The 17D-204 and 17DD yellow fever vaccines: an overview of major similarities and subtle differences


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The 17D-204 and 17DD yellow fever vaccines: an overview of major similarities and subtle differences

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ABSTRACT
Introduction: The yellow fever vaccine is a live attenuated virus vaccine that is considered one of the most efficient vaccines produced to date. The original 17D strain generated the substrains 17D-204 and 17DD, which are used for the current production of vaccines against yellow fever. The 17D-204 and 17DD substrains present subtle differences in their nucleotide compositions, which can potentially lead to variations in immunogenicity and reactogenicity. We will address the main changes in the immune responses induced by the 17D-204 and 17DD yellow fever vaccines and report similarities and differences between these vaccines in cellular and humoral immunity. This is a relevant issue in view of the re-emergence of yellow fever in Uganda in 2016 and in Brazil in the beginning of 2017.

Areas covered: This article will be divided into 8 sections that will analyze the innate immune response, adaptive immune response, humoral response, production of cytokines, immunity in children, immunity in the elderly, gene expression and adverse reactions.

Expert commentary: The 17D-204 and 17DD yellow fever vaccines present similar immunogenicity, with strong activation of the cellular and humoral immune responses. Additionally, both vaccines have similar adverse effects, which are mostly mild and thus are considered safe.

1. Introduction

The yellow fever vaccine is a live-attenuated virus vaccine that is considered one of the most efficient vaccines produced to date. The vaccine is one of the most applied vaccines worldwide, with more than 600 million doses distributed [1]. The vaccine was discovered by Max Theiler et al. in 1937 from a virus isolated from an African patient who was cured [2,3]. Theiler subjected the virus to 176 passages in monkey, mouse, and chicken cell cultures, which led to viral attenuation but maintained the virus’s immunogenicity [2,4–6]. This original strain was named 17D.

In 1940, the World Health Organization (WHO) created a virus seed lot system to avoid immunogenetic variations in the vaccine virus that could compromise vaccine safety. The lots consist of the preparation of a large number of virus stocks, which are currently used for the production of vaccines [7].

The original 17D strain generated the substrains 17D-204 and 17DD, which are used for the current production of vaccines against yellow fever [8,9]. Currently, there are six producers of yellow fever vaccines, but only 4 are prequalified by the WHO and international agencies: Bio-Manguinhos (Brazil), Sanofi-Pasteur (France), Pasteur Institute in Dakar (Senegal), and Institute of Poliomyelitis and Virus Encephalitis (Russia) [10].

The 17D-204 and 17DD substrains present subtle differences in their nucleotide compositions, which can potentially lead to variations in immunogenicity and reactogenicity [9]. Approximately 99.9% of the nucleic acid sequence is identical in both strains [6]. The 17D-204 substrain was produced after 235–240 passages in embryonated chicken eggs whereas the 17DD substrain was subjected to 287 passages. The 17D-213/77 vaccine originated from the 17D-204 vaccine and is currently in its 240th passage [6,11].

In this article, we will address the main changes in the immune responses induced by the 17D-204 and 17DD yellow fever vaccines and report similarities and differences between these vaccines in cellular and humoral immunity and adverse reactions.

2. Comparison between the immune responses induced by the 17d-204 and 17dd vaccines

The vaccine against yellow fever reproduces an acute viral infection and induces potent long-lasting humoral and cellular immune responses [1].

2.1. Innate immune responses induced by the yellow fever vaccine

Innate immunity is responsible for the initial control of infection with the yellow fever virus and induces an adaptive T and B cellular immune response with the formation of memory cells [12].
The 17D-204 vaccine stimulates the innate response through multiple Toll-like receptors (TLR), culminating in the activation of myeloid and plasmacytoid dendritic cells [1,8]. The TLRs involved are TLR 2, 9, 7, 8, and 3. The MDA 5 and RIG-I receptors, which recognize RNA molecules, are also activated [13,14].

Martins et al. studied 10 healthy individuals subjected to vaccination with 17DD. Neutrophil activation was observed with increased CD28 and CD23 expression on days 7 and 15 post-vaccination. Eosinophils were also activated with increased CD28 and human leukocyte antigen (HLA)-DR expression on day 30 post-vaccination. On day 7, there was an increase in the frequency of activated monocytes (CD14+CD16HI and proinflammatory monocytes (CD14+CD16HIHLA-DR+). Natural killer (NK) cells suffered a decrease on day 7, probably as a result of a regulatory effect, which also manifested in increased interleukin (IL)-10R (receptor) expression in a large proportion of the innate immune components on days 15 and 30 [12]. Immune system activation and modulation by innate immune responses occur simultaneously, as indicated by the positive correlation between the CD28 neutrophil levels and IL-10R expression [12].

In turn, NK cells play a central role in the initial response to the vaccine antigen by controlling viremia and contribute to dendritic cell maturation [14]. These cells are responsible for the increased expression of TLR 3 and 9 in the presence of IL-12; additionally, after contact with the 17DD vaccine, there is an increase in the expression of CD38 and CD16 on the surface of NK cells, which characterizes the intensification of cytotoxic activity [14].

Regarding the 17D-204 vaccine, a percent increase in CD86+ myeloid dendritic cells, plasmacytoid dendritic cells and CD14+CD16+ inflammatory monocytes was observed on day 7 post-vaccination[15], with increased production of interferon-gamma (IFN-γ)-inducible protein-10 (IP-10) and IL-1α. Additionally, the 17D-204 vaccine promotes the induction of a network of genes that mediate a proinflammatory antiviral response [1].

