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Review article

Vaccines for yellow fever disease: A narrative review

Sumira Malik[1], Jutishna Bora[1*], Richa Mishra[2], Nayan Talukdar[3], Archna Dhasmana[4], Sagnik Nag[5], Geeta Bhandari[4], Swati Priya[1], Sarvesh Rustagi[6], Seema Ramniwas[7]

1- Amity Institute of Biotechnology, Amity University Jharkhand, Ranchi, Jharkhand, 834001, India
2- Department of Computer Engineering, Parul University, Ta. Waghodia, Vadodara, Gujarat, 391760 India
3- Program of Biotechnology, Faculty of Science, Assam down town University, Guwahati, Assam, India
4- Himalayan School of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, Uttarakhand, 248140, India
5- Department of Biotechnology, School of Biosciences & Technology, Vellore Institute of Technology (VIT), Tirvalam Road, Tamil Nadu - 632014, India
6- School of Applied and Life Sciences, Uttaranchal University, 22 Dehradun, Uttarakhand, India
7- University Centre for Research and Development University of Biotechnology, Chandigarh, University, Gharuan, Mohali, Punjab

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ABSTRACT

Background: Yellow fever is a severe and often fatal illness caused by Flavivirus. Yellow fever is endemic in tropical and subtropical regions of South America and Africa. The present study has highlighted the urgent requirement for an effective vaccination drive to be implemented in areas with the potential of an endemic outbreak. Vaccination is the best way to prevent yellow fever, especially for people traveling to affected areas. The yellow fever vaccine is highly effective; protection usually begins by the 10th day after vaccine administration in 95% of people. The vaccine has been reported to last for at least 10 years, and a single dose is now considered to confer lifelong immunity against yellow fever disease by the WHO. The attenuated vaccine strain 17D of the yellow fever virus is commonly used in research laboratories to study the virus and develop antiviral therapies. The vaccine strain is weakend and does not cause human disease, making it safe for laboratory settings without requiring high-level microbiological containment facilities. In particular, the vaccine strain is used to evaluate the inhibitory effect of compounds on yellow fever virus Vero cells. Vero cells are a type of monkey kidney cells that are commonly used in virus culture and antiviral research. Researchers can screen many compounds using vaccine strains, and cells identify potential antiviral agents against the yellow fever virus. Despite these challenges, several vaccines have undergone preclinical and clinical testing on humans. The group of vaccines includes vaccines that are like viral particles, DNA-based vaccines, entire virus recombinant vaccines, vaccines with origins in incompetent replication, attenuated vaccines, and vaccines with origins in competent copy. In conclusion, developing an effective yellow fever vaccine is crucial in containing the upsurge of the disease. The current review discussed several challenges in creating such a vaccine, progress has been made in recent years, and the current outlook is promising. Further, appropriate post-authorization and surveillance may be crucial factors in monitoring the vaccine's safety and efficacy in real-world settings.

Introduction

Yellow fever virus (YFV) is primarily transmitted to humans through the bite of infected mosquitoes, the Aedas, Sabethes, and Haemagogus species. The virus is endemic in tropical regions, where it can cause periodic epidemics and sporadic cases. Yellow fever (YF) can cause significant death in areas where the virus is endemic. There are an estimated 200,000 cases of yellow fever and 30,000 deaths worldwide yearly, primarily in tropical

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* *Corresponding author:* Jutishna Bora
E-mail address: jbora@rnc.amity.edu

regions [1]. Yellow fever is caused by the YFV, which is enveloped, positive-sense, single-standard RNA virus belonging to the Flaviviridae family and the Flavivirus genus [2].

Phylogeographic studies have provided evidence that the YFV likely originated in Africa and was introduced to the Americas via the slave trade during the 15th and 16th centuries. Genetic analysis of YFV strains from different parts of the world has revealed distinct African genotypes, which are thought to represent the ancestral lineage of the virus. The subtropical climatic conditions in Central America and the prevalence of amenable insect vectors provided an ideal environment for transmitting YFV, which subsequently spread to non-endemic, more populous regions, including the coastal towns of eastern America. Yellow fever virus is classified into seven genotypes, with two primary YFV clades; the first involves four genotypes, and the two are from western Africa and South America. The diversification of YFV genotypes is believed to have occurred much longer than 47 decades ago. Their analysis estimated that YFV diverged from other flaviviruses around 1,000 years ago, and the seven known genotypes subsequently diverged from each over the centuries. The second branch, the central/ East African branch of YFV, is not exclusively comprised of three genotypes. While genotypes V, VI, and VII are primarily found there, including genotype IV [3].

The YF vaccine has been used for over 80 years and is based on the 17D strain of the YFV. These strains, known as 17D, were highly effective in inducing immunity to yellow fever while causing only mild or no symptoms in vaccinated individuals. Since the development of the 17D strain, several variants of the YF vaccine have been developed, including the 17DD (passage 195), 17D-204(passage 204), and 17D-213 strains. These variants differ, but all are based on the original 17D strain and provide lifelong protection against yellow fever after a single dose [4]. The World Health Organization (WHO) recommended a single dose of the YF vaccine to confer lifelong protection against the disease. However, some countries and organizations have implemented a booster dose recommendation every ten years for people at continued risk of yellow fever exposure. The side effects of the YF vaccine are mild and occur within ten days of vaccination. These may include local reactions at the injection site, such as pain, redness, or swelling, as well as systemic symptoms, such as headache, fever, muscle aches, and fatigue. However, while rare, serious adverse events have been reported following YFV vaccination. These can include hypersensitivity reactions, such as anaphylaxis, and rare but severe neurotropic or viscerotropic disease manifestations. These serious events are estimated to occur at a rate of about 1 per 100,000 doses administered and are more common in people over 60 years of age. The re-emergence of the YFV in low- and high-risk areas is a concern, and vaccination is a crucial strategy for preventing outbreaks. Achieving high vaccination coverage rates is essential for interrupting local transmission of YFV by reducing the number of susceptible individuals in a community [5, 6]. According to the WHO, a target vaccination coverage rate of at least 80% is recommended for achieving herd immunity against YFV in a given population. In high-risk regions where YFV transmission is ongoing, achieving high vaccination coverage is essential for prevention and control from the outbreaks. Several approaches can be used to improve vaccination efficiency in these regions, including strengthening routine immunization, mass vaccination campaigns, and targeted vaccination. These approaches will depend on the local context, including vaccine supply, healthcare infrastructure, and community attitudes toward vaccination.

