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Review

Live attenuated vaccines: Historical successes and current challenges

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ABSTRACT

Live attenuated vaccines against human viral diseases have been amongst the most successful cost effective interventions in medical history. Smallpox was declared eradicated in 1980; poliomyelitis is nearing global eradication and measles has been controlled in most parts of the world. Vaccines function well for acute diseases such as these but chronic infections such as HIV are more challenging for reasons of both likely safety and probable efficacy. The derivation of the vaccines used has in general not been purely rational except in the sense that it has involved careful clinical trials of candidates and subsequent careful follow up in clinical use; the identification of the candidates is reviewed.

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Introduction

This review is restricted to vaccines against human diseases caused by viruses although live vaccines have been successfully used against a range of human and veterinary viral and bacterial infections. For example the last case of Rinderpest occurred in Kenya in 2001 making Rinderpest vaccine arguably the most successful live attenuated vaccine to date on the basis that smallpox vaccine was not derived from variola and was therefore not strictly speaking an attenuated vaccine.

While live attenuated vaccines against human viral diseases have been very successful there are many recurrent issues. The safety and efficacy of certain mumps vaccines is questionable and the first rotavirus vaccine to be licensed was withdrawn when it became clear that it was associated with intussusception. Some vaccines must be used in a specific way if they are to be maximally useful. For example in tropical regions, where exposure to the virus occurs throughout the year, the live attenuated polio vaccine must be given in mass campaigns to reduce the susceptible population and interrupt transmission of the virus. In contrast in temperate climates transmission is seasonal. Thus routine immunisation at a set age is sufficient to

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reduce the pool of susceptible individuals during the low transmission period to such an extent that the virus dies out. Similarly measles vaccine used in mass campaigns has controlled measles by reducing the number of susceptibles and thus the circulation of virus. This bypasses the failure of the vaccine to work in the very young in the presence of maternal antibody. An imperfect vaccine can be used in such a way as to achieve disease control.

The list of vaccines considered here is not comprehensive but is chosen to demonstrate how they have been developed over the years, and that the principle challenge historically has been to identify a vaccine strain of the right properties when it appears. Safe and effective vaccines against chronic diseases such as HIV or HCV remain to be identified in part because the possibility of using a vaccine that causes serious disease is unacceptable. Successful vaccines against other diseases associated with chronic infection, such as varicella zoster have been developed. The characterisation of a live vaccine strain in terms of its probable attenuation is often fairly light until clinical trials begin, so that vaccines are developed by careful empirical clinical science rather than prior design.

There has been much discussion and scientific interest in vectored vaccines, for example using adenovirus or poxviruses as the carriers of a protective antigen. The approach has had limited practical success, although there are interesting developments in the flavivirus area that could come under this heading.

The first vaccine to be discussed in this paper is that against smallpox. Vaccinia is not considered by some an attenuated vaccine in the strict sense because it is a distinct virus from variola the causative agent of smallpox and not derived from it. On the other hand the process followed and all of the issues of potency, safety and quality control raised provide a forceful model for all live attenuated vaccines developed since. This includes the development of an anti-vaccine lobby.

Smallpox

Smallpox was declared eradicated in 1980 making vaccinia arguably the most successful human vaccine to date. It had significant side effects in most first time recipients and serious, sometimes fatal, effects in a proportion of individuals.

It was common knowledge in rural communities in the UK in the 18th century that individuals who had contracted cowpox were resistant to smallpox, and the farmer Benjamin Jesty deliberately inoculated farm hands as a protective measure. He never wrote an account of the experience nor did he challenge the recipients with smallpox as Edward Jenner did (Jenner, 1798). Variolation, first brought to the UK from Turkey by Lady Mary Wortley Montagu, involved the inoculation of material from a smallpox sore into the arm of the patient, and, if done correctly into the superficial layers of the skin, resulted in only 1% mortality rather than the up to 30–40% found for those infected naturally at the time. Jenner's experiment on the boy James Phipps was therefore not quite as appalling as it sounds to modern ears. Phipps was inoculated with material from a cow infected with cowpox, which resulted in a lesion indicating infection, and then seven weeks later with material from a smallpox pustule. The smallpox inoculation did not result in recognisable lesions and Phipps was clearly protected. Many other examples are given by Jenner (1798).

The later history of smallpox vaccine illustrates the need for quality control in vaccines (Baxby, 2001; Minor, 2012a; WHO, 1966, 2003). No potency assay was used until the start of the 20th century, when a test in rabbits was introduced. Titration on chorioallantoic membranes of chicken eggs was not introduced until growth in eggs had been demonstrated (Lazarus et al., 1937) so throughout the 19th century when vaccination was in theory compulsory in the United Kingdom, and later into the twentieth century, the vaccinator had at best a very rough idea of the quantity of active material in the vaccine. While the vaccine could produce satisfactory lesions and sores it did not follow that these were due to the virus, many being caused by bacterial contamination; the preparations might also be completely inactive. These issues were raised by Jenner (1798). There was also a practice of growing the vaccine in human arms as well as in cows with transmission from person to person. Transmission of syphilis and other diseases by vaccination sometimes occurred as a result. There was a certain amount of popular resistance to vaccination which was understandable in view of the fact that it might not protect you, could kill you from other causes and was in any case compulsory. As a result the debate that followed was highly partisan.

At the start of the 20th century however compulsion was dropped in the United Kingdom and the debate became more rational; evidence was presented that the vaccine worked in epidemics and data were produced on the nature and duration of the protection it afforded (McVail, 1902; Baxby, 2002). In 1965 WHO developed guidelines to provide criteria to be met by a satisfactory product (WHO, 1966) and in 1967declared the intent to eradicate the disease by use of vaccine. The guidelines clearly hint at the poor state vaccine production was in. There was limited control of the strains to be used (although there were clear differences between the clinical properties and adverse event profile of the different strains) and little harmonisation of the general means of production, or of efforts to minimise or at least detect contaminating organisms. In 2001 when there was revived interest in vaccine production because of the attacks on the World Trade Center in New York and the consequent fear of bioterrorism, the guidelines were revised (WHO, 2003) and smallpox vaccine production became modernised. There was no direct way to establish whether the new production methods gave rise to an effective product as the disease had been eradicated for 20 years, leaving governments with a difficult choice between the old reactogenic vaccine of which there were still small stocks of satisfactory potency, new material manufactured by the same process which involved scarification of calf flanks, or the new vaccines which were of high but different quality and unknown efficacy in the field. The problem remains. The basis of the effectiveness of the vaccine in use is unclear in modern virological terms.

Smallpox vaccine was developed by human challenge studies and careful clinical examination. Given that the first description of a virus as a filterable infectious agent was in 1898 (Beijerinck, 1898), 100 years after the start of smallpox vaccination, it is not surprising that the influence of virology on the early development of smallpox vaccines was minimal.

Poliovirus

The developing understanding of poliomyelitis has been described (Paul, 1971; Minor, 1992) and the essential features of its pathogenesis, including the gaps in understanding, have been the subject of reviews (Minor, 1997; Nathanson, 2008). Poliomyelitis has nearly been eradicated (Minor, 2012b) and the main tool in the global programme initiated in 1988 (WHO, 1988) has been the live attenuated vaccine developed by Albert Sabin and used since the early 1960s. Many other vaccines both killed (Salk, 1953) and live were explored in the 1950s and the inactivated vaccine of Salk in its modern form is playing an increasing role in the end game, mainly because the live vaccine is based on an attenuated form of the wild type virulent virus which can change. The development of a live attenuated vaccine depended on an adequate and widely accepted understanding of the pathogenesis of poliomyelitis which was not present in the early period.

Poliomyelitis was rare before the end of the 19th century when it began to appear in regular major epidemics. The first of these

were in Sweden and by 1912 much of the information required to understand the disease was in place. Poliomyelitis had been shown to be caused by a filterable agent or virus which could be transmitted to non-human primates. This model also allowed infectivity to be titrated (Landsteiner and Popper, 1909). Wickman had shown by careful epidemiological studies that most infections were silent and that as well as asymptomatic infection there was an additional systemic phase which might or might not precede the neurological disease of poliomyelitis (Paul, 1971). Kling among others had shown that infectivity could be found in intestinal contents as well as the nervous tissue of fatal cases (Paul, 1971). Thus Swedish scientists had data that strongly suggested that there were different phases of infection, at least one of which was harmless and probably involved replication in the gut. This is the accepted view today.