Gamma delta lymphocytes (γδ) are a subtype of T lymphocytes with a receptor (TCR) with a gamma and a delta chain, which is in contrast to most T lymphocytes that have alpha and beta chains. This variation allows these cells to play roles in both innate and adaptive immunity because the recognition of antigens is major histocompatibility complex (MHC) independent, leading to quick recognition of infected cells and cytokine production [16]. After immunization with the 17D-204 vaccine, the γδ lymphocytes are the first cells to produce IFN-γ, followed by CD4+ and CD8+ T lymphocytes [17,18]. The release of IFN-γ affects the maturation of dendritic cells and the polarization of CD4+ T lymphocytes toward a T helper type 1 (Th1) response [18].

Additionally, the 17D-204 vaccine activates the inflammasome, which is a multiprotein complex that is responsible for promoting the maturation of IL-1β and IL-18 and inducing pyroptosis. Pyroptosis is a programmed cell death that differs from apoptosis by the release of cytokines into the extracellular space, which attracts other immune system cells and thus activates the inflammatory cascade [19]. Dendritic cells increase IL-1β production by up to ninefold after contact with the vaccine virus [19], which is capable of regulating T helper lymphocyte activation, B lymphocyte proliferation and immunoglobulin production [13].

Campi-Azevedo et al. compared the cytokine profile produced by cells of innate immunity in children after vaccination with 17D-213/77 and 17DD. A prominent proinflammatory profile with high levels of IL-2 and tumor necrosis factor (TNF)-α production was observed by neutrophils and monocytes and TNF-α in neutrophils with the 17DD vaccine. On the other hand, the 17D-213/77 vaccine presented a profile with a regulatory tendency, with a higher expression of IL-4 and IL-10 by neutrophils [20].

In summary, both vaccines induce potent innate immunity responses by activating various types of dendritic cells and TLRs, leading to a mixed pattern of Th1 and Th2 response.

2.2. Adaptive immune responses induced by yellow fever vaccination

2.2.1. CD4+ and CD8+ T LYMPHOCYTES

After vaccination with 17DD, viremia reaches its peak on day 5, followed by a gradual reduction until day 7 [10,21]. Regarding the T lymphocyte populations, the 17DD vaccine induces a decrease in the CD8+CD62L+ T lymphocyte subpopulation on day 7 post-vaccination. Martins et al. analyzed peripheral blood mononuclear cells (PBMCs) following the primary vaccination and found distinct profiles between the CD4+ and CD8+ T lymphocytes. On day 7 post-vaccination, the CD8+ T lymphocytes exhibited early activation markers (CD69+), whereas the CD4+ T lymphocytes exhibited late activation markers (HLA-DR) that were expressed at high levels until 30 day. The CD8+ T lymphocytes were activated late compared to the CD4+ T lymphocytes [22]. Additionally, there was a reduced frequency of CD38+ cells on day 30 post-vaccination, which could be justified by the increased expression of IL-10R on day 15 in the CD4+ and CD8+ T lymphocytes [22]. IL-10 inhibits proinflammatory cytokine production and T lymphocyte proliferation [23].

Santos et al. studied a group of 8 individuals who were immunized with the 17DD vaccine for the first time and 9 revaccinated individuals. The authors found an increase in the frequency of CD8+CD38+ T lymphocytes (activation markers) in all volunteers. The revaccinated individuals presented greater levels of CD4+CD45RO+ and CD8+CD45RO+ T lymphocytes than the group of first-time vaccinated individuals, which suggested a quicker immune response after revaccination [24].

The immune system activation and modulation events generated by the yellow fever vaccine occur simultaneously. On day 7 post-vaccination, there was a positive correlation between CD4+HLA-DR+ and regulatory CD4+CD25high T lymphocytes. The regulatory CD4+CD25high T lymphocytes produce the anti-inflammatory cytokines IL-10 and transforming growth factor (TGF)-β [22].

Similarly, the 17D-204 vaccine induces quick expression of CD8+Ki-67+ T lymphocytes, which promotes a reduction in the BCL-2 level and favors apoptosis [1,19]. High proliferation of CD8+ T lymphocytes occurs during the first 15 days post-vaccination; these lymphocytes express HLA-DR and CD38 (representative of differentiation into effector cells) and account for 2–13% of all CD8+ T lymphocytes [25]. On day 30 post-vaccination, a loss of the effector phenotype is
observed, with reduced Ki-67 expression and transformation of memory cells expressing BCL-2, CD127, and CD45RA. The CD8⁺ memory cells are classified according to the expression of CD45RA and CCR7 into central memory cells (CD45RA⁺CCR7⁻), effector memory cells (CD45RA⁻CCR7⁺) and terminally differentiated effector cells (CD45RA⁻CCR7⁻). Most memory CD8⁺ T lymphocytes are of the CD45RA⁻CCR7⁺ type, which typically has lower proliferative potential. However, the CD8⁺ T lymphocytes specific for the vaccine virus present a high multiplication capacity [25–27]. Blom et al. analyzed 21 individuals vaccinated with 17D-204 to evaluate the kinetics of the CD8⁺ T lymphocytes and observed a transition of effector CD45RA⁺PD-1⁻ cells into memory CD45RA⁺PD-1⁻ cells (PD-1 is a surface marker that induces apoptosis). This transition occurred gradually from day 15 to day 90 post-vaccination [21,28]. CD8⁺ T lymphocytes present a broad and complex response to multiple epitopes, of which the most common is the HLA-A2 restricted epitope N54b (a nonstructural protein encoded in viral RNA genome) [28,29]. Similarly, there is a correlation between the magnitude of the activation of CD8⁺ T lymphocytes and the viral load (i.e., a higher viral load indicates a greater intensity of effector CD8⁺ T lymphocytes until saturation is reached) [21].

