiamen College, provides protection for at least 4.5 years [47].

5.4.1 Background of Development of Hepatitis E Vaccine

Hepatitis E, caused by a RNA virus, is an acute viral hepatitis that has sudden onset following an incubation period of 4–9 weeks. The clinical symptoms are similar to hepatitis A but present with greater severity [48], usually with manifestations of jaundice, fever, fatigue, loss of appetite, extreme fatigue, unconsciousness, liver failure, and sometimes death. In childbearing age women, patients with chronic liver disease, the elderly, and infants, the manifestations tend to be more serious; for example, hepatitis E infection in pregnant women may lead to miscarriage, premature birth, stillbirth, neonatal hepatitis by vertical transmission, or death. Infection will cause severe hepatitis in one third of pregnant women, with a fatality rate of up to 20% [49, 50]. Approximately 44–83% of patients with chronic liver disease are at risk of superinfection with hepatitis E, with fatality rate as high as 75% [51, 52]. Hepatitis E virus (HEV) is spread primarily through the fecal-oral route, with two

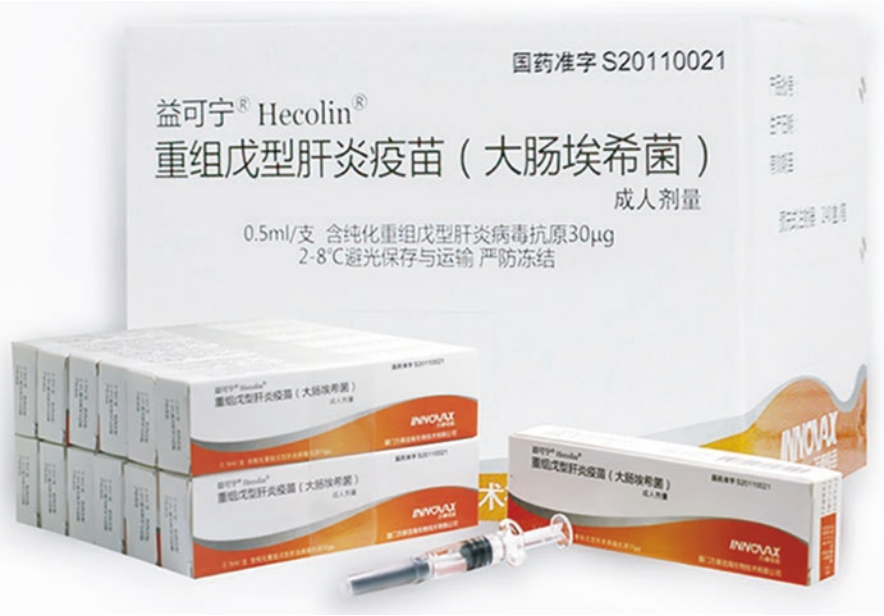

Fig. 5.5 Hepatitis E vaccine

common models [53]: spread by water polluted with feces causing large-scale outbreaks occurring mainly in underdeveloped countries and regions or spread by poor personal or public hygiene conditions, prevalent throughout the world.

Since the 1980s, hepatitis E has been prevalent in India and other developing countries in Asia, Africa, and Latin America. There was a big outbreak in Kitgum in northern Uganda in Africa in October 2007 [54] that had a cumulative number of cases of 10,196 and caused the death of 160 people. In the most serious involved areas of Madi Opei and Paloga, the incidence rates were 30.9% and 19.2%, respectively. The most affected populations are pregnant women and children aged 0 to 2-year-olds, having mortality rates of 8.2% and 8.7%, respectively.

Since 1982, hepatitis E has been notifiable in China, and thus far, there have been several outbreaks reported. The largest outbreak occurred from September 1986 to April 1988 in three prefectures, Hotan, Kashi, and Kezilesu in southern Xinjiang, covering 23 counties and lasting more than 20 months [55]. The epidemic had two peaks, a total of 119,280 cases, and caused serious harm and widespread concern in the community. In almost all provinces and autonomous regions of China, there are sporadic hepatitis E cases reported. Hepatitis E ranks first in acute sporadic viral hepatitis. A seroepidemiological survey in 63 disease surveillance sites in 13 provinces and autonomous regions in China showed that out of 31,120 people aged from 1 to 59 years old surveyed, the anti-HEV antibody prevalence was 17.2% [56], suggesting that HEV infection in Chinese is prevalent. The incidence of hepatitis E has increased year by year, from 9655 in 2003 to 29,202 in 2011 – an increase of 202% [57].