Unfortunately an alternative view emerged in the USA where Flexner focussed on the neurological aspects which are the most important features of the disease (Paul, 1971). Basing the model on work with flaviviruses he proposed that transmission was via the nose and involved infection of the olfactory lobes and subsequent transport through the brain to the lower spine where the damage occurred. The model was supported by the ability to transmit infection by instilling poliovirus into the nose of monkeys; this might also be an effective if artificial way of infecting a human. It was not clear how the virus travelled from person to person. The model would make vaccine development difficult as it involved unmediated access to the brain and is cited by some today as a prime example of the misleading nature of animal models of human diseases. Flexner's neurological model dominated the field for 30 years. Despite this, experimental vaccines were developed in the 1930s, based on the principles that Pasteur had used in developing rabies vaccine. One was allegedly a live attenuated vaccine, the other a killed non-replicating vaccine (Paul, 1971). One of them was acknowledged to cause disease in recipients in trials and there is reason to suspect that the other might have done so as well.

Development of a safe effective live vaccine depended on understanding the pathogenesis and virology of poliomyelitis. Albert Sabin, among others, contributed to the refutation of the neurological model when he showed that virus was to be found in large amounts in the gut of fatal cases and that such cases had no sign of viral replication or damage in the olfactory lobes of the kind found in the monkey model (Sabin and Ward, 1941). By the mid 1950s the current view of pathogenesis was in place (Bodian, 1955; Sabin, 1956), involving the infection of the gut following oral-faecal transmission, invasion of the local lymph nodes, viraemic spread to other still unknown peripheral sites and eventual invasion of the CNS. It had been shown that immunoglobulin protected against poliomyelitis and that serum antibodies were therefore a good and sufficient indicator of protection (Minor, 1997). Thus a virus strain which can induce neutralising antibodies in the blood and which is able to grow in the gut but not the nervous system is a candidate vaccine virus.

Polio occurs in three serotypes such that infection with one does not provide solid cross protection against another. Three vaccine strains were therefore required and a great deal of effort went into identifying non-neurovirulent viruses. The type 1 laboratory strain Mahoney was derived from a pool of isolates from clinically unaffected individuals and remains the standard laboratory strain. Li and Schaeffer passaged the virus in monkey testis and other cell cultures and produced several lineages, one of which, LSc, was eventually developed into the Sabin type 1 vaccine strain. The type 2 strain P712 was isolated from the stools of a healthy child from New Orleans and became the Sabin type 2 strain and the type 3 strain Leon was isolated from a fatal case in 1937 and had become a standard type 3 laboratory strain before development by Sabin into a vaccine. The passage history of these viruses to give rise to the Sabin vaccine strains has been recorded (Sabin and Boulger, 1973) but the key studies were directed to demonstrating their lack of neurovirulence and the stability of the attenuated phenotype when inoculated by multiple routes into a range of primate models (see for instance Sabin et al. (1954)). A colossal number of old world monkeys of various species as well as chimpanzees were used in these studies. Sabin also reported injecting the type 3 strain into human subjects, demonstrating that they did not seroconvert, and then feeding them the same strain and showing that an immune response was generated (Sabin, 1956). The result was the identification of one strain of each serotype among many studied considered suitable for widespread use. Each production batch was tested for lack of neurovirulence in monkeys although the test was not formalised and made meaningful until the late 1970s and early 1980s (WHO, 1983; Cockburn, 1988). The vaccine was put into a massive trial in millions of children in the USSR by Chumakov and licensed in the USA in 1960.

The incidence of poliomyelitis had fallen by about 95% as a result of the use of the inactivated polio vaccine developed by Salk. It was reduced still further by the introduction of the live Sabin vaccine, which was easier to give and could be relatively easily produced in large amounts. In the United Kingdom there were between one and 10,000 cases of polio per year in the 1950s but one to two per year by the end of the 1960s. While there were other issues including contamination of the vaccine with viruses derived from the monkeys that provided the cells in which it was grown (Shah and Nathanson, 1976) the main consideration in what follows is the vaccine itself.

It became apparent very soon that cases of poliomyelitis were temporally associated with the live vaccine and while it was hotly denied by Sabin the view was that in rare instances the vaccine could give rise to disease. The issue was obscured by the alleged difficulty of differentiating strains until molecular methods were applied although there was very little real scientific doubt (Nakano et al., 1966; WHO Report, 1981). There were recognised changes in the phenotype of virus excreted by vaccinees compared to the vaccine they were given including antigenic changes in the type 1 strain and increases in the virulence of the type 3 strain (WHO, 1969). Eventually molecular methods showed beyond reasonable doubt that the vaccine could cause poliomyelitis (Minor, 1980; Nottay et al., 1981). The viruses had been derived by passage of viruses that were, whatever their origin, known to be virulent in animals so the observation was not surprising. The frequency of the cases was so low as to be difficult to quantify at the time but a large study in the USA concluded that the incidence was about one in 500,000 first time vaccinees and much lower in the previously immunised (Nkowane et al., 1987); it could also occur in contacts of vaccinees proving that the virus could spread. The frequencies of vaccine associated poliomyelitis for the type 1 strain were about a tenth of those of the type 2 and type 3 strain combined while in contrast the virulence of the type 1 strain in monkeys was significantly higher than that of the other two serotypes (Marsden et al., 1980; Boulger et al., 1979). It was also known that hypogammaglobulinemic patients lacking humoral immunity were at greater risk of disease if given the vaccine but that some (estimated at about 1% exposed) would go on to excrete virus for periods measured in years instead of a few weeks (MacCallum, 1971). By the 1970s polio had ceased to be a public health problem in most developed countries although as the incidence was unchanged in the rest of the world vaccination had to continue.

The continued occurrence of vaccine associated cases meant that the monovalent components of polio vaccines were tested for safety batch by batch with the only available test, which involved monkeys. This was cumbersome, expensive and increasingly

ethically questionable. There was therefore interest in understanding what was being measured and establishing the molecular and virological basis of the attenuation of the Sabin vaccine strains of poliovirus. Leon, the virulent type 3 precursor of the Sabin vaccine strain was cloned and sequenced and compared to the Sabin vaccine strain itself. Depending on the origin of the vaccine studied, there were 10 or 11 differences. Creation and recovery of recombinant viruses showed that the monkey test detected two (Westrop et al., 1989) or three (Tatem et al., 1992) mutations that would attenuate the wild type strain, one in the 5′ non-coding region involved in the initiation of protein synthesis (Svitkin et al., 1990) the second in the capsid protein VP3 which made the capsid less stable and conferred a temperature sensitive growth phenotype (Minor et al., 1989) and the third in VP1 which was rapidly lost on culture or growth in vaccine recipients. The monkey test involves the injection of the virus directly into the spinal cord and therefore assesses neurovirulence in the pure sense and not necessarily as it would be manifest in vaccine recipients where the virus would have to move from gut to central nervous system by the blood stream. When the first two mutations were specifically reverted to the precursor sequence the resulting vaccine strain virus was almost but not quite as virulent as the wild type hinting at additional sources of slight attenuation (Westrop et al., 1989).

Similar studies were performed with type 2 strains where the vaccine was compared to an isolate from a vaccine associated case; here the results concerned the reversion of the vaccine to a paralytic phenotype, rather than attenuation of a wild type strain, and again two mutations were particularly identified, one again in the 5′ non-coding region, the other in the capsid protein VP1. Other mutations might also have an effect (Macadam et al., 1991, 1993). Finally studies were performed on the type 1 strain comparing the vaccine to the Mahoney strain from which it differed by 57 mutations. Here the result was more complex (Omata et al., 1986); some effect was seen with a mutation in the 5′ non-coding region and there was an effect of mutations in the capsid region, but these were individually less striking and less easily detected than for type 3 or type 2. In all strains the mutations in the 5′ non-coding region are in a single well ordered structure, domain 5, which forms a part of the Internal Ribosomal Entry Site (IRES). They are shown in Fig. 1. The thermodynamic stability of this structure correlates with the neurovirulence of the virus (Macadam et al., 1993, 2006).