International health regulations require proof of YF vaccination for travelers entering certain countries, particularly those with a risk of Yellow Fever transmission. These countries (DRC, Mauritania, Kenya, and China) have different requirements regarding YF Vaccination for travelers. For example, the Democratic Republic of Congo (DRC) requires proof of vaccination from all travelers arriving from countries with a risk of YF transmission. In contrast, Kenya only requires vaccination proof for travelers from endemic countries [4]. The WHO Eliminated Yellow Fever Epidemics (EYE) program recommends that all travelers over nine months receive YF vaccination before entering countries with a risk of YF transmission in Africa and South America. The EYE program also recommends the use of fractional dosing, which involves administering a smaller dose of the YF vaccine to increase the number of people who can be vaccinated and to conserve vaccine supplies.

Immunization & factors affecting immunization using YFV vaccines

The target population affected by YF is all age groups and gender, and to date, up to 200,000 cases have been reported annually, with a death number of 30,000/per year [7]. In the last two decades, most of the cases were reported in the four American countries: Peru, Bolivia, Brazil, and Colombia, having fatality rates of 54%, 18%, 16%, and 7%, respectively [8]. The CFR (case fatality rate) was estimated at 15/23 (death/infected cases) in 2013 by PAHO/WHO of the Peru and Colombia populations. This was significantly higher than last year's South American remote areas data. Hence, the researchers concluded that from the time period 2000 to 2013, a huge number of American populations, especially two countries: Brazil and Peru, were the most affected [9]. Besides that, the urbanized sectors of the mosquito Aedes aegypti be the vector for the transmission of the YF, which was totally different in the Haemagogus spp. and Sabethes spp. in the evergreen forest categorized as jungle YF [10]. Therefore, the transmission mode is from the primary host monkey to monkey, monkey to human, and globally. The protective measure taken by health care agencies is to prevent mosquito breeding and early vaccinations. Therefore, the person from the endemic native place or the traveler who gets infected or bitten by infected mosquitoes could be the target of yellow fever. The main drawback of YF treatment or vaccination is the variability of expression and effectivity. In the tropical region of Africa and America, viral acute hemorrhagic symptoms are the same in dengue or other viral infections like arenavirus. Within a week of biting, the next phase is crucial and toxic, with high fever causing organ failure and 50% death within 15 days.

The symptomatic person has poor survivability %, with complete supportive care due to incurable medication. Thus, early vaccination is the enduring shield against the spread of pathogen infection in society and viral protection; over one billion people are expected to be protected by 2026 [11]. A global strategy to eliminate yellow fever epidemics (EYE) 2017–2026.

Early vaccination is the only safe, affordable, and successful preventative measure. It strongly stimulates the host immune system and gives 99% of people who receive it in one dose effective immunity within 30 days [12]. On a global scale, the health agency CDC provides guidelines to implement in the target countries for improvised vaccinations. Under these guidelines, an individual of any gender ≥ nine months gets vaccinated, and visa allotment after vaccinations for specific countries. The YF 17D was the first attenuated live vaccine designed from the Asibi strain isolated in 1927 from Ghana yellow fever infected samples, and its sub-strains 17D-204, 17DD (17D-213) also used vaccine planning YF-VAX® Moreover, WHO makes the guidelines in Annex 5, TRS No 978 for the quality assurance of the live attenuated vaccine for safe immunization with significant efficiency against YF [13].

The factor affecting the vaccination is the immune response after vaccinations, especially for those with a medical history of acute hypersensitivity reaction having contraindicated outcomes. adversative outcomes are also observed in the individual having severe immunosuppression disorders or due to prolonged chemotherapy and suffering from anaphylaxis. Previous estimates of YF vaccination-related adverse events were based on U.S. government rep. It was thought that the age distribution of vaccine users at 13 United States-based travel clinics in 1998 was crucial in estimating this risk. Subsequently, vaccination is not recommended for infants aged 9 month or less or for lactating females. In-person≥60 years, severe immunoreactions reported after vaccinations, such as YF vaccine-associated viscerotropic disease (YEL-AVD), multiorgan failure, and post-vaccinal encephalitis. In the clinical study, the risk factor estimated in age 60-69 is 0.004% and 0.0075% ≥ 70 years. However, asymptomatic HIV-infected patients could experience antagonistic effects post vaccination. Overall, 17D vaccines are the only means of preventing YF disease. Immunization against YF is highly advantageous for travelers and those participating in YF outbreak management activities. Only those traveling to an area with a danger of YF should receive the vaccine 17D. It is crucial to carefully assess whether the person should have the vaccine, just like any other shot. Only those with clear exposure concerns to YF should receive the vaccine, and the contraindications to giving it should be carefully considered.

Vaccination protocol and strategies of YF vaccines development

After the successful isolation of the Asibi strain of the virus from a mild human case in Ghana in 1928, known as "Mr. Asibi", researchers dedicated much of the early 20th century to

developing a vaccine. Two approaches to creating a live, attenuated vaccine were considered during this period. The French viscerotropic virus (FVV) wildtype, discovered by Francoise Mayali in Senegal in 1928, was introduced into the mouse brain to create the French neurotropic vaccine (FNV) vaccine. On the other hand, the French neurotropic vaccine was developed using the wild-type Asibi strain and attenuated by passing it through chicken tissue to produce the 17D vaccine [14].

Development of the French Strain

After 128 passes through mouse brains, the first live-attenuated FNV variant was discovered, and by the 260th pass, it was ready for vaccination. The vaccine virus had lost its ability to transmit mosquito competence and cause viscerotropic (liver infection). Steps were taken to avoid both human transmission and return to virulence in mosquitoes, which is essential for a live attenuated vaccination strain based on a virus carried by mosquitoes. The widespread use of FNV in French-speaking regions of Africa significantly reduced the incidence of YF cases. However, passage through the mouse brain increased the virus's neurological potential, which increased the risk of post-vaccinal encephalitis in children. As a result, immunization is no longer recommended for adolescents under the age of 14. The FNV vaccine was discontinued in 1980 after the success of the 17D vaccine. Nonetheless, during the 1940s and 1950s, FNV was a highly effective vaccination for controlling YF in Africa [14].