CD8⁺ T lymphocytes play multiple roles, including direct cytotoxic action and secretion of antiviral cytokines, such as IFN-γ, TNF-α, IL-2, and macrophage inflammatory protein 1β [26]. The direct action occurs through the action of the perforin and granzyme A and B molecules. These cells acquire cytotoxic potential by day 12, which remains for up to 6 months post-vaccination [29]. Additionally, a single dose of the vaccine is capable of generating memory CD8⁺ T lymphocytes for 5–10 years regardless of reexposure to viral antigens [27].

In the 17D-204 vaccine, the CD4⁺ T lymphocytes initially decreased in association with the peak viremia but increased during the second week [30,31]. The activation of CD4⁺ and regulatory T (Treg) lymphocytes preceded the activation of CD8⁺ T lymphocytes [28]. These CD4⁺ T lymphocytes were predominantly IFN secreting and CD45RA⁻CX3c⁺ [32] and presented a significant expansion on day 14 post-vaccination; a transition from the effector phenotype to the central memory phenotype was observed after 1 month [32].

Campi-Azevedo et al. demonstrated a significant increase in effector T lymphocytes in volunteers 30–45 days after primary vaccination with 17DD compared to non-vaccinated individuals. When analyzing subjects whose vaccination had occurred 10–11 years ago, there was a decrease in the TNF-α levels secreted by CD4⁺ and CD8⁺ T lymphocytes and an increase in CD4⁺ IL-10⁺ T lymphocytes compared to the newly vaccinated group (30–45 days post-vaccination). Increased production of IL-10 and reduction of proinflammatory cytokines in this group suggests that, over time, there is a tendency to balance between proinflammatory versus regulatory response. Besides, the early effector memory CD4⁺ and CD8⁺ T cells as well as the classical memory B cells and the median PRNT (plaque reduction neutralizing titer) titers are decreased 10–11 years post-vaccination, which may indicate a fragility of the effector response in the time [33]. These data suggest that the non-booster recommendation may need to be reviewed. This is a controversial issue with disparate results in different studies. Wieten et al., in turn, concluded that the booster did not promote increases in frequency or phenotypic changes in specific CD8⁺ cells or increase in antibody titers [29]. However, the authors did not analyze the CD4⁺ T cell response and there was decay of antibody titers over time.

In a similar study, Kongsgaard et al. concluded that the response of CD8⁺ T cells from booster recipients was smaller compared to primary vaccination [34]. Both studies were performed with the 17D-204 vaccine. These disparate findings could be attributed to the vaccine strain, but are possibly more related to immunogenetic variations of the populations studied, given the study by Muyanja et al. who analyzed the immunological response to the 17D-204 vaccine in 2 distinct populations: the African and Swiss cohort [35]. The African cohort presented a cellular and humoral response significantly lower than that of the Swiss cohort. The authors attributed the fact to a chronic activation of the immune system present in the African cohort, being more exposed to viral, bacterial and fungal antigens. In the African cohort, T and B cell responses were boosted by a second vaccination [35].

Yellow fever vaccines induce a specific CD8⁺ T memory cell response with polyfunctional and high proliferative capacity. This is also found in other live virus vaccines, such as smallpox vaccine [26]. In inactivated vaccines, for example the inactivated influenza vaccine, the induction of a potent CD8⁺ T cell response has not been observed, with a more detectable increase in influenza-specific Th1 CD4⁺ T-helper cell response [36].

2.2.2. B LYMPHOCYTES

Studies show a balance between activation and modulation of the cellular immunity induced by the 17DD vaccine. The CD19⁺ B lymphocyte levels were decreased on day 7 post-vaccination for yellow fever, which led to a relatively larger proportion of T lymphocytes during this period [22]. On day 15 post-vaccination, an increase in B lymphocytes with an early activation phenotype (CD19⁺CD69⁺ cells), and increased IL-10R expression were observed, which favored the proliferation and maturation of B lymphocytes [20,37]. Increased frequency of IL5⁺ T cells was also observed on days 15 and 30 post-vaccination with 17DD, which facilitates the activation of B cells [37]. Conversely, regulatory markers, such as increased CD32 expression and decreased CD19⁺CD23⁺ cell percentages, were also observed. CD32 is a membrane receptor that inhibits the production of antibodies in the presence of IgG [20]. CD23 is a B lymphocyte-specific antigen that indicates cellular activation [38]. Campi-Azevedo et al. also demonstrated a significant increase in classical memory B lymphocytes in volunteers 30–45 days after their primary vaccination with 17DD compared to unvaccinated individuals [33].

Regarding the 17D-204 vaccine, Kohler et al. demonstrated a reduction in the number of B lymphocytes on day 7, followed by a subsequent increase until day 14 and then a gradual return to the pre-vaccination levels [31].

After re-encounter with antigen, as documented after vaccination with the inactivated influenza vaccine, memory B cells differentiate into plasma cells that secrete IgG antibodies and undergo secondary affinity maturation to influenza epitopes.
Compared to the inactivated vaccine, the live influenza vaccine induces a more intense B cell response [36].

### 2.3. Humoral response induced by yellow fever vaccination

Vaccination with 17D-204 produces neutralizing antibodies that can last 30–40 years [1,8,29,39,40]. IgM antibodies appear after day 7 and reach a peak after 2 weeks, followed by the emergence of neutralizing antibodies [27]. However, IgM antibodies can be found 3–4 years post-vaccination [41], and the seroconversion rates range from 89.7 to 98.2% [40,42].