These findings identified the principal molecular features that the monkey test detected; later when transgenic mice expressing the human polio receptor were developed it was shown that the same mutations were effective in mice (Chumakov et al., 1992). Moreover for type 3 at least the percentage of revertants detected at position 472 correlates well with the virulence or attenuation of commercial batches of vaccine in both monkeys and transgenic mice (Chumakov et al., 1991). In both animal models direct inoculation into the spine circumvents any other aspect of pathogenesis and it was left to studies in vaccine recipients to try to establish the significance of the mutations in the human subject.

After vaccination virus excretion is generally thought to occur for an average of 30 days during which the virus adapts to the host. The sequence of events for the type 3 strains isolated from two children given vaccine containing all three Sabin vaccine strains is shown in Fig. 2, focussing on the known attenuating mutations and the temperature sensitive phenotype attributable to the mutation in VP3 (Minor et al., 1986; Minor, 2012a, 2012b). Both children were immunised as part of the routine UK schedule at about three months of age; they excreted type 3 virus for 73 and 50 days respectively. The mutation in the 5′ non-coding region was lost completely and almost immediately, by 48 h in child 1 and by day 3, the time of the first stool produced, in child 2. This suggests

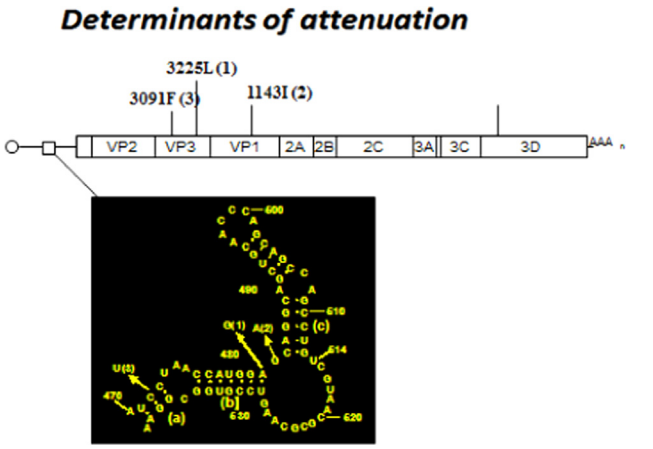

Fig. 1. Mutations affecting the virulence of the three Sabin vaccine strains of poliovirus. Each has a mutation in Domain V of the 5′ non-coding region and each at least one mutation in the capsid region. The types concerned are shown in paranthesis.

that this mutation so severely handicaps the virus that it cannot replicate in the human gut to any great extent. However after this the excreted virus was essentially unchanged for 11 days in both subjects, before a number of simultaneous alterations occurred: the excreted virus became a recombinant between type 3 and type 2 (in other children the excreted virus can be a recombinant between type three and type 1 (Cammack et al., 1988), the temperature sensitive phenotype was wholly or partially lost, and some indication of changes in antigenic sites could be detected. Other differences may also occur including a second recombination event a few weeks later. It seems likely that the changes are a response to the initiation of an immune response which the virus escapes through increasing its replicative fitness by suppressing the temperature sensitive growth phenotype and by recombining with other viruses as well as changing antigenically. This would imply that the immune response in the gut at this stage is not very strong as it can be avoided largely indirectly; it would also imply that the temperature sensitive phenotype is not seriously selected against in the absence of an immune response because the virus has replicated unchanged for 11 days. Moreover the loss of the temperature sensitive phenotype tends to be associated with indirect mutations rather than direct reversion. The type 2 strain reverts in a broadly similar manner but the type1 strain reverts less rapidly and less completely, even in the 5′ non-coding region (Dunn et al., 1990). This is consistent with the view that the type 1 strain is not so handicapped in its replication in the gut (or as above in the CNS of animal) and is therefore not under heavy selection for improved fitness.

The type 1 strain is the most virulent of the three types in animal models (Marsden et al., 1980; Boulger et al., 1979; WHO, 2012) yet it causes the lowest frequency of vaccine-associated poliomyelitis and the identification of the attenuating mutations responsible has been the most difficult. If there is little selective pressure to increase its fitness further the type 1 strain should be more stable in vaccinees than the other types. If there are more mutations each with a lesser effect then selection to high virulence will also be more difficult because more mutations will be needed. Thus paradoxically a more virulent virus can be both more effective and less virulent in the vaccinee particularly if it contains many weakly attenuating mutations. The development of a live attenuated vaccine can therefore be an extremely subtle and complicated process and is difficult to approach on a purely rational basis. In contrast the type 3 strain infects recipients given trivalent vaccine less often than the others, the mutations have a more readily detectable effect in animal models and the 5′

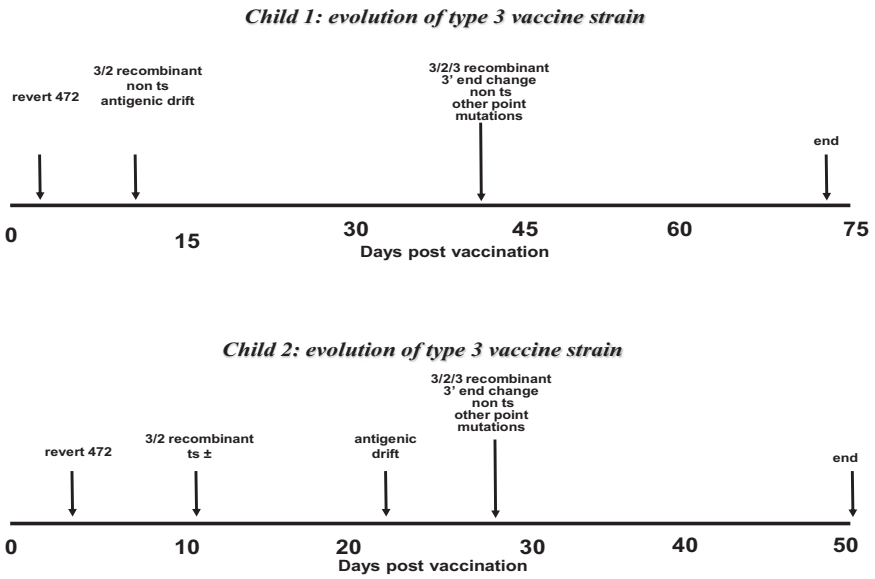

Fig. 2. Changes in the virus isolated during the excretion of type 3 polio vaccine after the first immunisation in two infants aged about three months. Child 1 excreted type 3 poliovirus for 73 days and child 2 for 50 as shown by the horizontal time bar. Changes in known attenuating mutations and phenotypes and other phenotypic and genotypic changes are indicated.
Reprinted from Minor (2012b). The polio eradication programme and issues of the endgame. J. Gen. Virol. 93:457–474.

non-coding mutation at least is selected against more strongly in the human gut. This is consistent with strong selection against a strong attenuating effect giving a genetically unstable vaccine.

Poliomyelitis has almost been eradicated from the world thanks to the programme initiated by the World Health Organization in 1988 (WHO, 1988, 2014a). Wild type 2 virus was last isolated from a case in October 1999 apart from an incident involving of OPV batches which were probably sabotaged (Deshpande et al., 2003). At the time of writing wild type 3 virus was last isolated in 2012 (WHO, 2014a), and the last case of any type in India was in January 2011, which given the social conditions is a colossal achievement. The last case of wild type 1 poliomyelitis in Africa to date was in August 2014. An outbreak in Syria in 2014 seems to have been brought under control as there have been no cases for six months, another extraordinary achievement given the lethal conditions prevailing. Currently Pakistan has most of the world's cases of wild type poliomyelitis although there are some cases in Afghanistan. There is a real if fragile possibility that poliomyelitis caused by wild type virus is about to disappear. The success of the programme so far is due to the use of the live attenuated vaccines in mass campaigns so that transmission of the wild type virus is broken. However the vaccines can also revert to virulence in vaccine associated cases, and in healthy recipients the viruses change freely by mutation and recombination in response to events. Therefore in regions where vaccine coverage is poor, and the immunised and non-immunised mix in conditions of sub optimal hygiene, it is not surprising that viruses can be selected that will transmit freely from one person to another and that such viruses cause poliomyelitis. They are termed circulating vaccine derived polio viruses (cVDPV) and there are many instances of their occurrence, although given the amount of vaccine used they occur at a low frequency (Kew et al., 2002). Virus excreted by hypogammaglobulinemic individuals becomes highly virulent but does not seem to be as transmissible as cVDPVs although there is no obvious reason why they should not become so. These viruses are termed immunodeficient vaccine derived polioviruses (iVDPVs). The vaccine therefore poses a problem for the final eradication of polio and this is the final issue. cVDPVs may be eradicated by vaccinating properly; not all vaccinated individuals give rise to transmissible strains. Chronic excreters of iVDPVs usually stop eventually but some can clearly continue for decades (MacCallum, 1971; MacLennan et al., 2004) and remain a problem to be solved.