Hepatitis E is not only prevalent in developing countries with poor hygiene conditions; in the most recent 10 years, sporadic hepatitis E cases have been reported in developed countries in Europe and in the United States, Japan, and Australia, with most cases being indigenous. In immunocompromised groups such as those with HIV infection, organ transplant recipients, and others, chronic hepatitis E infection is common.

5.4.2 Hepatitis E Vaccine: Development

As early as the 1990s, GlaxoSmithKline, the University of Oxford, and other institutions invested in research and development of hepatitis E vaccine. However the first approved was a hepatitis E vaccine (Yikening), developed by Professor Xia in Xiamen University.

Starting in 1998, Professor Xia led his team to develop hepatitis E vaccine. In December 2004, Yikening received clinical trial approval, and in 2005 a clinical trial was started. By October 2012, Yikening was officially approved [58].

Because HEV is difficult to culture in large scale in vitro, it is difficult to use the traditional inactivated approach and live attenuated approach to create a vaccine. A subunit vaccine or DNA vaccine made by a genetic engineering approach could be more appropriate. Xia's team adopted a genetic engineering approach to develop a recombinant subunit vaccine. Through a series of experiments, they found that VLPs can be expressed by *Escherichia coli* bacteria. This VLP antigen is immunogenic and able to induce high titers of neutralizing antibodies, potentially serving as a candidate antigen for a hepatitis E vaccine. On this basis, Xiamen University and Xiamen Innovax Biotech Corp (Xiamen Wantai) worked together to develop a vaccine and established an HEV vaccine production process using HEV VLPs in a series of preclinical and clinical research studies. They broke the long-standing concept that an *Escherichia coli* expression system is not appropriate for complex antigen production and thus laid the foundation for large-scale production of hepatitis E vaccine. Their new technology was later applied by Xiamen University and Xiamen Wantai to develop a human papillomavirus vaccine.

During the development of hepatitis E vaccine, governments at all levels provided support through the 863 plan science and technology funding, such as the National Tech Program, to ensure that the vaccine could successfully overcome development barriers. The traditional view that "a prokaryotic system does not express virus like particles" was debunked by the success of this product. Previous genetic engineering approach used yeast, insect, or mammalian cells, all of which are eukaryotic.

Between 2005 and 2007, Phase I and II clinical trials were completed [59], and they showed that hepatitis E vaccine had good safety and immunogenicity profiles: 505 volunteers received 1279 doses of HEV, with no serious adverse reactions observed. Fever (4.2%) and fatigue (2.7%) were the most common systemic reactions, and itching (4.1%) and redness (2.3%) were the most common local reactions.

From 2007 to 2009, Xiamen University, Xiamen Wantai, and Jiangsu CDC jointly completed a Phase III clinical trial that had 120,000 subjects, confirming the safety and efficacy of the vaccine [60]. During the clinical trial, adverse reactions were mild, and no vaccine-related serious adverse events were observed, again showing that the vaccine had a good safety profile. One month after completing the vaccination schedule, the seroconversion rate (IgG) was 98.69% (95% CI, 98.35–98.97%), with antibody titers increasing 139.27-fold (95% CI, 134.01–144.74), confirming that the vaccine was highly immunogenic. One year after vaccination, no hepatitis E cases were observed in the hepatitis E vaccine group, and 15 hepatitis E cases were observed in the hepatitis B vaccine group (control group), yielding a protection rate of 100% (95% CI, 72.1–100.0%). In 2010, Xia published the Phase III trial in *The Lancet* with Dr. Holmberg, of the US CDC Division of Viral Hepatitis, commenting that "the clinical trial convincingly confirmed the safety and efficacy of the hepatitis E vaccine, and is a major breakthrough in the prevention of hepatitis E in the world."

