EARLY INACTIVATED WHOLE-CELL BACTERIAL VACCINES

Three parenteral inactivated whole-cell bacterial vaccines to protect humans against cholera, typhoid fever, and plague, originally developed at the end of the 19th century, were used with little modification for three quarters of a century thereafter. In each instance, the isolation of the causative agent in pure culture was followed shortly thereafter by the development of vaccine candidates.

Cholera Vaccines

In 1896, Wilhelm Kolle (29) recommended the use of agar-grown, heat-inactivated whole *V. cholerae* organisms as a parenteral immunizing agent. This nonliving vaccine was markedly simpler to prepare and to standardize than Haffkine's live parenteral vaccine. By 1911, Haffkine was also utilizing inactivated vibrios as a vaccine with 0.5% phenol as a preservative (30). Kolle-type vaccines were first used on a large scale during the 1902 cholera epidemic in Japan (31). In the 1960s and early 1970s, randomized, controlled field trials carried out in Bangladesh (32–34), the Philippines (35), Indonesia (36), and India (37) documented that killed whole-cell cholera vaccines can confer significant short-term protection in older children and adults.

Typhoid Vaccines

In 1896, Richard Pfeiffer (38) and Almroth Wright (39) independently reported that a vaccine against typhoid fever could be prepared by inactivating cultures of typhoid bacilli with heat and preserving them in phenol (40). By 1915, killed whole-cell parenteral typhoid vaccines had become widely used by the military in Europe and the United States. Systematic use of the vaccine in the U.S. army, in 1912, was followed by a diminution of approximately 90% in the incidence of typhoid fever (41). Thus, epidemiological data suggested that the vaccine was protective (42), although rigorous controlled field trials of efficacy of the parenteral heat-phenol typhoid vaccine were not carried out until the 1950s (43). Controlled field trials sponsored by the World Health Organization (WHO) in the 1950s and 1960s demonstrated that the heat-phenolized vaccine conferred about 50% to 75% protection against typhoid fever (42,44–46).

LIVE VIRUS VACCINES

Following their success with the development of a vaccine against anthrax, Pasteur and coworkers turned their attention toward the problem of rabies (47,48). Although unable to cultivate the virus as they could a bacterium, they nevertheless established that the infectious agent resided within the spinal cord and brain of infected animals. Pasteur and his team inoculated nerve tissue from a rabid animal submeningeally into rabbits and removed the spinal cord after the rabbits died; they were able to pass the infection from rabbit to rabbit in this manner. Roux discovered that if the spinal cords were desiccated for 15 days, they lost their ability to induce rabies. Spinal cords dried for fewer than two weeks were less attenuated, whereas minimally dried spinal cord clearly contained virulent virus. Pasteur's group prepared a vaccine that consisted of dried spinal cord suspended in saline. Their immunization schedule involved daily inoculations for 14 days, commencing with material from spinal cord that had been dried for 14 days and progressing on the successive days to the use of cord dried for less and less time. This was continued until, after two weeks, the final inoculation was with minimally dried cord, which contained virulent virus. Needless to say, this vaccination procedure was quite controversial, even among the members of Pasteur's group. What is extraordinary is how Pasteur and colleagues identified the tissues wherein the rabies virus resides and how they managed to achieve attenuation yet retain immunogenicity.

In the late 1920s and early 1930s (49,50), Max Theiler, a South African physician working at the Rockefeller Foundation, developed an attenuated strain of yellow fever virus by repeated passage of the wild-type Asibi strain in minced chick embryo tissue from which the head and spinal cord had been selectively removed to minimize the amount of nerve tissue. Somewhere between the 89th and 114th passages, the virus lost its neurotoxicity. Theiler adapted this attenuated virus, strain 17D, to grow in chick embryos. In the 1930s and 1940s, this attenuated virus vaccine set a standard for safety, immunogenicity, and efficacy that continues to draw admiration today. Strain 17D remains one of the best all-around vaccines ever developed. It has been safely given to hundreds of millions of adults and children and provides long-term protection. This is an amazing feat, but particularly so when one considers that the vaccine was developed in an era before modem tissue culture techniques and concepts of viral genetics had evolved. Max Theiler received the Nobel Prize in 1951.

"SUBUNIT" AND "EXTRACT" VACCINES

The early diphtheria and tetanus toxoids should be regarded as the pioneer subunit vaccines. In each instance their development followed a similar course. Discovery of the etiological agents of diphtheria by Edwin Klebs (51) and Frederick Loeffler (52) and of tetanus by Shibasaburo Kitasato (53) was followed by the demonstration that *Corynebacterium diphtheriae* and *Clostridium tetani* elaborate potent exotoxins. Inoculation of broth cultures of these bacteria through porcelain filters resulted in sterile filtrates that were toxic for animals, leading to syndromes characteristic of human disease (54). The production in horses of specific antitoxins for passive protection and treatment came next (Fig. 3), and this was followed by the

Figure 3 An immunized horse being bled for serum as a source of diphtheria antitoxin at the CSL, Parkville, Victoria, Australia, 1920. *Abbreviation*: CSL, Commonwealth Serum Laboratories. *Source*: Photo courtesy of CSL.