Polio vaccines illustrate the balance that must be struck between the ability to grow and the inability to cause disease as well as the fact that once an individual is infected with a live attenuated virus the situation is in a real sense out of control. The long period over which poliovirus is excreted is a factor in this but it makes it possible to investigate what happens to the virus over time.

Yellow fever

Yellow fever virus is the archetypal flavivirus and is transmitted by the mosquito *Aedes aegypti*, as first hypothesised by Carlos Finlay in 1881 and shown by experimental transmission to army volunteers by Walter Reed in 1900 (Frierson, 2010). The virus causes yellow fever which can have fatality rates of 20–80% depending on the circumstances; while none of Walter Reed's 14 volunteers died a repeat of the experiment in Cuba by John Guiteras in 42 individuals resulted in severe disease in 8 and three deaths (Frierson, 2010). Many other laboratory and field workers became infected in the course of their studies (including Max Theiler who developed the vaccine in use today) and several died. Max Theiler fortunately survived. Yellow fever is now confined mostly to low and middle income countries but was initially also present in Europe and the Southern States of America including Texas and Florida; Cuba was particularly affected. While there has been much concern over the neurotropism of vaccine strains the primary cause of death from yellow fever is viscerotropic disease resulting in jaundice.

In 1927 the virus was transmitted to rhesus monkeys from a Ghanaian called Asibi and in a separate study from a Senegalese called Mayali. The Asibi isolate gave rise to the 17D vaccine lineage which is the only one in use today, while the Mayali isolate was used to develop the French Neurotropic Vaccine strain (FNV) that was successfully used well into the 1960s when the high incidence of vaccine associated encephalitis it induced made it unacceptable. The monkey model made it possible to demonstrate that human

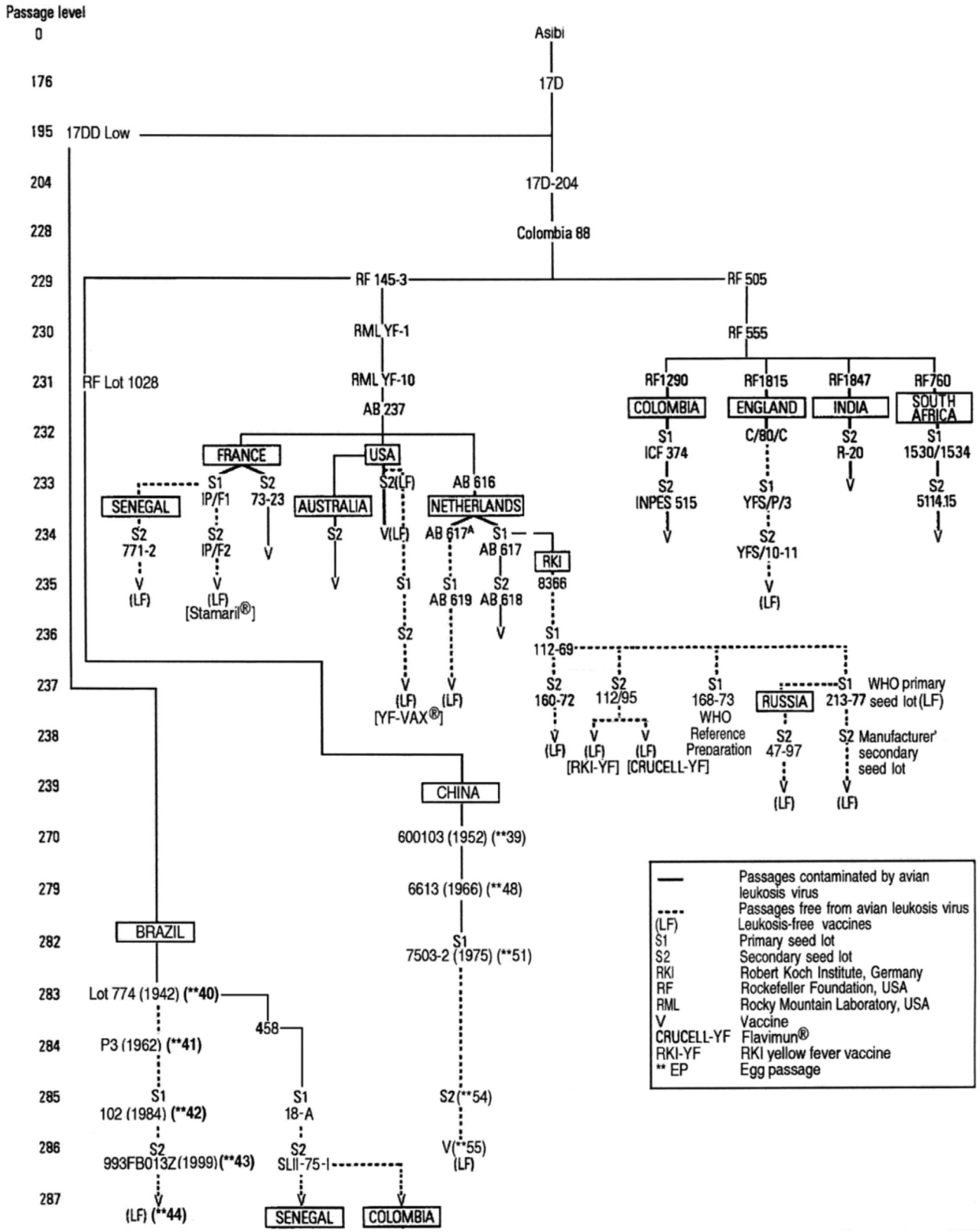

Fig. 3. Genealogy of yellow fever vaccine strains. All strains are derived from the Asibi strain and the 176 strain derived from it by passage. The divergence of the different seed strains is shown.
Reprinted from WHO (2010). Recommendations to assure the quality safety and efficacy of live attenuated yellow fever vaccines. Technical report series 978, Annex 5; pp. 241–314.

serum from recovered cases was protective and that the killed virus preparations of the time were not effective as vaccines. It later played a crucial role in the development of vaccines particularly with respect to the viscerotropism or neurotropism of virus strains (WHO, 2010). In 1930 Theiler reported that mice could be infected with yellow fever by the intracerebral route

producing a more usable model than the rhesus monkey. Passage in mouse brain resulted in a virus of increased neurovirulence but decreased viscerotropism in monkeys and the FNV strain passaged more than 100 times in mouse brain was the first yellow fever vaccine to be used in clinical trials in 1931 (Theiler and Smith, 1937). For some time after this the FNV strain was used in the USA and UK in conjunction with human immune serum to further attenuate it. In France and Africa the vaccine was given by scarification without serum but in conjunction with small pox vaccine.