To further determine the duration of protection, Xia's team conducted a 4.5-year follow-up study and found that among 60 subjects infected with HEV, 7 were from the study group and 53 were from the control group, for a protection rate of 93.3% (95% CI, 78.6–97.9%) [47]. In the study group, HEV IgG antibody was positive in nearly 90% subjects 4.5 years after immunization, while only 9% of the control group were positive. These data confirmed that the hepatitis E vaccine can induce excellent and persistent immunity, significantly reducing the risk of hepatitis E. The results were published in the *New England Journal of Medicine*. Dr. Eyasu Teshale from US CDC said: "A hepatitis E vaccine could become a powerful new tool in the prevention and control of HEV transmission and disease" and suggested "now is the time to answer these remaining questions and establish the public health applications of a hepatitis E vaccine."

5.4.3 Significance of Hepatitis E Vaccine

After 14 years of efforts and more than 500 million yuan invested, hepatitis E vaccine, "Yikening," was officially approved in October 2012. This is the first approved hepatitis E vaccine in the world and so far the only hepatitis E vaccine with regulatory approval for market authorization. Hepatitis E vaccine is a major, innovative scientific achievement in China – a breakthrough in genetically engineered vaccines. The approval of hepatitis E vaccine changed the unfortunate situation that no vaccines were available against hepatitis E. Now hepatitis E is a vaccine-preventable disease, paving the way to prevention and control of hepatitis E.

The success of Yikening is a major breakthrough in the field of biomedical innovation. Through the research on Yikening, Xiamen University and the Xiamen Innovax Biotech established a vaccine development platform that will make an important contribution to increase the availability of vaccines in the world, especially in developing countries. We believe that in the future there will be more Chinese new and innovative drugs approved to the benefit of human beings.

5.5 Inactivated Polio Vaccine from Sabin Strains

On January 14, 2015, the CFDA website announced the "approval of the first Sabin inactivated poliovirus vaccine (IPV) in the world," announcing that after 30 years of research and development, the innovative Sabin-IPV, which is made from the attenuated Sabin strains of poliovirus, was formally approved. The successful development of this innovative vaccine will play a vital role in ensuring the eradication of polio in China. As a product with major significance, it not only fills a gap in the field of IPV production in China but also improves eradication of polio in China and in the world – especially in developing countries.

Sabin-IPV, a national Class 1 new drug, was independently developed by the Institute of Medicine and Biology, China Academy of Medical Sciences, retaining complete intellectual property rights. There was no Sabin-IPV in China prior to this development, and there was no stand-alone Sabin-IPV licensed anywhere in the world. The development of this vaccine demonstrated advanced vaccine development capacity in China. Sabin-IPV is one of the best choices for use in the final stages of polio eradication.

5.5.1 The Eradication of Diseases

Polio is a disease that is as old as smallpox that has been observed since the beginning of recorded human history. The earliest known image of polio is possibly the portray in an Egyptian Stele between 1300 and 1500 BC, depicting a young priest with one atrophic leg, an image consistent with polio.

Polio is a highly infectious disease caused by poliovirus serotypes I, II, or III, mainly affecting the gray and white matter in the anterior horns of the spinal cord. Poliovirus infections can cause permanent damage to the gray matter, with subsequent weakness of the muscles innervated by affected nerves and flaccid paralysis of the limbs. Polioviruses historically typically infect children under the age of 5 years; it has a clinic presentation of fever, followed by stiff neck and vomiting. About 1 out of every 200 people infected with polio become paralyzed, with respiratory muscle paralysis and death in severe cases. Because the disease is common in infants and young children, it is also called infantile paralysis. The disease is preventable but has no effective treatment. Once limb paralysis occurs, lifelong disability and even death follow. Humans are the only natural host of the poliovirus. Poliovirus spreads primarily by the fecal-oral route but also by nasopharyngeal droplets.

In 1916, an outbreak of polio occurred in New York resulting in more than 9000 cases and 2343 deaths. During the same year, a total of 27,000 cases and 6000 deaths occurred in the United States, most cases being among children. Since then, outbreaks became more frequent in the twentieth century. The most serious epidemic in the United States was in 1952. Polio had also been endemic in China, and there were polio case records as far back as 1882. By 1938 there were 14 provinces and municipalities that reported sporadic cases. In 1955, China's MoH included

polio into the list of notifiable infectious diseases, and as surveillance improved it became clear that outbreaks were increasing and that endemic areas were expanding. In 1955, the first large-scale polio outbreaks in China were reported in Nantong, Jiangsu Province, and Qingdao, with incidence rates of 32.1/100,000 and 50/100,000, respectively. The annual number of cases ranged from 20,000 to 43,000, with a peak in 1964 when 43,156 cases were reported, for an incidence of 6.21/100,000. The majority of children who got polio suffered lifelong disability. People lived in extreme fear – the common disease known as "polio" made children suffer seriously [61].