Development of 17D strain

The 17D vaccine was developed by passaging the wild-type Asibi strain 18 times in minced mouse embryos, followed by 58 passages in the minced chicken embryos. Finally, 128 courses in minced chicken embryo with the spinal cord and brain removed. After 176 passages in chicken tissues, the 17D vaccine strain was identified and found to have the desirable characteristics of a live attenuated vaccine that is highly immunogenic and attenuated. The 17D strain had lost its ability to cause neurotropism, viscerotropism, and mosquito competence. The vaccine was highly immunogenic, with less than 1000 infectious units required to elicit protective immunity against the vaccine virus. The first human immunization tests were published in 1937, and the vaccine was quickly developed as a vaccine produced in embryonated chicken eggs. Significantly, the 17D vaccination was used by several sources, and the vaccine's passage level was increased considerably, sometimes up to 400 passages. However, the long transit histories occasionally led to over-attenuation, which reduced the vaccine's immunogenicity. As a result, a seed-lot method was developed in 1945, using "primary seed" viruses to produce "secondary seed" viruses. The latter was utilized to produce vaccine lots for human immunization. Over the previous 75 years, the seed-lot technique has generated about 650 million vaccine doses from 13 different producers. The vaccine is a freeze-dried substance reconstituted into a single dosage of 0.5 ml and administered through intramuscular or subcutaneous routes. There is no upper limit on the amount of virus that can be included in a single dosage; each dose must contain at least 1000 international units (IUs). A dosage typically comprises between 4000 to 1 million IUs. Despite research showing that within 30 days after vaccination, 99% of adult vaccine recipients had seroprotective levels of neutralizing antibodies, current data reveals that this percentage is less than 90% in children. A booster dose was once every ten years until recently. However, a WHO study recommended that most patients stop receiving booster doses due to evidence of persistent, possibly lifelong protective immunity. This change took effect in July 2016. However, not all nations have adopted this advice; for instance, Brazil still uses 10-year boosters. The 17D vaccine is always referred to as a "legacy" since it was created in the 1930s before modern vaccine creation methods were established. In the twenty-first century, this does result in specific challenges. Since cell culture was not developed while the vaccine was being produced, the virus was initially titrated using a 50% mouse intracerebral fatal dosage (MICLD50). The viral titration was then changed to utilize plaques (pfu) in the Vero cells of the monkey kidney. But it soon became apparent that pfu to MICLD50- s ratios and viral plaque testing differed between manufacturers. As a result, IUs were used to standardize vaccinations from various manufacturers. Protection measurement is the other important issue. To achieve protection against disease, vaccination requires the development of immunity. In some cases, passive protection can be achieved through transmitting antibodies. In the case of the 17D YFvaccine, it has been shown that a neutralizing antibody titer of 0.7 by log neutralization index (LNI) can provide this passive protection in non-human primates. In this

test, the virus can be present at different (10-fold) concentrations while the antibody level stays the same, producing a seroprotective titer where the viral titer is reduced by 100.7. We now utilize 50% plaque reduction neutralization tests (PRNT50), in which the virus amount is constant but the antibody concentration changes (usually approximately 50 pfu). Yet, data from a hamster trial demonstrate that a PRNT50 titer of 1 in 40 is seroprotective, even though LNI and PRNT50 tests have never been directly compared in controlled testing. The hamster experiment results might not apply to people, though. The current approach for vaccine approval uses non-inferiority studies in clinical evaluation when a "new" vaccination is superior to a licensed vaccine.

17D lineages

The seed-lot method is still in use today, and the vaccine is being made using 1940s-era technology in embryonated chicken eggs. The vaccination virus, however, exists as three different substrains. Asibi's 204th passage in chicken tissue served as the source for the 17D-204 strain, the vaccine virus, created between passages 234 - 238. The 17DD substrain was made from chicken tissue from the 195th passage of Asibi and through a unique passage history to become 17D-204, which was then utilized during passages 285-288. Lastly, the 17D-213 strain is employed at passages 238–239 and was developed from 17D–204 at passage 235 by the Robert Koch Institute in Germany. The three substrains cannot be regarded as having the same genotype or phenotype since they have slightly distinct genomic sequences, as would be predicted. Notably, the phenotype of a flavivirus is known to be influenced by the different envelope protein glycosylation sites [15]. However, it should be noted that there is no evidence to suggest that the attenuation or immunogenicity of the vaccinations differs [16]. While vaccines have been evaluated for non-inferiority in clinical assessments at doses required to immunize humans, the vaccine viruses have never undergone thorough head-to-head comparison testing. Research has been conducted to identify the molecular causes of the attenuation of the 17D vaccine. Attenuation has been studied utilizing genomic sequence comparisons of the 17D-204, 17DD, and 17D-213 vaccinations from various manufacturers to identify the common mutations in all three vaccination lineages because the original passage 176 attenuated variant is no longer available. In the 30 untranslated regions, the total standard of alterations in 4 nucleotide changes has been found in the 3' untranslated region **(Table 1)**. The structural proteins have nine amino acid substitutions: one in the membrane and eight in the envelope (E) protein. The non-structural (NS) proteins include 11 amino acid changes, four in NS2A, two in NS5, and one in NS1, NS2B, NS3, NS4A, and NS4B. To be clear, not all vaccine manufacturers submit their goods for prequalification; therefore, the lack of prequalification does not imply that a vaccine is worse than one that has.

Immune responses mediated by YF-17D

Vaccination with YF-17D triggers diverse adaptive immune responses, encompassing the generation of cytotoxic T cells, a combination of T helper 1 (Th1) and Th2 cell profiles, and strong and enduring production of neutralizing antibodies that can endure for as long as 40 years following vaccination. However, until recently, there was limited understanding of the interplay between YF-17D and the innate immune system, as well as the implications of these interactions for fostering the adaptive immune response.