Neutralizing antibodies have the function to neutralize the viral particle and prevent its entry into the cell. They are considered the main mechanism of protection and are directed to complex epitopes found at the virion surface [43]. After vaccination, they arise in about 6 days, peaking within 2 weeks [44]. PRNT is the gold standard method to detect neutralizing antibodies, while ELISA analyzes the total of neutralizing and non-neutralizing antibodies. In order to evaluate protection, dose–response studies in rhesus monkeys showed log₁₀ neutralization index (LNI) >0.7 as a protector marker against yellow fever. There are no human studies. However, the US FDA considers the above value as protective. The PRNT test, which uses a known concentration of virus and several serum dilutions, replaced the LNI. In general, 1:10 and 1:20 titers are often used as cutoff in most studies [40].

Antibodies confer protection through various mechanisms, including direct cytotoxicity, complement-mediated lysis of infected cells, viral fusion inhibition, neutralization of virus binding to the cell receptor, and Fc-γ receptor-dependent viral clearance [45].

Melo et al. studied 238 healthy volunteers submitted to vaccination against yellow fever with the 17DD vaccine. In that study, 100% of the individuals developed a protective humoral response, with PRNT titers ≥1:120 [6]. Melo et al. also measured the PRNT titers in individuals who were vaccinated 5 and 10 years earlier; the results showed decreases in the PRNT titers of 83 and 87%, respectively, compared with recently vaccinated individuals, with PRNT titers in the range of 20–320, although they remained positive [6].

Reinhardt et al. detected neutralizing antibodies in all volunteers submitted to vaccination against yellow fever with 17D-204 during the second week post-vaccination, with average titers of 1:88. After 10 years, the antibody titers decreased by 18%, nevertheless showing high neutralizing antibodies titers 10 years after primary vaccination [30].

Camacho et al. published a randomized, double-blind, placebo-controlled study comparing the 17D-204 and 17DD vaccines. A total of 1087 volunteers were recruited, and the seroconversion rates were the same in approximately 98% of the participants. The mean and median antibody titers were also similar between the vaccine groups. The antibody titer was measured with the PRNT and seropositivity was defined as neutralizing antibody titer equal to or higher than 630 mIU/ml [46].

These data show that there are no significant differences in the humoral immunity triggered by the yellow fever vaccine strains 17DD and 17D-204.

### 2.4. Cytokines and chemokines

Cytokine production occurs early and strongly from day 5 to 7 post-vaccination with the 17DD vaccine. Simultaneous to the peak viremia, there is a peak in the production of the proinflammatory cytokines IFN-γ, TNF-α, and IL-2 [47]. In turn, the regulatory cytokine levels gradually increase, with a peak on day 30 (IL-4 and IL-5; the exception is IL-10, whose increase occurs early on day 3, followed by a decrease on day 5 and another increase between days 6 and 7). The decrease in the IL-10 levels during peak viremia should allow the development and maturation of antigen-presenting cells and increased MHC expression [20].

Querec et al. studied the early changes in cytokines in 15 individuals vaccinated with the 17D-204 vaccine on days 0 to 21 post-vaccination. The authors observed a significant increase in the chemokine IP10 (CXCL10) and IL-1α during all periods [15].

According to Martins et al., the CD4+ and CD8+ T lymphocytes exhibited increased expression of CXCR3 [22], which is a chemokine receptor involved in the Th1 response (particularly the recruitment of T lymphocytes) on day 15 post-vaccination with the 17DD vaccine [48]. However, on day 30 post-vaccination, increased expression of CCR2 by CD4+ and CD8+ T lymphocytes characterized a Th0 response with a mixed pattern of cytokines [22]. This pattern was persistent and was found up to 1 year post-vaccination [19].

The innate immune cells also presented a mixed pattern of cytokine production, with increased CCR3 (Th2), CCR5 (Th1), and CCR4 (Th0) by neutrophils on day 7 post-vaccination with 17DD [12].

### 2.5. Gene expression

Vaccination with the 17D-204 vaccine quickly induces modulation of genes that orchestrate the innate and cellular immune responses. The gene expression peak occurs at 7 days post-vaccination [19].

Querec et al. studied 15 individuals vaccinated with 17D-204 and found 65 genes modulated by the yellow fever vaccine. Of these 65 genes, 44 were identified. The main genes involved were related to the production of interferon and the antiviral response of the innate immune system, such as IFN-α, STAT1 (transcription factors that regulate type I interferons), TLR7, RIG-I, MDA-5 (associated with viral recognition), and genes related to complement activation, ubiquitination or ISGylation (protein modification through the addition of IFN-stimulated gene products) [15,19].

Scherer et al. analyzed the gene expression of polymorphonuclear cells of 20 individuals vaccinated with 17D-204 to determine the gene signature of the yellow fever vaccine. This study identified 615 genes that were induced or suppressed during the period from 4 to 7 days post-vaccination [49]. These genes were related mainly to immune biological processes, such as protein synthesis, apoptosis,
signal transduction, and transcription control. The main genes induced were associated with interferon, such as OAS1, OAS2, MX1, MX2, ISG15, and IFIT3 [49]. Figure 1 illustrates the main immunological changes caused by yellow fever vaccines. Figure 2 summarizes this main immunological events plotted on a timeline.