In 1932 it was shown that yellow fever virus could be grown in chick embryo tissue and both the Asibi and FNV lineages were passaged extensively in unsuccessful attempts to lessen their neurovirulent phenotype. Cultures in which the neurological tissue had been removed were then used and after 100 passages in this culture type in addition to the previous 76 passages in chick tissue the neurovirulence of the Asibi strain was reduced. The strain is referred to as 17D. Attempts to repeat the process with FNV or unpassaged Asibi virus failed and the isolation of the strain that went on to be the basis of all yellow fever vaccines in current use therefore seems to have been pure chance (Frierson, 2010); no stocks of the Asibi passage 176 virus (17D) are known to exist today. The 17DD strain diverged at passage 195 and is currently used in Brazil. The 17D 204 strain originated at passage 204 and its derivatives are used in the rest of the world, in lines obtained from Colombia; one group of 17D 204 derived viruses is used in China, a second in a group of countries including the USA, France and originally Australia and the Netherlands. The Netherlands virus in turn was sent to the Robert Koch Institute in Germany where a stock was made that became the WHO seed (213-77) and reference preparation (168-73); this is sometimes referred to as the 213 lineage. A third group of 17D 204 viruses was used in Colombia, England and India. The situation is summarised in Fig. 3 (WHO, 2010).

Passage is clearly central to the history of yellow fever vaccine, and the 17D lineage originated from virus grown in cell cultures prepared from chick embryos where the neurological tissue had been removed. Yellow fever vaccines are currently produced in embryonated hen's eggs, a method developed in the late 1930s (WHO, 2010; Frierson, 2010). It is difficult to establish from the existing record which of the passages giving rise to the strains shown in Fig. 3 were in cell cultures from chick embryos from which the neurological tissue had been removed, in cell cultures from whole chick embryos, or in embryonated hen's eggs. It is known that the WHO preparations 168-73 and 213-77 were manufactured in embryonated eggs at the Robert Koch Institute.

All of the vaccines in current production and use are considered to be of equally high safety and efficacy but the biological reason for this is not clear. There are differences in the sequence of the different seeds and lineages shown in Fig. 3 which demonstrate that the viruses are distinct, whatever the clinical significance of the differences (Santos et al., 1995; Galler et al., 1998; Hahn et al., 1987; Rice et al., 1985; Dupuy et al., 1989). Given the origin of the attenuated strains, passage might be expected to affect the phenotype. Rapid changes on passage of the Asibi strain in HeLa cell culture systems leading to an attenuated phenotype have been reported repeatedly over the years (Hearn et al., 1965; Barrett et al., 1990) and the properties of yellow fever virus are clearly as changeable as any other virus. In 1941 there were reports of encephalitis in recipients of a vaccine a few passages on from the parent strain (Fox et al., 1942); the conclusion was that some change had been introduced by passage and that the number of cultures between parent strain and vaccine should be controlled and restricted. The seed lot system was introduced in which a master seed is used to generate a working seed that is used in turn to generate the vaccine itself. Thus passage is restricted to controlled levels and vaccine production should be more reproducible. By 1943 the original seed material had been distributed and passaged in various laboratories and manufacturing sites world-wide and there were significant differences between the respective vaccines in their phenotype in monkeys (Fox and Penna, 1943). In recent years it has been shown that the WHO seeds and reference (213-77 and 168-73 in Fig. 3) differ in the monkey neurovirulence test from other seeds such as that originally used in England (RF 1815 in Fig. 3) or currently in France or Senegal (S1 IP/F1 and S2 771-2) (Minor, 2011). This is attributed to the loss of a glycosylation site in the WHO materials. Its significance for human safety or efficacy is unknown and the relationships between the laboratory markers, the genomic sequences and the clinical properties of the vaccine in general remain to be established. However one phase 3 trial reported that the immune responses to vaccines made from the WHO seed were superior to those from vaccines made from one of the other 17D 204 groups (Pfister et al., 2005).

Yellow fever vaccine is a classical product. It is known to be effective, and in fact had a major effect on the disease in Western Africa in the 1950s as a result of well executed mass vaccination campaigns before efforts moved on and the disease returned in the late 1980s; vaccination in South America is known to be effective with a different lineage of vaccine. The vaccine has also been considered extraordinarily safe. Given the origin of the attenuated phenotype in extensive in vitro passage, the serendipitous occurrence of the desired phenotype at passage 176, the rapid changes in the laboratory phenotype on limited passage of yellow fever virus in novel culture systems, the need for a seed lot system arising from the observation of adverse events in humans and the genetically heterogeneous nature of the different vaccines in use it is reasonable to wonder why a live attenuated, genetically unstable vaccine should be so satisfactory in use.

The situation has been further complicated by reports of serious adverse events, specifically vaccine associated neurotropic disease (YEL-AND) and vaccine associated viscerotropic disease (YEL-AVD). In the 1940s cases of encephalitis linked to vaccination with 17D vaccines were reduced by the introduction of the seed lot system. In the 1950s however there were still a number of such cases in infants (produced by vaccines made according to the seed lot system), and WHO recommended that infants below 6 months of age should not be vaccinated. All except one of the known cases recovered fully, and the strain from the fatal case was identical to the vaccine strain antigenically and by the molecular markers available at the time (Jennings et al., 1994) although it was more virulent in intranasal mouse neurovirulence tests. YEL-AND was therefore always a known but rare adverse event whose origin is not clear. Since 2000 however there have been increasing numbers of reports particularly in the elderly. The incidence varies depending on the study from 0.19 to 0.8 per 100,000 doses in Europe and America (WHO, 2010). Its cause has not been established, specifically whether it is a property of the virus or the host; it is associated with both 17D 204 and 17DD lineages at similar rates so far as can be seen.

YEL-AVD or vaccine associated viscerotropic disease, was first reported in 1975 in Brazil (Barrett and Teuwen, 2009), and up to 2009 51 cases had been identified. There is no evidence that the syndrome is caused by changes in the vaccine virus in recipients (Engel et al., 2006) and host factors such as immunodeficiency are usually quoted. A cluster of five cases, four fatal, was reported in 2007 in Peru; the viral genome from one of the fatal cases was sequenced and shown to be identical to that of the vaccine virus; the root cause of the incident is unknown (Whittembury et al., 2009). The incidence of YEL-AVD ranges from 0.004 to 0.21 per 100,000 for episodes other than the Peruvian cluster where the incidence was 7.9–11.7 per 100,000. The range in frequencies

suggests that there may be a problem of ascertainment (WHO, 2010).

The incidence of the adverse events is low but the events themselves can be very serious or fatal. The reports of YEL-AVD raised the serious proposal that a major campaign of immunising against yellow fever in Africa should be abandoned; the risks of yellow fever were rightly considered greater and the programme went ahead. It is difficult to know how to proceed when a vaccine that has been used successfully and safely for decades suddenly raises concerns. One possibility was that the adverse events were associated with vaccines of particularly high titre, and this revealed another issue. The titre of yellow fever virus in vaccines was defined in WHO requirements in terms of mouse lethality, the specification being that it should be no less that 3 $\log_{10}$ mouse LD50. In practice manufacturers calibrated their mouse test against a more convenient validated and accurate cell culture assay. This introduces two sources of possible disagreement between manufacturers, namely the inaccuracies and variability of the mouse LD50 test and the similar variability of the cell culture assay. A study showed that both were very significant (Ferguson and Heath, 2004) so that doses of products assayed in different laboratories were not comparable. Even such a basic parameter as the amount of vaccine given was thus not known. The matter has now been corrected by the preparation of an International Reference preparation calibrating the internal controls used in assays, and the definition of the dose in a common International Unit (Ferguson and Heath, 2004; WHO, 2010). The effect of dosage on the incidence of adverse events is not fully understood but it is clear that if it is a factor it is not the only one.

A better understanding of yellow fever vaccine in its interaction with the vaccinee would help in the assessment of risks but this is not the only live vaccine where knowledge is imperfect.