Adaptive immune response to YF-17D. Vaccination with YF-17D leads to an acute viral infection characterized by transient viral replication peaking around 5 to 7 days before diminishing. The primary safeguard against yellow fever virus infection is believed to be neutralizing antibodies [16, 17], and vaccination has been shown to confer protection in over 90% of recipients. YF-17D prompts a swift and specific production of neutralizing antibodies, primarily of the IgM subclass, within seven days post-vaccination, reaching peak levels at two weeks [17]. Interestingly, during the initial 4-6 weeks, IgM antibody titers surpass those of IgG antibodies, persisting for at least 18 months. Meanwhile, IgG neutralizing antibodies develop more gradually and can endure for up to 40 years. The mechanisms driving such a sustained antibody response remain unknown, as do the cellular and molecular processes responsible for the initial prolonged IgM response. While T cells are considered to play a crucial role, only a limited number of studies have explored T cell responses to YF-17D [18]. Human $CD8^+$ T cells responsive to YF-17D have been found to recognize epitopes from E, NS1, NS2b, and NS3 proteins [19]. Recent research in humans has confirmed the expansion of effector $CD8^+$ T cells following

immunization with the live attenuated YF-17D vaccine. This was monitored through the expression of activation markers CD38, HLA-DR, and Ki67, as well as the downregulation of intracellular B cell lymphoma 2 (BCL-2) in T cells in the blood [20]. The downregulation of intracellular BCL-2 is a characteristic feature of activated effector $CD8^+$ T cells. By analyzing HLA-DR- and CD38-expressing $CD8^+$ T cells at multiple time points, the magnitude and kinetics of T cell responses post-immunization could be evaluated. The peak of the $CD8^+$ T cell response occurred on day 15 post-immunization, with 4-13% of peripheral $CD8^+$ T cells co-expressing CD38 and HLA-DR. Consequently, immunization with YF-17D triggers a substantial expansion of the activated $CD8^+$ T cell population [20]. The number of activated $CD8^+$ T cells decreases after day 15, returning to normal levels by day 30 post-immunization.

Innate immune response to YF-17D. The concept that the initial innate immune response to YF-17D might play a crucial role was introduced through a 1945 study demonstrating partial protection in monkeys challenged with virulent virus 1–3 days after YF-17D administration, before the onset of antibody production [21]. Additionally, simultaneous or near-simultaneous inoculation with YF-17D and dengue virus was found to delay the onset of dengue fever. It is now established that the innate immune system significantly influences the strength and nature of the adaptive immune response. Dendritic cells (DCs) play a pivotal role in sensing microbial stimuli and orchestrating the adaptive immune response through various pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I) receptors, C-type lectins, and nucleotide-binding oligomerization domain (NOD)-like receptors [22, 23].

Despite a growing understanding of the role of the innate immune system in modulating adaptive immune responses, it was unclear until recently whether successful empirically derived vaccines achieve their immunogenicity by activating TLRs. A few years ago, it was discovered that YF-17D activates multiple DC subsets, including myeloid DCs and plasmacytoid DCs, through TLR2, TLR7, TLR8, and TLR9, leading to the production of potent pro-inflammatory cytokines, including interferon-α (IFNα), by plasmacytoid DCs. The exact viral components triggering these TLRs remain unknown, but it is presumed that viral nucleic acids activate TLR7 and TLR8. Intriguingly, robust induction of IFNα production by plasmacytoid DCs following YF-17D stimulation requires TLR-mediated activation of the mammalian target of rapamycin (mTOR) pathway, which regulates various cellular processes. Inhibiting mTOR activation in antigen-presenting cells resulted in decreased type I interferons and impaired antigen-specific CD8+ T cell responses post YF-17D immunization [24, 25].

Yellow fever-17D infects human DCs but replicates minimally in these cells. This limited replication is sufficient for the presentation of endogenous epitopes and those from proteins encoded by foreign genes inserted into the YF-17D vector through recombinant DNA technology. Activation of multiple TLRs results in a mixed TH1- and TH2-type cytokine profile. Successful vaccines like YF-17D achieve their immunogenicity, in part, by signaling through multiple TLRs expressed on distinct DC subsets. This raises the possibility that synthetic vaccines activating the appropriate combination of TLRs may mimic the immunogenicity of YF-17D. The question of whether engagement of these TLRs by YF-17D in human DC subsets also leads to a mixed TH1 and TH2 cell profile remains unanswered. YF-17D serves as a model vaccine, offering insights into the immunological principles that induce robust and persistent protective immune responses [26, 27]. This knowledge is crucial for designing new vaccines against global pandemics and emerging infections, with recent efforts utilizing systems biology tools to delve further into the mechanisms by which YF-17D induces adaptive immune response.

Fractional dose of yellow fever vaccination

The worldwide supply of the YF vaccine is inadequate to ensure full-dose vaccination for the millions at risk during outbreaks. With an abundance of live-attenuated 17D YFV in the existing single-dose vials, the adoption of dose sparing could significantly multiply the available vaccine doses. Fractional-dose YF vaccination is now recognized as an immediate solution, as it has been verified to provide short-term protection in outbreak scenarios.

The WHO's recommendation for dose sparing was grounded in two clinical studies. The first study, conducted in the Netherlands, was a randomized controlled trial focusing on non-inferiority. It

demonstrated that the 0.1-ml fractional dose administered intradermally was comparable to the standard 0.5-ml dose administered subcutaneously. The assessment of seroprotection involved measuring an 80% plaque reduction neutralization in the least diluted serum, spanning from 2 weeks to 1 year post-vaccination. The fractional dose (0.1 ml) contained 3200-IU live attenuated 17D YFV. Initially, we hypothesized that intradermal administration of the YF vaccine would be more immunogenic than the subcutaneous route due to the direct targeting of antigen-presenting cells in the papillary dermis. Surprisingly, despite the lower vaccine dose, the percentage of participants who demonstrated viremia via reverse transcriptase polymerase chain reaction (RT-PCR) after intradermal vaccination was as high as in the standard full dose group (50% in both groups). This observation can be explained by the rapid dissemination of live vaccines throughout the body following injection, regardless of the route, to reach their target cells, similar to a natural infection [28, 29].

The second clinical study, which formed the basis for the WHO recommendation, was also a randomized controlled non-inferiority trial, featuring a rational dose-finding design incorporating de-escalating doses of the yellow fever vaccine. The reference dose was 27,476 IU, with de-escalation to a minimum of 31 IU per vaccine dose, all administered subcutaneously in a 0.5 ml volume. In this study, the lowest vaccine dose was 587 IU, demonstrating non-inferiority to the reference dose. Surprisingly, the two lowest doses, 158 IU and 31 IU, still elicited seroconversion in 88.5% and 67% of participants, respectively. Crucially, among those in the lowest dose groups who underwent seroconversion within the initial month after fractional-dose vaccination, 98% maintained protective neutralizing antibody titers even 10 months later. Additionally, in this study, the occurrence of viremia after vaccination did not seem to correlate with the administered vaccine dose, although there was a slightly reduced and delayed viremia peak after 587 IU. It's worth noting that only 10% of participants had detectable viremia by RT-PCR, constituting a limitation in the study [30].