2.6. Immunity in children

Seroconversion is usually lower in children than in adults [9]. An observational study of the 17DD vaccine showed seroconversion rates of 67–94% with differences between age groups; the lowest values were observed in children 9–11 months of age [50]. A study conducted in Ghana showed seroconversion rates of 68–79% with 17DD vaccine in children 9 months of age, 4 weeks post-vaccination. A similar trial carried out in Mali, but with the 17D-204 vaccine, showed higher seroconversion rates of 95.1–98.3% [51]. This can be explained by the variation in the amount of viral particles in each vaccine [4.34–4.56 log_{10} plaque-forming unit (PFU) in the 17DD vaccine and 4.5–4.7 log_{10} PFU in the 17D-204 vaccine] [51]; however, both had a viral particle concentration above the WHO recommended minimum of 3.0 log_{10} PFU [11].
Factors that may be associated with the lower seroconversion rates in children include immune system immaturity, the presence of maternal antibodies, and the combined application of the yellow fever and MMR (measles, mumps, and rubella) vaccines [52]. Recently, Clarke et al. conducted a study to evaluate safety and immunogenicity of the simultaneous application of inactivated poliovirus vaccine along with measles-rubella combined vaccine and yellow fever vaccine [53]. There is no report on which strain of the yellow fever vaccine was used. The seroconversion rates ranged from 95 to 97%; however, the median antibody titers were much lower in the combined application of the vaccines when compared to the isolated application of the yellow fever vaccine [64 (95% CI 45–64) × 128 (95% CI 91–128)] [53]. This is an important finding with regard to the recent WHO recommendation about not being necessary booster vaccination anymore [54], as such low titers would probably not guarantee a life-long immunity.

Luiza-Silva et al. studied 60 healthy children aged 9–43 months who received the 17DD vaccine. Serum analyses 30 days after vaccination showed increased synthesis of IL-12 and TNF-α by neutrophils and monocytes in the seroconverted children. Additionally, a decrease in IL-4 production can be considered to promote a proinflammatory environment. Conversely, the group that did not achieve seroconversion presented cytokine patterns that were predominantly regulatory, with increased synthesis of IL-5 and IL-10 by CD8+ T lymphocytes [55]. The group that did not achieve seroconversion was subjected to revaccination, and a change in the cytokine profile was noted, with increased proinflammatory cytokine production.

The cytokine profile correlates with the humoral response. High neutralizing antibody levels are associated with a proinflammatory pattern (IL-12 produced by neutrophils and monocytes and IFN-γ produced by neutrophils), whereas the median levels are associated with a balanced pattern of inflammatory and regulatory cytokines [55].

Cytokine production is due in large part to innate immune cells, which are represented by neutrophils and monocytes. Differences in these cell types were noted between the groups that achieved and did not achieve seroconversion; in the latter group, the synthesis of cytokines by neutrophils and monocytes was impaired. Interestingly, 100% seroconversion was achieved with revaccination, resulting in a change in this scenario, with additional synthesis of cytokines by neutrophils and monocytes [55].

Campi-Azevedo et al. compared the immunogenicity of the 17DD vaccine and the 17D-213/77 vaccine (derived from the 17D-204 strain) in 80 children. Both strains showed a balance between the production of inflammatory and regulatory cytokines, with the 17DD vaccine showing a predominance of IL-12 and the 17D-213/77 vaccine showing a slight predominance of IL-10 production [20]. The innate immune response was more intense in the group vaccinated with 17DD, resulting in high synthesis of IL-12 and TNF-α. In turn, the 17D-213/77 vaccine presented greater IL-12 production by CD8+ T cells. There were no differences in the production of neutralizing antibodies between the groups. However, the subgroup with higher neutralizing antibody titers in response to the 17DD vaccine had a proinflammatory cytokine profile, which was in contrast to the 17D-213/77 vaccine group in which a regulatory profile predominated. Similar to the observations in the study of Luiza-Silva et al., individuals who did not achieve seroconversion presented a production deficit of proinflammatory cytokines; this scenario was reversed by revaccination with 17DD [20].

These small variations are probably due to genetic changes arising from viral passages in embryonated chicken eggs [9]; however, both vaccines are highly immunogenic.

### 2.7. Immunity in the elderly

Immunosenescence is aging of the immune system, which contributes to a lower response to antigens, vaccines, and infections in the elderly. The adaptive immune response is the most affected, with a decrease in the T lymphocyte repertoire and an increase in late memory and effector cells [56]. Other changes include decreased B lymphocyte levels and increased NK cell and myeloid dendritic cell levels [56]. These changes are responsible for a greater risk of adverse reactions in the elderly, including yellow fever vaccine-associated viscerotrophic disease (YEL-AVD), whose incidence is 2.3–3.2:100,000 in those older than 70 years of age [57].

Myaji et al. conducted an observational and prospective study to investigate adverse effects related to the 17DD yellow fever vaccine in 828 elderly individuals. The results showed the occurrence of only mild adverse effects in approximately 15% of the participants [58]. These results corroborate the findings of Thomas et al., who assessed studies using pharmacovigilance databases through a systematic review but did not find a large number of serious adverse events in the elderly. In the reported cases, another vaccine in addition to the yellow fever vaccine had been applied concomitantly in approximately 50% of the cases [59].

Roukens et al. conducted a prospective controlled study to analyze the humoral response and viremia after vaccination with 17D-204. In that study, young volunteers (18–28 years) were compared to elderly individuals (60–81 years) [57]. On day 10 post-vaccination, 77% of the young people presented neutralizing antibodies compared to only 50% of the elderly. There was a significant difference in neutralizing antibody titers between the groups. On average, these titers were 2.9 UI/mL higher among the young people on day 10, although the titers reached levels similar to those observed in the elderly on day 28. The elderly group presented higher viremias for more prolonged periods [57].