Measles

In developed countries measles is considered a trivial disease of childhood but in developing countries in the absence of vaccination the death rate can be as high as 30%; the situation in 19th century London was similar. In the absence of global vaccination programmes it is estimated that 6 million children would die of measles per year, mostly of pneumonia, other respiratory complications or diarrohea. The vaccines developed in the 1960s to 1980s are therefore life saving additions to immunisation programmes. Measles is a complicated disease and a normal infection with recovery within weeks causes prolonged immune disruption over a period of a year or more, which for example affects the response to tuberculosis and some immune mediated syndromes (Moss et al., 2004). Killed measles vaccines were developed in the 1960s using a process involving formalin treatment similar to that used for the manufacture of inactivated polio vaccines; some at least involved aluminium hydroxide adjuvants which may have been a factor in what followed. The protection they gave declined in the medium to long term and when immunised individuals were then exposed to wild type measles they developed a serious disease with an atypical rash and a high rate of lung involvement which could require hospitalisation (Fulginiti et al., 1967). No deaths were recorded. The susceptibility to atypical measles persisted for many years; one case was reported 15 years after immunisation (Fulginiti and Heller, 1980). Initially the aberrant response was attributed to the finding that the formalin treatment had destroyed the immunogenic properties of the fusion protein (Norrby et al., 1975). The consequent absence of antibodies meant that while cell free virus would be neutralised the virus could avoid neutralising antibodies by spreading from cell to cell by cell fusion. Later studies in non-human primate models concluded that the syndrome resulted from priming for an inappropriate non-protective type 2 CD4 T-cell response which meant that non-protective but biologically active anti F protein antibodies were induced more rapidly than in naïve animals; there was no lack of antibodies against the F protein (Polack et al., 1999). Atypical measles raises issues of the suitability of some types of killed vaccines even today. Similar more serious reactions were also recorded with vaccines against Respiratory Syncitial virus, another paramyxovirus, where deaths occurred (Kim et al., 1968). However despite the subtleties of the immune response the serological response to measles as measured by neutralising antibody is accepted as the best marker of protection from infection. Protective levels have been defined (Chen et al., 1990).

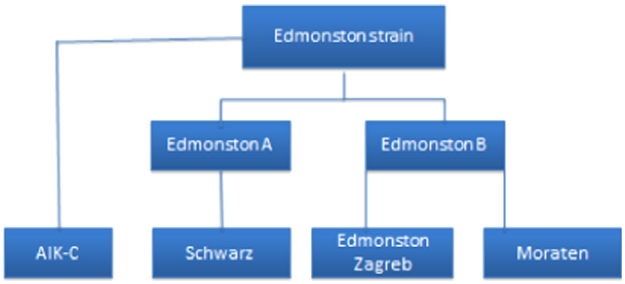

Fig. 4. Genealogy of measles vaccines derived from the Edmonston strain.

Wild type measles causes fatal acute encephalitis in some instances. It can also cause subacute sclerosing pan encephalitis (SSPE) up to 10 years after the original infection, as a result of chronic virus persistence in the brain of victims. Various genes of SSPE strains particularly the fusion gene are deleted or modified (Schmid et al., 1992). The role of live measles in SSPE was not discovered until after the development of the live attenuated vaccines and might have caused concern over vaccine use. The vaccines have in fact prevented many deaths and have never been implicated in SSPE. Measles vaccines however are clearly a potential minefield.

The first isolation of measles virus was described in 1954 (Enders and Peebles, 1954) and the virus is named the Edmonston strain after the child concerned. Isolation used primary human kidney and primary human amnion cells and the virus was subsequently passaged 12 times in embryonated chicken eggs and 19 times in primary chick embryo fibroblast to produce the first candidate measles vaccine. The Edmonston B vaccine was derived from this strain by a further five passages in chick embryo fibroblasts at 36–37 °C. It was associated with fever and was initially given with immunoglobulin to reduce its virulence further; production lots in cell culture were said to be less reactogenic and were licensed for use with or without immunoglobulin. Other strains were developed from the Edmonston strain, and an outline of some of the resulting vaccines still in global use is given in Fig. 4. In later years there were attempts to develop a convincing animal model and it was shown that the isolation of the virus in the usual cell types such as Vero or human diploid cells gave rise to a virus that would not cause measles in primates to the same degree as unpassaged virus (Kobune et al., 1996; van Binnendijk et al., 1994). Isolation in peripheral blood lymphocytes or the marmoset B cell line B95a gave viruses that were virulent, and also improved the isolation rate, suggesting that the viruses are more like the wild type, and cells infected in disease are similar to the lymphocyte lines (Kobune et al., 1990).

The Moraten strain was derived from Edmonston B by 40 further passages in chick cell culture at 32° (Hilleman et al., 1968); the Schwarz strain by 85 further passages of Edmonston A in chick cells (Schwarz, 1962) and Edmonston Zagreb by passage of Edmonston B in the human diploid fibroblast line WI38 (Ikic et al., 1970, 1972). The AIK-C strain was derived from the original Edmonston B by growth at low temperature in chick cells (Hirayama, 1983; Makino, 1983).

Other strains were developed independently from other isolates: CAM 70 from the Japanese Tanabe isolate (Ueda et al., 1970), Leningrad 4 (the most globally widely distributed measles vaccine because of its use in WHO programmes) from a Russian isolate, (Smorodintsev et al., 1960) and Changchun 47 and Shanghai 191 from two independent Chinese strains (Bankamp et al., 2011). Comparisons of the sequences of the different isolates have been published which show their relationships (Bankamp et al., 2011).

While the phenotype of the candidate vaccine strains arose from passage on unusual cell substrates, mainly chicken embryo fibroblasts, the question of how a suitable vaccine was recognised remains; for example the Edmonston B strain was passaged in chick cells but was initially considered too virulent, causing fever at an unacceptable rate whereas the Moraten and Schwarz strains arose from additional passages on the same cell type. Buynak et al. (1962) compared the phenotypes of the wild type Edmonston isolate, an independent wild type isolate from Philadelphia and the Moraten strain in monkeys and in vitro cell culture. The results were useful in identifying the individual strains but of limited predictive value for a novel vaccine. The egg passaged viruses, both Moraten and Edmonston, formed distinctive plaques in chick embryo fibroblasts whereas the Philadelphia strain formed plaques only in monkey cells; plaque morphology was distinctive. None of the viruses caused clinical signs in monkeys when inoculated by either the subcutaneous of intracranial routes. Histologically the Philadelphia strain produced lesions when inoculated intracranially and the original Edmonston strain produced similar lesions but in only 60% of the animals. The Moraten strain did not give lesions. The results did not give a clear indication of the suitability of any of the strains as vaccines but implied that they were all to some degree attenuated. The obvious conclusion is that the only way to determine the suitability of the strains was by clinical trial which is what was done; this was also the route followed by the Japanese in assessing the AIK-C strain where several candidates were used and compared for their clinical side effects (Makino, 1983). Little supportive in vitro laboratory data is provided in the literature.

While the vaccines in use are life saving and do not have unacceptable side effects they all cause fever to some extent and occasional rash about seven days post-immunisation; this resembles mild measles. At some stage they have been accused of more serious side effects including inducing autism and Crohn's disease (Duclos and Ward, 1998; Afzal and Minor, 2002; Afzal et al., 2006). These linkages have not been supported by closer examination but indicate the mystique of measles and the extent to which it is not fully understood.

Measles vaccines are live viruses given by injection so that in the presence of maternal antibody the vaccine is neutralised and fails to immunise. In developed countries vaccination is delayed until about 12 months of age so that maternal antibodies are at undetectable levels for all recipients. The decay of maternal antibodies varies between individuals so that some children may be unprotected for a long time which is a serious risk in developing countries where mortality rates are high. There have been attempts to immunise younger babies by using higher titre vaccines or other strains; immunisation by mucosal routes by intranasal administration has also been investigated. One observation that resulted was that the mortality in recipients of standard dose Schwarz strain was less than in recipients of high titre vaccines. Death was by causes unrelated to measles and the effect was particularly seen in girls and in countries where the death rate was already high. It was suggested that the standard vaccine had some beneficial side effect on the immune system (Aaby et al., 1993).

In the end the issue of controlling measles with a vaccine that is not necessarily effective in the main target group of very young children was solved by giving the vaccine in mass campaigns regardless of vaccination history (Sabin, 1991). This generates herd immunity and reduces exposure of susceptible infants. It is an example similar to that of polio where a vaccine with a flaw is used in a novel way that makes it effective.