5.5.2 Emergence of Vaccines

Because of fear of polio and the significant threat of outbreaks, many scientists work on the prevention and treatment of polio. Microbiologist Jonas Salk isolated the virus in 1952 in the University of Pittsburgh and developed the first injectable vaccine containing three serotypes (IPV), which was approved in the United States in 1955. After the vaccine was in use, the number of cases in the United States declined from 35,000 in 1953 to 5300 in 1957. For several years Dr. Salk's IPV was the standard polio prevention measure; indeed, in Finland, Iceland, Holland, and Sweden, IPV vaccination successfully blocked the spread of wild poliovirus. At about the same time, Albert Sabin, a virologist at the University of Cincinnati, worked to develop a polio vaccine and ultimately successfully developed oral polio live attenuated vaccine (OPV). Because OPV was simpler to administer, less expensive, and more convenient to transport compared with IPV, most countries replaced IPV with trivalent OPV (Sabin strain) in 1963 as the main polio preventive measure [62].

The Chinese government has long attached great importance to the prevention and control of polio. In 1959, according to an agreement on science and technology between China and the former Soviet Union, China MoH sent Fangzhou Gu and Dexiang Dong from the China Academy of Medical Sciences, Zhongquan Wen from Beijing Institute of Biological Products, and Jingwu Jiang from Chengdu Institute of Biological Products to the Soviet Union to study polio vaccine manufacturing technology (Fig. 5.6). The Institute of Medical Biology of the Chinese Academy of Medical Sciences was approved to produce polio vaccine in Kunming and to conduct research on enteroviruses. The project team recommended the use of OPV based on the pros and cons of OPV and IPV, China's large population, and China's developing economic condition. This pioneering work represented key steps for the later control and eradication of polio in China. In March 1960, the first lot of 5 million doses of monovalent OPV for type I, type II, and type III was produced in pilot batches, and the vaccine was demonstrated to be effective after evaluation in about 4 million children in a clinical trial. In 1963, to promote the use of poliovirus vaccine in rural areas, the Institute and Shanghai Xinyi Pharmaceuticals jointly developed OPV in pill formulations. In 1965, China began to gradually promote the use of OPV in China, leading to decreases in the number of cases by 60%

Fig. 5.6 Fangzhou Gu and other comrades learning in the Soviet Union

in the 1970s compared with the 1960s. In the 1980s, China strengthened EPI, further reducing the incidence of poliomyelitis. Since October 1994, no cases caused by indigenous wild polio have been reported in China.

On very rare occasion, OPV can cause vaccine-associated paralytic poliomyelitis (VAPP) and can mutate into vaccine-derived polio viruses (VDPVs) that can circulate in nature and cause polio. In addition, although also rare, patients with primary immune deficiencies can become long-term excretors of VDPVs leading to prolonged spread of polioviruses. The last case of wild type II polio was seen in 1999, and the disease caused by the type II OPV strain has exceeded the amount of disease caused by wild poliovirus. Because IPV cannot cause VAPP and VDPVs, IPV has become an essential weapon to finally eradicate polio.

5.5.3 Development of IPV in China

Research and development of IPV from Sabin strains began in the last century in China. Sabin-IPV development received support from the National 863 Plan, major new drug development major science and technology projects, the Bill and Melinda Gates Foundation (BMGF), and the Special Major Science and Technology Funding of Yunnan Province Biological Vaccine. This support effectively promoted Sabin-IPV development. Sabin-IPV is represented in two national patents, "culture method for attenuated strain IPV" (Patent No.: ZL2004 10040721.1) and "post-processing method of IPV production from attenuated strains" (Patent No.: ZL2004 10040720.7), retaining independent intellectual property rights.

While studying in the Commonwealth Serum Laboratory (CSL) of Australia in 1983–1984, Shude Jiang, who was among the first generation of biology scientists

in the Institute and who had been engaged in OPV research for more than 20 years, began the development of IPV using Sabin strains. In 1987–1990, Jiang guided the graduate student Liang Nong to conduct a study called "comparison of IPV from attenuated and virulent viruses," which showed that the seroconversion rates in rabbits were both 100% whether vaccinated with IPV from Sabin strain or IPV made from wild (Salk) strains. In 1988–1989, Jiang explored the production processes for Sabin-IPV that used a micro-carrier technology and Vero cell lines from the National Institute of Health and Environmental Protection in Holland.