Similarly, Schulz et al. when analyzing the elderly immune response to 17D-204 vaccination, found a late viremia (days 7–10) compared to young individuals, probably due to a slowed viral clearance. The elderly also had a transient reduction in neutralizing antibody titers [60]. The number of CD8+ T cells was also reduced in the acute phase. CD4+ T cells, on the other hand, showed late expansion until day 14 post-vaccination, which may characterize an exaggerated or prolonged response, facilitating undesirable adverse reactions. There was maintenance of protective levels of antibodies after 3 years; however, the population of IFN-γ+ CD40L+ CD4+ T cells was significantly smaller compared to younger ones, with lower polyfunctionality [60]. These results suggest that, in the
elderly, the immune response is deficient, and that this group would probably benefit from a booster vaccination.

3. Adverse reactions

The 17D-204 vaccine is quite safe, and serious adverse reactions are rare (1 per 250,000 or 500,000 vaccinations) [1]. Yellow fever vaccine-associated viscerotropic disease (YEL-AVD) and neurotropic disease (YEL-AND) are part of this spectrum. The former consists of a clinical picture very similar to yellow fever, with multiple organ failure and high lethality (approximately 60%). In a recent review, 62 YEL-AVD reports were identified that met the Brighton Collaboration criteria. Of these, 35 died [61]. The neurotropic disease is characterized by involvement of the central nervous system, such as encephalitis and Guillain-Barré syndrome. The most likely explanation for these adverse reactions is individual genetic susceptibility because no mutations have been found in the vaccine virus [1,62]. Cases of YEL-AVD in thymectomized individuals and polymorphisms in the OAS1 and OAS2 genes and in the CCR5-RANTES axis have been reported [62]. The main defect seems to focus on innate immunity because in all cases, individuals have high neutralizing antibody titers and a strong cellular immune response.

Pulendran et al. reported the case of a 64-year-old man who developed YEL-AVD two days after receiving the 17D-204 vaccine. Serum analysis detected viral RNA up to 33 days post-vaccination, whereas viral clearance in healthy individuals occurs within 7 days on average. The persistence of the viral RNA was not due to changes in the adaptive response, which was quite consistent. In this case, there was a polymorphism in the CCR5 chemokine receptor and its ligand RANTES, which are responsible for the migration of effector T lymphocytes and CD14+CD16bright monocytes to tissues; this polymorphism may have compromised the innate immune response [63].

Silva et al. studied the serum of a young 23-year-old female who presented with YEL-AND after vaccination with 17D-204 [64]. On day 8 post-vaccination, the patient had evolved with encephalitis, rhabdomyolysis, and hepatitis. Polymorphonuclear cells in the peripheral blood were analyzed, and the results were compared to healthy volunteers. The patient in question showed a greater NK cell level and less CD16 expression in monocytes. Additionally, there was a higher rate of activated CD4+ T lymphocytes and a low level of regulatory T lymphocytes [64]. The B lymphocytes were also more activated compared to the healthy volunteers, but reduced CXCR3 expression was observed in the CD4+ and CD8+ T lymphocytes. The proinflammatory pattern also manifested in the production of cytokines, with increased IL-12 and TNF-α and low production of IL-10, IL-4, and IL-5 [64].

In order to assess the chance of an individual developing YEL-AVD, Seligman et al. analyzed 64 cases in which gender and age were known. The greatest risk factor associated with YEL-AVD was the presence of thymoma (OR = 140.0 95% CI = 34–540), men over 55 years old (OR = 10.0 95% CI = 6.4–17), patients with autoimmune diseases (OR = 6.0 95% CI = 3–12), and young women (OR = 2.8, 95% CI = 1.6–4.9) [65].

A randomized, placebo-controlled study compared the reactogenicity of the 17D-213/77 vaccine with two virus seed lots of the 17DD vaccine [7]. A total of 1087 volunteers were vaccinated and observed for 30 days for viremia analysis and measurement of liver enzymes. No serious adverse events occurred. The most common adverse reaction was pain at the injection site. Fever, myalgia, and headache also occurred with similar frequencies between the groups. The rate of adverse events ranged from 17.8 to 21.7% in the vaccine group and was 14.3% in the placebo group [7].

Serious adverse events following immunization are quite rare. In large part of the reports it is difficult to establish the causal link with the vaccine, and it is easier to detect the temporal relation between the administration of a vaccine and its adverse effects. Some well-documented serious adverse events are Guillain-Barré syndrome, whose estimate would be 1.6 per million cases following influenza vaccination; apnea in preterm newborns, which has been associated with inactivated polio virus vaccine, hepatitis B virus vaccine and diphtheria-tetanus-whole-cell pertussis; and immune thrombocytopenic purpura, whose incidence following MMR vaccine is 1: 40,000 cases [66].

Table 1 summarizes the above comparisons for both yellow fever vaccines.

4. Conclusion

The 17D-204 and 17DD yellow fever vaccines present similar immunogenicity, with strong activation of the cellular and humoral immune responses. The vaccines induce the production of neutralizing antibodies and confer protection that manifests as a strong and polyfunctional cellular response with amplification potential. Additionally, both vaccines are considered safe since they have similar adverse effects, which are mostly mild. The vaccines 17DD and 17D-204 show subtle differences between them.

5. Expert commentary

Yellow fever vaccines have been in use for many decades, with excellent results and countless lives saved.

In 2016, there was a large yellow fever epidemic in Angola, and the yellow fever vaccine stock was shortly exhausted culminating with the interruption of routine immunization in this endemic area of Africa. Moreover, the yellow fever outbreak in Brazil also required a large amount of yellow fever vaccine doses to cover the areas at risk. Considering the limited capacity of worldwide producers to supply these increasing demands, alternative strategies have become needed. Amongst these measures, the use of fractionated doses and reevaluation of booster dose requirement were implemented. The use of fractionated doses have been originally addressed by two studies carried out in Brazil by Martins et al., 2013 and Campi-Azevedo et al. [47,70]. Together, these studies provided supporting evidences that, upon primary vaccination, ten-fold lower subdose of yellow fever vaccine induced similar immunological and virological pattern. These findings led to the World Health Organization and the Center for Disease Control (USA) to design
Table 1. Comparison between the 17D-204 and 17DD yellow fever vaccines.