Many of the effects of measles vaccine for good or ill are still unclear. The vaccines have greatly reduced mortality from measles and in many areas including the Americas indigenous measles has been eradicated although cases due to imported virus occasionally occur. It is not necessary to understand how the beneficial effects are generated, but it is an insecure position should some controversy arise. If the cell substrate on which the vaccine is produced should become suspect the position is difficult for a vaccine where the cell passage history is crucial, as is the case with measles. Reverse transcriptase activity was reported in vaccine produced in embryonic chick cells raising the possibility of contamination with a retrovirus which might have had serious consequences for vaccine recipients (Boni et al., 1996). The obvious solution was to produce vaccine on a different substrate but as the attenuation of the virus depended on cell passage and only the Edmonston Zagreb strain was already produced on a different cell substrate (human diploid cells) in relatively small amounts, this might well have ended in a non-immunogenic or unacceptably virulent vaccine or no vaccine at all. In fact the reverse transcriptase activity was associated with defective retrovirus particles endogenous to the chick embryos at the stage of development at which they are used for production and is still present in most of the vaccines used today. It is not thought to pose a hazard (Robertson and Minor, 1996; Duclos and Ward, 1998).

Mumps

Compared to measles, mumps is not a serious infectious disease in public health terms, rarely resulting in death. However it is a major cause of aseptic meningitis in unvaccinated populations and the parotitis, orchitis and mastitis that are associated with disease are common, painful and unpleasant, occasionally leading to sterility in post adolescent males. Mumps vaccine is usually given in combination with measles and rubella vaccines as a triple immunisation. Mumps is thought to grow poorly compared to other viruses by those who work on it, there is no reliable marker of protective immunity as there is for measles or polio and the different assays of antibody level correlate poorly and give generally low titres (Pipkin et al., 1999; Yates et al., 1996). Antibodies cross reacting with other paramyxoviruses including parainfluenza are common (Christenson and Bottiger, 1990). There is no convincing animal model (Parker et al., 2013) although the disease was transmitted between rhesus macacques in the 1930s (Johnson and Goodpasture, 1934). Many strains of mumps have now been sequenced (Jin et al., 2005) and wild type strains can be classified into different genetic lineages as with other types of virus including polio and measles. Like these viruses mumps is so far as is known antigenically monotypic; one vaccine should protect against all mumps strains. There is evidence that when the virus is grown on a new cell substrate many mutations are introduced before the virus grows optimally and while this is true of other viruses to some extent mumps is a particularly extreme case (Afzal et al., 2005). It is possible that the right cell substrate to imitate natural infection has not yet been identified which raises the questions of what cells the virus grows in in vivo, why it is as infectious as it is given its laboratory properties and whether variation in the virus occurs between different in vivo compartments.

The Jeryl Lynn vaccine strain was developed in 1966 from virus isolated from Maurice Hilleman's daughter, who had developed unilateral parotitis on March 30th 1963 (Buynak and Hilleman, 1966). Virus was isolated and passaged in chick embryo amniotic

cavity and passaged further on chick embryo fibroblasts and tested in a number of institutionalised children; virus at passage 12 produced parotitis in a proportion of recipients while virus at passage 17 did not. Passage 12 virus also produced higher titres of neutralising antibody than passage 17 illustrating the trade-off between attenuation and efficacy. Monkeys were inoculated by the intrathalamic, intraspinal and intramuscular routes; while they seroconverted with respect to neutralising antibodies there were no neuronal lesions or clinical signs with either passage level. No cell culture system could distinguish the vaccine at different passage levels from virulent viruses. In short the vaccine was developed by testing in children without much help from pre-clinical laboratory studies. Later studies showed a 95% protective efficacy in children; production was on chick embryo fibroblasts.

The Urabe strain was developed in Japan by six passages in chick amniotic cavity, cloning in quail cell cultures and further passage in eggs (Yamanishi et al., 1970). Six virus clones were tested in children for neutralising antibody and side effects, and one clone was selected and designated UrabeAm9. Production was on chick embryo fibroblasts. The Rubini strain was isolated from the urine of a child with mumps, given two passages in human diploid cells followed by 13 passages in eggs and then further passages on human diploid cells on which it was eventually produced commercially (Gluck et al., 1986). The Leningrad Zagreb strain originated in the Institute for Influenza in Leningrad, where it was first isolated by 15 passages in guinea pig kidney cultures, five passages in Japanese quail fibroblast cell cultures, and additional passage in guinea pig kidney cells before adaptation to chick embryo cells in which it was grown for production (Beck et al., 1989). Other strains have been developed and used (Mrazova et al., 2003; Boxall et al., 2008).

The Jeryl Lynn vaccine causes mild parotitis in about 1% of recipients as does the Urabe strain (Balraj and Miller, 1995). However the Urabe strain also causes aseptic meningitis in a proportion of recipients about 24 days post-immunisation; the affected children recover without long term sequelae. The precise frequency has been difficult to determine because cases were defined in part by the isolation of Urabe strain virus from CSF samples obtained by lumbar puncture. This is an invasive procedure and the clinical threshold for performing it is very variable. It is not clear whether the Urabe strain would be found in CSF of totally healthy vaccine recipients. The initial findings suggested a frequency of aseptic meningitis of 1 in 100,000 in recipients of the Urabe vaccine, but in the UK this was revised to 17 per 100,000 leading to suspension of the use of the Urabe strain although the licence was not withdrawn. Figures were higher in other countries and Japan stopped immunising against mumps altogether. No increase in the rate of aseptic meningitis was seen in recipients of the Jeryl Lynn strain although the rate of parotitis was similar to that seen with Urabe. While the Urabe strain can be isolated from parotitis cases it appears that Jeryl Lynn cannot.

The Leningrad Zagreb strain was produced in India to supply the WHO global immunisation programme. It was used in a campaign in Brazil in 1997 in which 105,098 doses were administered to children in five areas over a period of about 11 weeks. Fifty five cases of aseptic meningitis occurred in the weeks following the campaign, giving a rate of 28.7 cases per 10,000 person weeks, compared to 2.4 in the same period in the previous year. While it seems reasonable to conclude that the cases were linked to the vaccine (da Cunha et al., 2002) there was no virus isolation or characterisation and the obvious conclusion was disputed by the company, who subsequently performed a study in Egypt where no link was reported (Sharma et al., 2010). There were reports of mumps caused by the same strain (Bakker and Mathias, 2001; Kaic et al., 2008) and of a limited outbreak where strains from cases were shown to be derived from the Leningrad Zagreb strain by sequence (Gilliland et al., 2013). The vaccine was highly immunogenic.

The Rubini strain was used extensively in Switzerland and Portugal and Singapore (Schlegel and Vernazza, 1998; Afzal and Minor, 1999; Goh, 1999). Outbreaks of mumps followed but in these cases the issue was primary vaccine failure where the vaccine was insufficiently immunogenic to afford protection with a single dose. The vaccine was over attenuated which has also been observed with other vaccine strains (Mrazova et al., 2003; Boxall et al., 2008).

The balance between attenuation and immunogenicity is clearly very hard to get right for mumps. However the Jeryl Lynn strain has not been associated with either aseptic meningitis or primary vaccine failure although the duration of immunity following a single immunisation in early life has been questioned (Cheek et al., 1995; Miller et al., 1995).

The genomes of isolates from Urabe associated aseptic meningitis were subjected to sequencing and a polymorphism at residue 1081 of the HN RNA was associated with the adverse event (Brown and Wright, 1998) implying that changes in the virus replicating in the human host may be of significance in contrast to yellow fever. According to existing regulations mumps vaccine seeds must be tested for neurovirulence by intracranial inoculation of monkeys; it has been shown that this will not distinguish the vaccine from the corresponding aseptic meningitis isolate, and in fact while there are differences between different vaccines and wild type strains there is no clear correlation with the phenotype in humans (Afzal et al., 1999). A more sensitive and acceptable form of this test has been developed in neonatal rats but again while it will distinguish strains it does not differentiate the vaccine from the meningitis isolate (Rubin et al., 2005). It is possible that more sophisticated methods including deep sequencing may help understanding in particular of the various subpopulations found (Sauder et al., 2006).