Hence, the findings from the observational study conducted by Ahuka-Mundeke and colleagues, assessing the efficacy of fractional-dose yellow fever vaccination in a Kinshasa cohort participating in an emergency vaccination initiative, were eagerly anticipated. Individuals at risk were administered a fractional dose of 0.1 ml, sourced from six distinct vaccine batches, each with a minimum batch potency of 2700 IU per dose. During this campaign, 10-dose vials were utilized, enabling the extraction of up to 50 vaccine doses from a single vial. Children under two years old and pregnant women were given the standard vaccine dose. For individuals seronegative before vaccination (n = 493), 98% experienced seroconversion one month after receiving the fractional dose, irrespective of age or gender. However, the geometric mean titers (GMT) were lower in the 2- to 5-year-old age group and exhibited a peak in the 13- to 50-year age group. Importantly, this study also demonstrates that valid concerns regarding the cold chain and the repeated use of vials can be effectively addressed in emergency vaccination campaigns [31].

The utilization of fractional-dose YF vaccination is gaining increased acceptance as a strategy to conserve doses during periods of vaccine scarcity, as long as the essential minimum potency criterion is satisfied. The recent findings indicating prolonged protection are promising, suggesting that the initial short-term seroresponse likely correlates with the long-term seroresponse. However, it's essential to note that the study population evaluating long-term protection is limited and may not consistently reflect the demographics of populations residing in YF-endemic regions.

Immunization mediated through yellow fever vaccine- in elderly & children

Data on longer-term humoral immunity for at least eight different cohorts of individuals who received the total dosage of the YF vaccination in both endemic and non-endemic regions of the world are available, and there were no significant differences between them. Sero-positivity rates peaked at over 90% in all cohorts' first five years following immunization. However, except for a small cohort of healthy volunteers in the Netherlands, where 97% (34/35) of those who received the vaccine at ten years were seropositive when tested with the Plaque Reduction Neutralizing Test (PRNT)80, seropositivity rates at ten years post-vaccination ranged from 67% to 88% [32-34]. In a trial of 595 children in Colombia and Peru who received the YF vaccine alone or combined with a dengue vaccine, the seroconversion rate was 99.8-100%. These percentages were similar to those observed in Mali (95–98%) among children who

received the YF vaccine and the meningococcal A (Men A) vaccine simultaneously or sequentially. However, in Ghana's same Men A vaccine research, children only attained 68–79% seroconversion rates lowing YF immunization. When the cohorts were followed up at 2–6 years after vaccination, the same trend of lower rates of detectable antibodies between the two populations was still evident from the Men in a research [35]. Children in Ghana had seropositivity rates as low as 68-79% at 2-3 years after vaccination; however, after six years after vaccination, the percentage had climbed to 83%, as opposed to 95-98% seropositivity among children in Mali at 4.5 years after immunization[34].

Yellow fever-live attenuated vaccine (YF-LAV) may have severe contraindications in children below six months of age owing to their underdeveloped immune systems. Children can also suffer from the serious adverse events of YF-LAV through passive immunization from their mothers (in case pregnant or lactating women are vaccinated). This advice is based on the argument that the vaccine's neurotropism in early newborns implies a higher risk. Nevertheless, the WHO advises making a risk-benefit analysis when there are outbreaks and when a location is endemic for YF since the advantages of immunization can outweigh the risk of passing the attenuated virus to the fetus. The fact that this YF-LAV is formulated using embryonated chicken eggs can result in severe adversities and anaphylaxis in children allergic to chicken egg proteins [36].

There is a high risk of severe adverse events (SAEs) post-vaccination in older adults, that is, those who are aged above 60 years. This prevalence is more pronounced in people aged above 70 rather than in the age group 60-69. According to some reports, Passive monitoring databases identified only a small number of cases of allergy, viscerotropic disease, and neurotropic disease linked to the YF vaccination in individuals over the age of 60, which is consistent with the prevalence ratio meta-analysis. Another study found that the reporting rates of serious adverse events, including viscerotropic disease and neurological events, were significantly higher among individuals aged 60 years or older compared to those aged 19-29 years (non-elderly) after yellow fever vaccination [37].

Limitation of current vaccines and the emergence of next generation vaccines

Despite the widespread success of YF vaccination over the past 75 years, it remains a significant public health concern in areas where the virus is endemic or sporadic. Particularly during emergencies like the 2016 outbreak in Angola, there has been a noticeable shortage of the YF-LAV [36]. The current method of manufacturing YF-LAV involves slow and labor-intensive processes, relying heavily on a limited supply of virus-free eggs [38]. This production method only yields a modest number of doses per egg, exacerbating the gap between supply and demand. Given the challenges faced by global manufacturers in meeting the increasing demand for the vaccine, alternative strategies need to be explored. One potential solution is to consider fractional dosing and reassessing the need for booster doses [39]. However, as of 2019, fractional dosing of YF-LAV did not meet the requirements of the International Health Regulations, and thus was not approved by the WHO except in the context of large outbreaks. Additionally, there are other gaps that need to be addressed, such as ensuring vaccination for immunosuppressed individuals. YF-LAV contains a live virus, making it unsuitable for use in immunosuppressed individuals due to the risk of uncontrolled virus replication and vaccine-related complications [40].