<table>
<thead>
<tr>
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<th>17D-204</th>
<th>17DD</th>
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<tbody>
<tr>
<td>Humoral response</td>
<td>Seroconversion Equivalent seroconversion rates, approximately 98%</td>
<td>Equivalent seroconversion rates, approximately 98%</td>
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<td></td>
<td>GMT (geometric mean titers) 17.6 IU/ml [46]</td>
<td>17.7 IU/ml [46]</td>
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<td>observation: this was a head-to-head study comparing 17D and 17DD vaccines</td>
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<td>PRNT titers 30 days 17D vaccines GMT titers: 9.81 IU/ml [29]</td>
<td>65% had a titer ≥1:10 [6]</td>
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<td>Innate response</td>
<td>Stimulation of multiple toll-like receptors (TLRs), culminating in the activation of myeloid and plasmacytoid dendritic cells</td>
<td>Neutrophil activation on days 7 and 15 post-vaccination. Eosinophils were also activated on day 30 post-vaccination. On day 7, there was an increased frequency of activated monocytes. Decrease of the CD8+CD62L+ T lymphocyte subpopulation on day 7 post-vaccination. The CD8+ T lymphocytes exhibited early activation markers (CD69+), whereas the CD4+ T lymphocytes exhibited late markers activation (HLA-DR), which remained at high levels until day 30. Increase of effector T lymphocytes 30–45 days post-vaccination.</td>
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<td>T lymphocyte responses</td>
<td>Proliferation of CD8+ T lymphocytes in the first 15 days’ post-vaccination, with expression of HLA-DR and CD38. On day 30 post-vaccination, there was a loss of the effector phenotype and transformation in memory cells</td>
<td>CD4+ T lymphocytes initially decreased associated with the peak viremia, followed by an increase in the second week.</td>
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<td>CD4+ T cells present a biphasic appearance with transient increase on day 2, decrease on day 3 and reappearance on days 4–7. Secretion of IL-2, TNF-α and IFN-γ [31].</td>
<td>CD4+ T cells are activated earlier than CD8+ and are closely related to modulator events mediated by Treg [22]. There is an increase in progressive CD8+ T-cell level up to the 30th day post-vaccination, with 70% of these cells being identified as memory cells [24].</td>
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<td>CD8+CD62L− T cells naive undergo clonal expansion and differentiate to acute-phase T cells on 12–14 days after vaccination. They develop into late differentiated T cells on days 90–180 with cytotoxic potential preserved. [29,31]</td>
<td>CD8+CD62L− T cells naïve undergo clonal expansion and differentiate to acute-phase T cells on 12–14 days after vaccination. CD8+CD62L− T lymphocytes exhibit early activation markers (CD69+), whereas the CD4+ T lymphocytes exhibited late markers activation (HLA-DR), which remained at high levels until day 30. Increase of effector T lymphocytes 30–45 days post-vaccination.</td>
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<td>B lymphocyte responses</td>
<td>Decrease in the number of B lymphocytes on day 7 followed by a subsequent increase until day 14, after which there was a gradual return to pre-vaccine levels.</td>
<td>Balance between activation and modulation. CD19− B lymphocytes were quite decreased on day 7 post-vaccination. On day 15, there was an increase in the early activation B lymphocytes. Mostly mild.</td>
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<tr>
<td>Adverse effects</td>
<td>Mild: headache, myalgia, local pain, and fever. Serious reactions are rare (1 per 250,000 or 500,000 doses)</td>
<td>Mostly mild.</td>
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<td>Hypersensitivity reactions 1.8 per 100,000 doses [87]</td>
<td>0.76 per 100,000 doses [68]</td>
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<td>YEL-AND 4–8 per million doses [67]</td>
<td>5.6 per million doses [69]</td>
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<td></td>
<td>YEL-AVD 3.1–3.9 per million doses [61]</td>
<td>0.19 per million doses – probably underestimated [61].</td>
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a field study of fivefold fractionated dose in Angola [71]. Based on these results, dose fractionation has become a strategy used only in emergency situations if there is a lack of yellow fever vaccine stocks. It is an off label use of the vaccine [72]. In this scenario, it would be interesting to review the non-necessity booster recommendation since the magnitude of the CD8 response is proportional to the amount of antigen present [21].

Additional gaps still need to be overcome, such as the vaccination of immunosuppressed individuals. Yellow fever vaccines contain live virus; therefore, their use is contraindicated in immunosuppressed patients because of the risk of uncontrolled viral replication. Due to ethical considerations, few controlled studies of such vaccines that include immunosuppressed individuals have been performed.

In the literature, there exist observational and retrospective studies involving HIV-positive individuals; the largest such investigation examined a cohort of 364 HIV-positive individuals [73] who underwent 17D-204 immunization. In this cohort, only 9 subjects did not achieve protective titers. Individuals with high viral loads and lower CD4 levels were more likely to exhibit lower antibody production. All 14 subjects in the subgroup with CD4 < 200 cells/mm³ had protective antibody titers. There were no reports of serious adverse events. The yellow fever vaccine is considered to be effective in HIV-positive individuals but exhibits mildly impaired immunogenicity in such individuals relative to non-HIV-infected individuals. This can be explained by an aberrant activation of the B cells or low T-helper response promoted by HIV infection. Antiretroviral therapy may improve the protective response to the yellow fever vaccine by reducing the activation of the immune system and improving immune cellular response [74]. Similar studies have produced similar results, leading to a recommendation to vaccinate HIV-positive individuals who are exposed to high-risk areas and have CD4 levels greater than 200 cells/mm³. Ideally, these individuals should be on highly effective antiretroviral therapy and/or have undetectable HIV RNA [74].