In 1990–1999, the Institute of Medical Biology obtained a loan from the World Bank to build a modern OPV production line. The OPV production line was developed in compliance with European good manufacturing practice (GMP) standards. As the responsible technical lead, Jiang used the production line to conduct research on Sabin-IPV. In 2000, the research project received its first support from a special science and technology project funding source, "Yunnan province new drug research special fund," which provided a valuable 1.3 million yuan to open the door for Sabin-IPV development. By 2005, the production technology of Sabin-IPV had matured, and a Sabin-IPV that is stable, safe, and consistent was developed. That year, the Institute submitted an application to the CFDA for a clinical trial.

In May 2007, CFDA approved the clinical trial application. A total of 1830 subjects participated in the Phase I, II, and III trials, which showed that the vaccine had good safety and immunogenicity, inducing immunity against infection by all serotypes of poliovirus. The Phase III clinical trial showed that positive antibody rates for type I were 100% and 95.87% after vaccination with Sabin-IPV and Salk-IPV, respectively; for type II were 96.09% and 92.46%; and for type III were 99.30% and 98.40%, respectively.

During the development of Sabin-IPV, the Institute received great support from international experts. After completing Phase II clinical trials, in order to select an appropriate vaccine dose, neutralization tests were conducted to determine the level of neutralizing antibodies against Sabin strains (attenuated strain), pre- and postvaccination, by NIFDC. Because polio type II has been eradicated, it is impossible to use wild virus to carry out neutralization testing. CFDA commented in the clinical trial application approval letter (2011 L01484) for the Phase III clinical trial: "the applicant should be aware of the importance of cross neutralization (against wild virus) to the evaluation of vaccine effectiveness." After receiving that feedback, the Institute went to Geneva to participate in a polio virus research meeting, and during the meeting the Institute communicated with coordinator Dr. Roland Sutter, an expert from WHO on polio vaccine development and immunization strategies. With Dr. Sutter's coordination, testing was completed by the US CDC with support from Dr. Mark Pallansch. The results showed that serum antibody induced by Sabin-IPV had a good protective effect on different strains, could prevent infections by different polio viruses, and had immunogenicity no less than the imported Salk-IPV. This international cooperation effectively promoted the development of Sabin-IPV, and the results were recognized by scientists worldwide.

NIFDC further cooperated with WHO and the British NIBSC to establish a standard Sabin-IPV vaccine D antigen assay method. The Institute is actively involved in the process. Each year, the WHO invites experts from the Institute to Geneva to participate in an annual meeting about global polio vaccine development and uses and to present at the meeting.

On January 14, 2015, after 30 years of extremely hard and challenging work, Sabin-IPV obtained new drug certificate and was the world's first stand-alone Sabin-IPV produced on an industrial production scale. On June 30, 2015, Sabin-IPV was launched, and on July 1, the world's first dose of China's Sabin-IPV was administered to a 2-month-old baby as the first polio dose in Daguan Community Health Service Center in Kunming City in Yunnan Province.

5.5.4 Significance of Sabin-IPV

A small volume of 0.5 ml vaccine represents the heroic efforts of 30 years of hard work by several generations of biologists, all with a common goal of "making polio into history" (Fig. 5.7). Sabin-IPV changed the concept of "made in China" to "developed in China" in the vaccine field and represents a Chinese dream for the Chinese vaccine industry. Sabin-IPV fills a gap in the field of attenuated IPV in China, with the research achieving an advanced global level. Sabin-IPV will have a huge impact on polio eradication in China and the world, especially in developing countries. The successful licensure of Sabin-IPV demonstrated that China, with one-fourth of the world's population, is able to rely on its own technical capacity to eradicate polio and to contribute to the final eradication of polio worldwide.

Sabin-IPV is safe to produce, and its price has been lessened by using a large-scale micro-carrier fermentation technology that lowers production costs and is especially suitable for developing countries. The successful licensure of Sabin-IPV will also bring great economic benefits to developing countries.

Fig. 5.7 Sabin-inactivated poliovirus vaccine

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