While the YFV-17D is known for its strong immunogenicity, concerns persist regarding its safety. The vaccine's effectiveness is undisputed, and high immunogenicity. However, this live attenuation also introduces most of its contraindications and inherent risks. Severe adverse events of YF vaccination are rare but can lead to hospitalization, long-term disability, or even death [36]. These SAEs are typically categorized as either vaccine-associated neurotropic disease (YEL-AND) or vaccine-associated visceral disease (YEL-AVD), which manifests as multi-organ involvement similar to YF itself. Clinical data on SAEs indicate that their development is primarily influenced by host characteristics rather than vaccine viruses reverting to a pathogenic phenotype. Neurological manifestations are the most common SAEs observed, particularly in infants under six months of age. YEL-AND encompasses a range of neurological issues such as acute disseminated encephalomyelitis, bulbar palsy, Guillain-Barré syndrome, and meningoencephalitis. YEL-AVD

was formally identified in 2001, with a high mortality rate among affected individuals. While factors like sex and age are important risk parameters, many cases have been reported in otherwise healthy young children and adults [41]. Increased monitoring following the initial documentation of YEL-AVD has led to a rise in reported cases across different continents, with retrospective analysis indicating instances dating back to the 1970s [41]. The incidence of SAEs associated with the YFV-17D vaccine is comparable to that of other vaccines like oral poliovirus vaccine (OPV) and RotaShield, both of which were withdrawn globally due to safety concerns. Preliminary vaccine failure appears to be more common in children vaccinated with YFV-17D compared to adults, with seroconversion rates ranging from 69% to 85.8% in some cases [42].

Developing new YF vaccines using advanced technology is crucial to address the potential for future outbreaks without facing the risk of vaccine shortages. Ideally, a next-generation yellow fever vaccine should overcome the limitations and contraindications associated with current production methods while maintaining high levels of immunogenicity and safety. To evaluate protective efficacy, two commonly used surrogate markers are the log10 neutralization index (LNI) of ≥0.7 or a neutralization titer of ≥1:10.4. Currently, various yellow fever vaccine candidates are in different stages of pre-clinical or post-clinical development. These include inactivated vaccines, mRNA vaccines, plasmid-vectored DNA constructs, virus-like particles (VLPs), recombinant vaccinia constructs, and plant-produced subunit vaccines. One approach involves creating an inactivated yellow fever vaccine candidate using embryonic chicken fibroblasts or Vero cells from monkey kidneys, involving the 17D strain and subsequently inactivating it with formalin or beta-propiolactone (BPL) [17]. However, no inactivated vaccine has been licensed yet. XRX-001 is one such inactivated vaccine candidate, produced by purifying and inactivating YF-VAX cultivated in Vero cells containing BPL and adsorbed to Al(OH)3 [17]. Another candidate, developed by Bio-Manguinhos/FIOCRUZ, involves cultivating the 17DD substrain in serum-free Vero cells, showing scalability and preserving antigen structure [43]. Recombinant vaccinia viruses have been engineered to produce both structural and non-structural proteins of the 17D strain, inducing the synthesis of neutralizing antibodies and offering protection against YFV [44]. A plasmid-based infectious cloning system has also been developed to produce plasmid-launched live-attenuated vaccines (PLLAV), showing promise in terms of safety and similarity to the 17D vaccine [45]. Additionally, DNA vector vaccines and multivalent virus-like particle vaccines have been explored. The use of mRNA technology, proven effective in recent COVID-19 vaccines, has also been researched, with efforts underway to develop mRNA-based vaccines for YF, Lassa fever, and rabies. The Coalition for Epidemic Preparedness Innovations (CEPI) has supported the development of mRNA-based vaccines for these diseases, utilizing a "printing" method for mRNA.

Some scientists have utilized plants as a means of producing vaccines. They successfully expressed the gene responsible for the viral protein YF E in Nicotiana benthamiana plants [46]. This involved incorporating the YF E gene into an engineered version of Clostridium thermosolum lichenase (LicKM) and inserting it into the pGR-D4 vector. The plasmids were then transferred into Agrobacterium tumefaciens using electroporation, which were subsequently introduced into Nicotiana benthamiana plants via vacuum infiltration. The resulting E protein was purified from the Nicotiana plants and used in pre-clinical trials. Both E and E-LicKM proteins were effective in eliciting the production of neutralizing antibodies in mice, with 70% of the mice being protected from YFV.

However, there is still a need for a comprehensive understanding of the molecular mechanism underlying the attenuation of the 17D strain. This lack of understanding has hindered the progress in developing second-generation 17D vaccines and other live attenuated vaccines produced using various platforms. Encouragingly, the successful application of reverse genetics, chimeric vaccines based on the 17D backbone, and plasmid-launched live-attenuated vaccines (PLLAV) has sparked optimism for the development of next-generation yellow fever vaccines. Currently, regulations set by the WHO mandate the use of only live attenuated 17D vaccines, cultivated in chick embryos, and tested for safety in non-human primates (NHPs). These regulations need to be reassessed to facilitate the efficient development of new candidate vaccines for the global market.

Tolerability and safety of yellow fever vaccines

Studies indicates that the YF vaccine is generally well-tolerated among adults, with infrequent reports of serious adverse events [46]. In initial trials, mild reactions were observed in 10-15% of vaccine recipients, occurring five to eight days post-vaccination, while more severe reactions were noted in only one to two percent of cases [47]. These reactions range from localized pain and redness at the injection site to symptoms like headache, nausea, vomiting, and diarrhea. Although systemic reactions are reported in less than 0.2% of cases, it is suggested that they may be more prevalent than currently recognized [48].

Adverse reactions to the YF vaccine, typically mild, may manifest as headache, muscle pain, low-grade fever, and discomfort at the injection site [49]. Clinical trials have shown that around 25% of those vaccinated experience mild adverse events [50-52]. Severe reactions to the YF vaccine are uncommon. Anaphylactic reactions, characterized by severe allergic responses involving multiple organ systems, are estimated to occur in 0.8 per 100,000 vaccinations, primarily among individuals with allergies to components like eggs or gelatin [53]. Yellow fever vaccine-associated neurotropic disease, which encompasses conditions like post-vaccinal encephalitis, Guillain–Barré syndrome, and autoimmune diseases affecting the central or peripheral nervous system, are estimated at approximately 0.4 per 100,000 vaccinations [54]. Yellow fever vaccine-associated viscerotropic disease, clinically resembling naturally acquired yellow fever, occurs in around 0.3 per 100,000 vaccinations [54]. Advanced age and a history of thymus disease are identified as risk factors for systemic adverse events following YF vaccination [55-57].

Adverse events following immunization (AEFI) are manifestations that occur after vaccine administration and are believed to be linked to the vaccine [58]. These events are monitored through a passive surveillance system called the Vaccine Adverse Event Reporting System, managed by the CDC and the US Food and Drug Administration (FDA). Adverse events following immunization related to yellow fever vaccination are typically mild and nonspecific [49]. Notably, studies have proposed a higher occurrence of local inflammatory events in female vaccine recipients compared to males. However, this gender-related effect was not observed concerning the response to the yellow fever vaccine booster [59].