With respect to transplant recipients, even fewer studies exist; such investigations include only case series, the largest of which involved 19 cases [75]. No adverse effects were reported, but no antibody measurements were performed. Observational studies of individuals using immunosuppressive drugs have been conducted; however, these investigations have involved small samples. For instance, Oliveira et al. evaluated 31 patients diagnosed with inflammatory rheumatic diseases who were using immunosuppressants [76], such as methotrexate, leflunomide, infliximab, hydroxychloroquine, and rituximab. Overall, 87.1% of these patients had protective titers of neutralizing antibodies. In that study, no serious adverse effects were observed. These investigations point to the need for larger studies of immunosuppressed individuals to assess safety. However, it is known that risks and benefits should be weighed when deciding whether to vaccinate an immunosuppressed patient because there is no effective treatment for yellow fever, which is highly lethal. The development of an inactivated vaccine would probably reduce the severe side effects; however, still there are no information about the long-lasting protective immunity. Ongoing animal studies [77–81] have shown that such vaccines result in lower levels of neutralizing antibodies than live virus vaccines but high survival rates in viral exposure tests.

High hydrostatic pressure has been described to abolish yellow fever virus infectivity and eliminated the ability of the virus to cause disease and represent an alternative strategy to produce safe vaccine to be use on immunosuppressed patients. However, pressure-inactivated 17DD vaccine virus elicited low level of neutralizing antibody titers although exhibited complete protection against an otherwise lethal challenge in murine model [81].

Innovative insights have also gained strength as nonviral DNA-based vaccine formulations. Preliminary reports have characterized the DNA-based vaccine expression and its immunological properties, suggesting that DNA-based vaccine candidates should be considered for further developmental studies [82].

6. Five-year view

In the next 5 years, studies on inactivated yellow fever vaccines will likely become consolidated with the development of phase II, III, and IV studies. Safety and immunogenicity issues, such as the number of doses required for immunization and the duration of protection, should be addressed.

Observational studies with representative samples that analyze the cellular and humoral immune responses of immunosuppressed individuals who receive 17D-204 or 17DD vaccination will help pave the way for additional scientific knowledge and will support difficult decisions regarding whether to vaccinate immunocompromised individuals residing in or traveling to endemic areas.

Considering the expansion of yellow fever virus circulation in areas at risk of transmission, alternative strategies has become urgent to overcome the increasing need for yellow fever vaccine supply worldwide. The reevaluation of booster dose requirement and the use of fractionated doses have been implemented.

Key issues

- The yellow fever vaccine is a live attenuated virus vaccine that is considered one of the most efficient vaccines produced to date.
- The original 17D strain generated the substrains 17D-204 and 17DD, which are used for the current production of vaccines against yellow fever.
- The 17D-204 and 17DD substrains present subtle differences in their nucleotide compositions, which can potentially lead to variations in immunogenicity and reactogenicity.
- The vaccine against yellow fever reproduce an acute viral infection and induce potent long-lasting humoral and cellular immune responses.
- In the 17D-204 and 17DD vaccines, the CD4+ T lymphocytes initially decreased in association with the peak viremia but increased during the second week. The activation of CD4+ and Treg lymphocytes preceded the activation of CD8+ T lymphocytes. These CD4+ T lymphocytes were predominantly IFN-secreting and presented significant expansion on day 14.
The immune system activation and modulation events generated by the yellow fever vaccine occur simultaneously. Yellow fever vaccines produce neutralizing antibodies that can last 30–40 years. The seroconversion rates range from 89.7 to 98.2% in adults and 67–94% in children. The 17D-204 and 17DD vaccines are quite safe, and serious adverse reactions are rare (1 per 250,000 or 500,000 vaccinations). Yellow fever vaccine-associated viscerotropic disease (YEL-AVD) and neurotropic disease (YEL-AND) are part of this spectrum.

The 17D-204 and 17DD yellow fever vaccines present similar immunogenicity, with strong activation of the cellular and humoral immune responses. The vaccines induce the production of neutralizing antibodies and confer protection that manifests as a strong and polyfunctional cellular response with amplification potential.

Current gaps that should be shortly addressed based on the increasing demand for yellow fever vaccine supply worldwide include validation studies for using fractionated doses, especially regarding the duration of protective immunity in a long term fashion.

A critical issue regarding protective immunity induced by yellow fever caused by wild type virus still remains to be elucidated.

Systematic studies focusing on particularities of immune response amongst children and adults are required to support the need of booster doses to guarantee long lasting protecting immunity induced by yellow fever vaccine.

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Declaration of interest
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References
Papers of special note have been highlighted as either of interest (●) or of considerable interest (●) to readers.


● Important to compare head-to-head 17D and 17DD vaccines.


● Study that reports the events in innate immune post-vaccination.


● Defines the signature of the immune response to 17D-204 yellow fever vaccine.


● Very interesting article which compares the cytokine signature of 17D-204 and 17DD vaccines.


- Important to clarify the profile of memory CD8+ T cell.


- Very interesting article that advocates the booster of the yellow fever vaccine.


- Interesting article that compares cytokine signatures with neutralizing antibodies levels.