The other vaccines were also subjected to sequencing. The Jeryl Lynn strain was shown to be a mixture of two very different strains from the same genetic lineage (Afzal et al., 1993) suggesting that the original infection involved two distinct viruses, that contamination had occurred during the isolation and passaging of the virus or that the viruses had diverged on passage. The number of differences may be thought to make the last explanation unlikely. The two strains could explain the results of the original clinical trials if the proportion were related to clinical phenotype and changed on growth. Secondly the Rubini strain, reportedly isolated from the urine of a child in Europe, was shown to be very closely related to the laboratory Enders strain and distinct from other available European strains (Yates et al., 1996). The most likely explanation seemed to be that it arose by laboratory contamination.

Neither of these findings affected the continued use of the respective vaccines the decision being based solely on their clinical performance. They illustrate again the difficulty of working with mumps virus.

Mumps vaccines have a major effect in preventing mumps. It is disturbing that it is not always clear why, or what properties make them safe and effective and, in the last analysis, even where they came from.

Rotavirus

Rotavirus is a member of the family reoviridae, the virion being composed of 11 segments of double stranded RNA in a complex capsid. Two of the proteins VP4 (P) and VP7 (G) are targets of neutralising antibodies; the P protein must be cleaved for the virus to be infectious. The nomenclature is based on serology and the genotype of the P protein; thus G1 P7[5] has a G protein that is

serologically group 1 and a P protein that is serologically group7 and genetically group 5. The rationalised nomenclature of GxPy was adopted by the working group of the ICTV in 2011; in the references cited here the order is reversed (PyGx). The segments can re-assort readily and there are at least 14 G genotypes and 23 P genotypes. In humans the commonest isolates are G1P8, G2P4, G3P8 and G4P8 but G9P8 and G9P6 are also isolated. The spectrum of isolates and disease changes year on year and is very complex and country dependent (Santos and Hoshino, 2005).

Infections and diarrhoea caused by rotavirus occur world-wide independent of health or hygiene status because the virus is excreted in such colossal amounts and is so hardy that disinfection by conventional methods is more or less impossible. However, the consequence of infection is very different depending on the region and country and before vaccination in low or medium income countries, rotavirus caused 500,000 deaths per year. Irrespective of the subtype of virus or the location the first infection is the most severe; subsequent infections occur but are not life-threatening to the same extent. Virus excretion at some level is prolonged, typically 30 days.

In developed countries the mortality rate is very low as a consequence of available levels of health care. However treatment such as rehydration is only effective if given in time, which with the heath care infrastructures in low and medium income countries can be difficult. Treatment has had a major effect on deaths from diarrhoea in developing countries but mostly for non-rotavirus disease (Glass et al., 2005). The introduction of effective vaccines into existing programmes where high coverage has been achieved is likely to be one of the most cost effective interventions.

The strategy for developing live attenuated vaccines against rotavirus has been mixed and efforts continue. Vesikari developed a vaccine based on an isolate of a calf rotavirus designated RIT4237, which was a G6P1 type. (Vesikari et al., 1984). This classical Jennerian approach of taking an agent that resembled the human virus of interest while not being derived from it gave a vaccine that worked well in Finland, but failed when tried in developing countries (De Mol et al., 1986). This was attributed to the competing effects of other enteric infections; live polio vaccine was also shown to reduce take of the rotavirus vaccine. Kapikian used rhesus monkey rotavirus MMU 18006, a G3P3 strain, as the base but constructed reassortants with MMU 18006 containing the human G1, G2 and G4 segments to produce the tetravalent vaccine Rotashield. This was the first rotavirus vaccine to be licensed globally in 1998 but was later withdrawn from the market when it was associated with a low incidence of intussusception in recipients where the gut invaginates potentially leading to blockage. The product remains licensed and there is still debate about the significance of the syndrome, which may have occurred in those who were destined to develop it anyway at some time, the vaccine being the precipitating factor.

Three other vaccines were subsequently licensed: Rotarix, licensed in Europe in 2006 and in the USA in 2008, Rotateq, licensed in the USA in 2006 and the Lanzhou strain of lamb rotavirus, licensed in China and widely used there since 2000. Rotarix is a single human strain (G1P[8]). Rotateq is a pentavalent mixture of human/bovine re-assortants (G1P7[5], G2P7[5], G3P7 [5], G4P7[5] and G1P1A[8]). The Lanzhou strain is a monovalent G10P[12] (Fu et al., 2010). Other vaccines include a monovalent attenuated neonatal human strain containing one bovine segment (G9P[11]) manufactured by Bharat in India and other multi- or monovalent strains in development in many other regions of the world. The nature of the optimum vaccine is clearly still a matter of debate with no clear winning strategy. It is not clear whether multivalent vaccines are needed or whether a monovalent vaccine could effectively prevent the high mortality associated with the first infection by taking the place of the first attack as some clinical trials suggest; full protection against infection and disease is strain specific however. Guidelines for the production of oral rotavirus vaccines have been developed by WHO (2007) based on the licensed and effective vaccines in use.

The development of vaccines against rotavirus have thus followed a familiar pathway of careful clinical evaluation and unpredicted events.

Vectored vaccines

Vectored vaccines are usually taken to be constructed from a carrier virus such as an adeno or pox virus which has been modified to carry a gene from a virus of interest. Thus when the recipient is given the vector the gene will be expressed and protective immune responses including antibodies and T cell responses will be generated. In some cases the relevant gene is obvious as for the haemagglutinin of measles or the G protein of rabies virus, although in practice there may be more to protection than an immune response to a single antigen. Vectored vaccines have been explored in a number of instances by established manufacturers (Plotkin et al., 1995; Priddy et al., 2008; Watkins et al., 2008) and many biotech companies and spinouts. While the approach has generated licensed gene therapy products and a rabies vaccine used to immunise wild fox populations (Pastoret et al., 1995) no licensed human vaccine has resulted. This may be related to the completeness, duration and protective efficacy of the immune response generated, commercial concerns about the scale of production required to meet global requirements and the availability of easier options that are a priori more likely to succeed, including the production of live attenuated vaccines or the non-replicating equivalents. The strategy could lead to generic solutions to vaccine design if it worked which has not been clearly demonstrated.

A different category that most would not think of as a vectored vaccine has been successful however. Yellow fever virus is a flavivirus for which there is a highly safe and effective if rather poorly understood live attenuated vaccine as reviewed above. Other human flaviviruses include the agents of Japanese encephalitis and Dengue fever both of which are major human pathogens in certain areas of the world. The strategy followed is to replace the capsid proteins of the 17D derived yellow fever vaccine with the equivalent region of either JE or one of the four serotypes of Dengue virus or other flaviviruses to give a replicating virus expressing a novel antigen (Monath et al., 2002). The assumption is that the attenuated phenotype of yellow fever does not depend on the capsid region or that replacing it with a foreign capsid region will itself be attenuating. The JE vaccine Chimerivax based on this principle has been licensed in Thailand and Australia, although it is not extensively distributed at the time of writing (WHO, 2002). The equivalent Dengue construct is in clinical trial (WHO, 2005, 2014b, 2014c; Capeding et al., 2014). The approach is virologically less disruptive than the usual vectored vaccine approaches; most of the machinery remains the same or at least highly homologous to the natural infection and the vaccine may therefore be more likely to succeed.

A similar thought process could be applied to live attenuated influenza vaccines which use the core of an attenuated virus onto which the current seasonal surface proteins are grafted by reverse genetics. Again most would probably not think of this as a vectored vaccine.

Discussion and conclusions

Live vaccines against viral diseases are one of the most cost effective health interventions currently available. Their use has

eradicated one infectious disease of humans and poliomyelitis is close to becoming the second. Measles has been controlled in the Western hemisphere and in much of the developing world by the use of live viral vaccines and rotavirus vaccines may be the best way to reduce rotavirus mortality in the world at least in the short term until better health care infrastructures are developed. Vaccines are shown to be successful by clinical experience and widespread use with monitoring of adverse events and efficacy as an on-going process.

The understanding of where they came from and why they are successful is generally poor. The failures of vaccines relate to adverse events such as causing the disease they are meant to prevent, or lack of efficacy and there is no clear way to tell where on the spectrum a given live vaccine lies other than by using it and acting on the results. For this reason a live vaccine against HIV or any other virus that causes a lethal disease is problematic, even if it seems possible that it would be the most likely to succeed. Once a live vaccine is used it is out of control so far as the patient is concerned and that can be a challenge.

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