Table 1. Amino acid substitutions and nucleotide changes between Asibi and 17D YF strains (Hansen & Barrett 2021).

Proteins	Nucleotides	Genes	AA Number
	1127	E	G52R
	1491	E	T173I
	854	M	L36F
	1482	E	A170V
	1572	E	K200T
	1887	E	S305F
	2193	E	A407V
	2112	E	T380R
	1870	E	M299I
Non-structural Proteins	3860	NS2A	M118V
	4022	NS2A	T172A
	3371	NS1	I307V
	4007	NS2A	T167A
	4056	NS2A	S183F
	6023	NS3	D485N
	4505	NS2B	I109L
	6876	NS4A	V146A
	10,142	NS5	E836K
	7171	NS4B	I95M
	10,338	NS5	P900L
3'Untranslated Region Nucleotide changes	10,418		U → C
	10,847		A → C
	10,367		U → C
	10,800		G → A

Table2 . Vaccines undergoing or completed a clinical trial in children and elderly people.

Name of Vaccine	Clinical trial ID	Status	The age group targeted	Target immune cell	Comment	Reference
Booster Vaccine for Yellow Fever (BoVY)	NCT05332197	On-going (Phase 3)	Children (1-9 years)	CD4 and CD8 T cell responses are elicited	In the presence of neutralizing antibodies, booster vaccine doses are not required; booster doses are assessed based on endemic or non-endemic regions.	[60]
Non-Inferiority Fractional Doses Trial for Yellow Fever Vaccine (NIFTY)	NCT04059471	On-going (Phase 4)	Children and adults (9 months to 60 years)	B and T lymphocytes; changes in serum biomarkers, like TNF, INF-γ, IL-2, IL-4, IL-5, IL-10, IL-8/CXCL-8, MCP-1/CCL-2, MIG/CXCL-9 and IP-10/CXCL-10	Recurrence of Yellow fever outbreaks in 80% of the population can be prevented through booster vaccination.	[61]
Yellow Fever Vaccine (YEFE)	NCT02991495	Completed (Phase 4)	Children and adults (9 months to 60 years)	CD4+ T and B cells	The correlation between vaccine viremia and immunogenicity is assessed	[62, 63]
Yellow Fever 17DD Vaccine	NCT03725618	Completed (Phase 4)	Children (9-23 months)	Virus-neutralizing antibody-producing T and B cells	Male participants had a slightly greater immunologic response to the vaccine	[64]
Yellow Fever Vaccine (17DD)	NCT03338231	Completed	Adult, Elderly	T and B lymphocytes	The minimum vaccination dose required is evidenced as 1000IUwhich is on par with WHO	[65]

Figure 1. Pathogenesis of yellow fever disease.

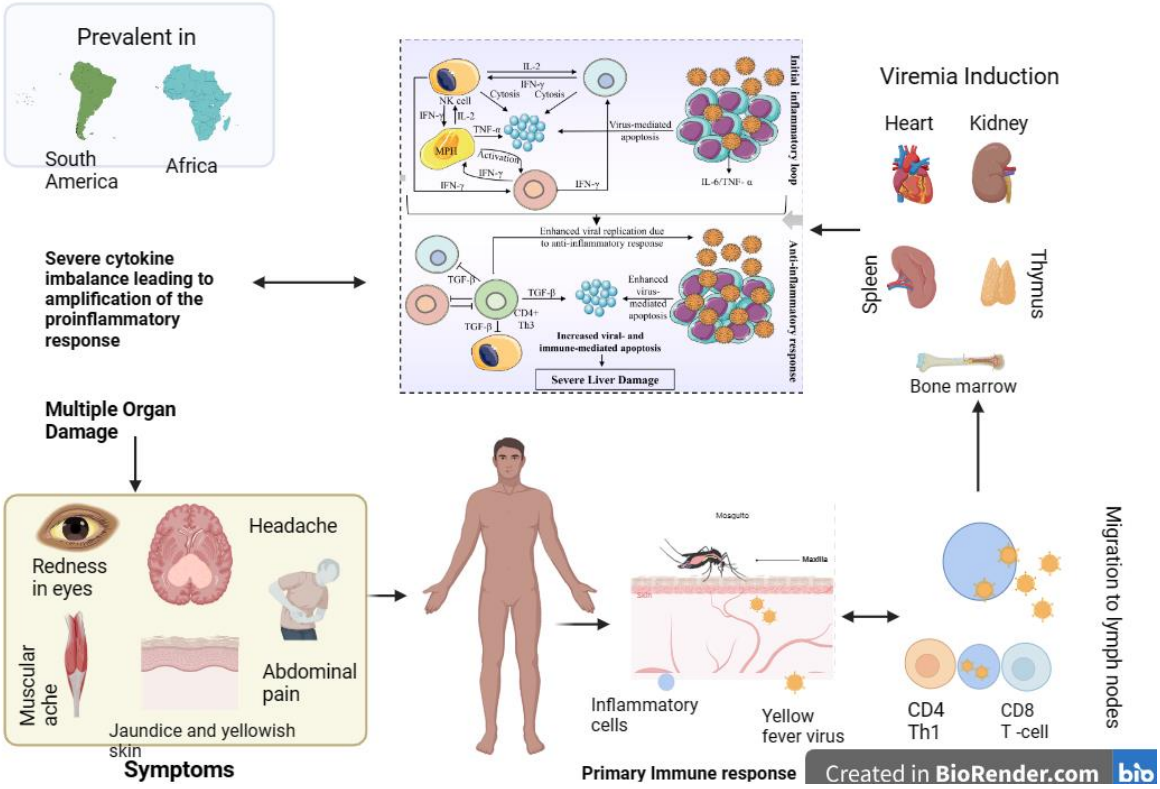


Figure 2. The critical immunological events are depicted on a timeline.

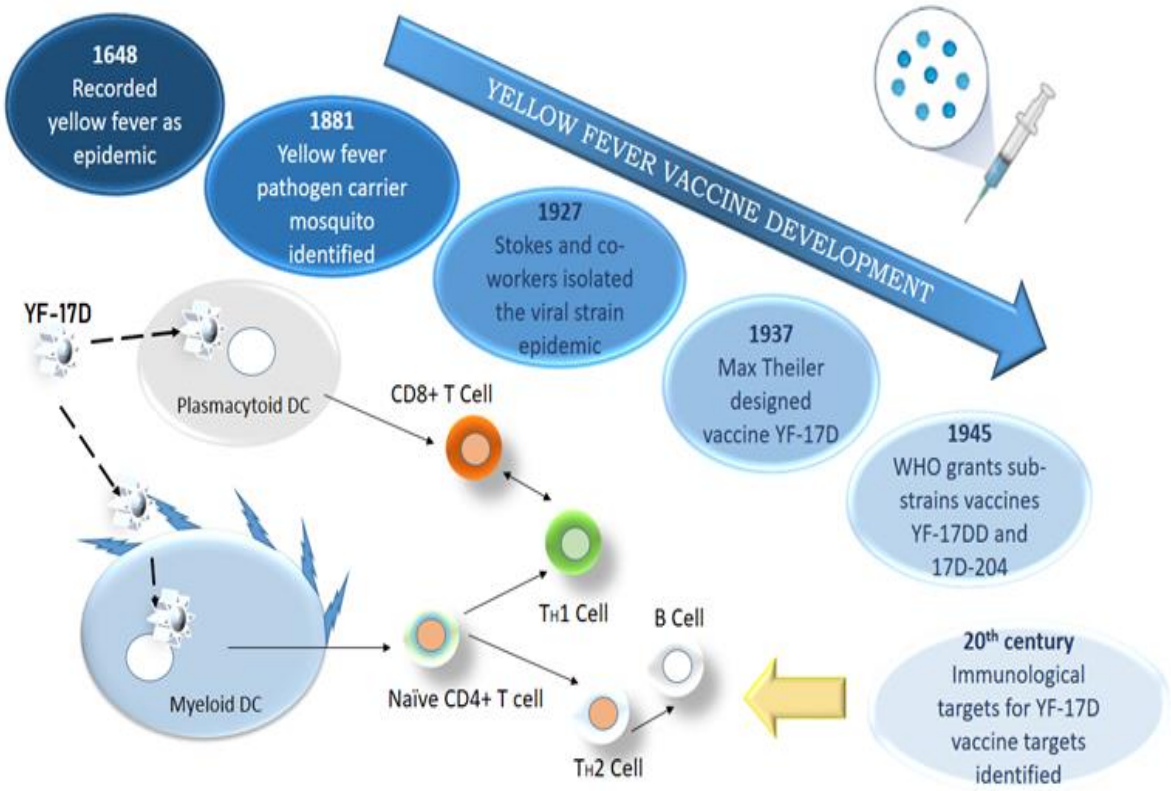
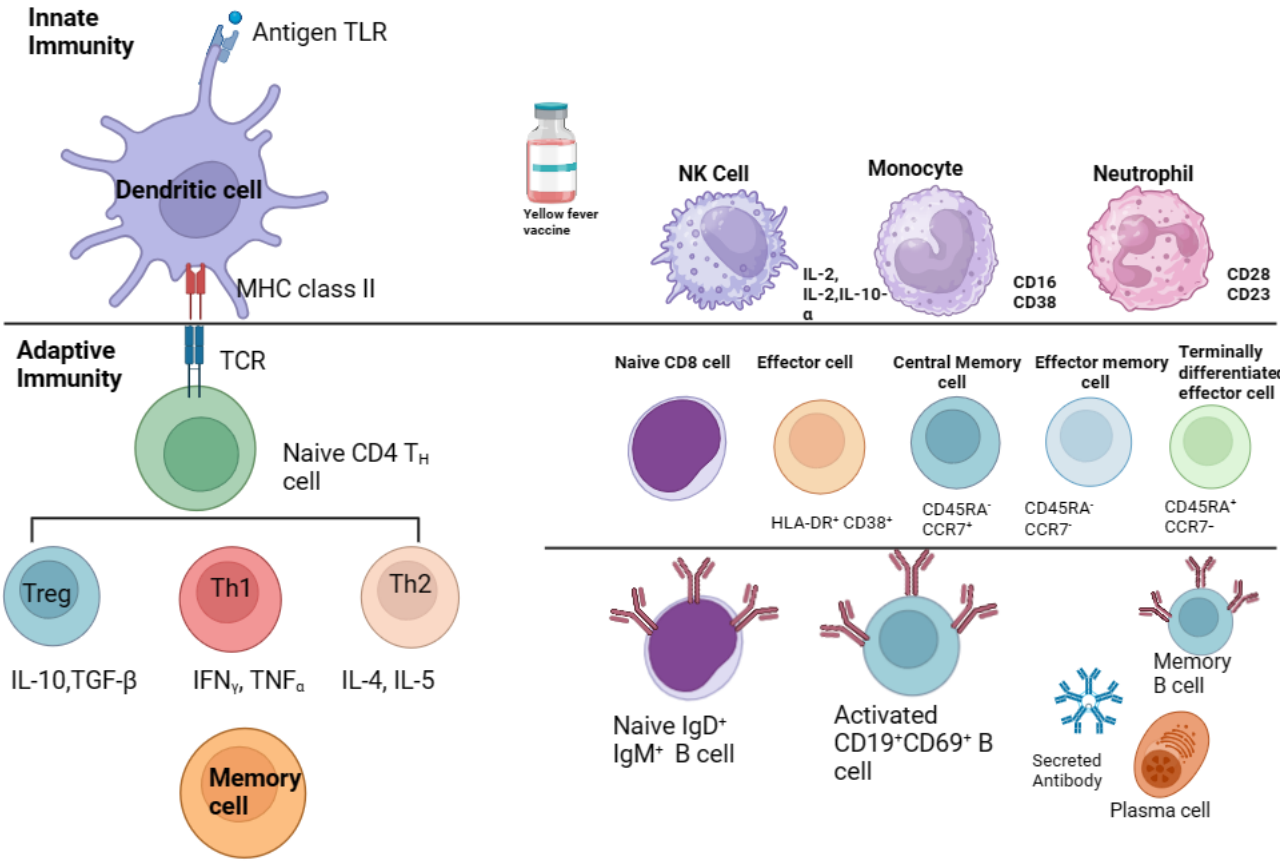


Figure 3. The primary immunological responses induced by the yellow fever vaccine



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