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## LETTER TO THE EDITOR

# Yellow fever vaccine viremia following ablative BM suppression in AML

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Yellow fever vaccine (YFV) has been used for over 70 years in endemic areas. It is produced from 17D attenuated viral strain and is rarely associated with serious adverse events (SAE), such as anaphylaxis and YFV-associated neurotropic and viscerotropic diseases.[1]

Immunosuppressed patients and patients at extremes of age are at higher risk of developing YFV-associated adverse events (AE).[1,2] The vaccine is not recommended for infants less than six months old or for persons with altered immune status such as thymic disorders, AIDS and immunosuppressive therapies based on a presumed increased risk for YFV-associated SAE.[3] YFV should be deferred in persons with a history of cancer or transplant recipients until immune recovery,[4] as suggested by two reports describing the lack of YFV AE in immune-recovered BM recipients.[5,6]

Mechanisms for YFV-associated neurotropic and viscerotropic diseases are still not completely elucidated. It is likely that immune response may not contain 17D replication, which usually follows the first vaccine dose.[3] Indeed, a low-level viremia has been described in primary recipients, starting 3–7 days after vaccination, persisting for 1–3 days and decreasing as IgM Abs are produced.[7]

We report the case of a patient with AML who started chemotherapy just 7 days after yellow fever (YF) vaccination. 17D viral load was monitored by RT-PCR, as well as clinical and laboratory abnormalities. Levels of neutralizing Abs (NA) against 17D were also quantified.

The titration of NA against YF was performed at Fiocruz, Rio de Janeiro, using a Plaque Reduction Neutralization Test[8] in serial twofold dilutions starting at 1:5, in 50 μL aliquots of heat-inactivated (56 °C for 30 min) serum, in 96-well tissue culture plates.[9] A positive *in house* monkey serum sample with YF Ab content calibrated by a WHO International Reference Preparation, with 1115 mIU/mL was the reference for each set of tests,[10] and was repeated every 10 samples. After incubation at room temperature for 1 h, a suspension of Vero cells was added and the plates were incubated for 3 h. The medium was then discarded and the cells overlaid with 100 mL of medium containing carboxymethylcellulose. After incubation for 7 days at 37 °C in 5% $CO_2$, cell monolayers were fixed with 10% formalin, stained with 0.04% crystal violet and plaques counted. The mean Ab content at the 50% end point of the standard was calculated through linear regression. The mean titre of the standard sera determined the levels of protection in each sample.

Total RNA was extracted from 140 μL of plasma using QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) and eluted in 60 μL of elution buffer. cDNA was obtained through a reverse transcriptase reaction using 10 μL of the extracted RNA, 300 ng of random primer (Amersham Biosciences, Piscataway, NJ, USA); 10 U/μL of Super Script TM II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) in a buffer solution with 0.25 U/μL of ribonuclease inhibitor (Invitrogen) and 0.5 mM deoxyribonucleotide triphosphates (Invitrogen), at final volume of 20 μL. The reaction was incubated at 45 °C for 90 min. Five microlitres of cDNA was added to 20 μL of TaqMan Master Mix (Applied Biosystems, Foster City, CA, USA) and was amplified by RT-PCR using the following primers and probe: (YF-NS5_F) 5′-GCACGG ATGTAACAGACTGAAGA-3′; (YF-NS5_R) 5′-CCAGGCCGAACCTGTC AT-3′ and (YF-NS5Probe) 5′-FAM-CGACTGTGTGGTCCGGCCCATC-3′-TAMRA.[11] The product was amplified using optical detection system layout of BioRad ICycler for 45 cycles at the following settings: 10 min at 95 °C, followed by 45 cycles of 15 s for 94 °C and 60 s for 60 °C.

An asymptomatic 39 year-old man was diagnosed with AML after routine blood test. BM aspiration demonstrated 76% blasts, urging chemotherapy initiation. He had been vaccinated against YF for the first time 7 days before chemotherapy was started. Detailed clinical and laboratory follow up for possible YFV-associated AE was performed, and 17D viral load was monitored by RT-PCR, daily for the first 19 days after vaccination (Table 1). He remained clinically stable, without any neurological or hepatic abnormalities that could resemble YFV-associated neurotropic or viscerotropic disease. 17D viral load, as measured by RT-PCR, decreased progressively from the first measurement, with undetectable levels on day 16. NA against 17D were measured on day 28 after vaccination, and surprisingly indicated protective levels.

This is an unusual case of YF vaccination followed by chemotherapy-induced severe immunosuppression. The 17D viremia was documented for 15 days after vaccination, and was not associated with detectable AE. Surprisingly, protective NA titers were detected 1 month after the vaccine, indicating memory B lymphocytes may have been preserved despite ablative BM suppression.

The use of 17D in immunosuppressed patients is a common concern in clinical practice and several points remain unknown. In a recent review, 17D AE in vulnerable populations of children, pregnant women, older persons, HIV positive patients and in individuals taking immunosuppressive medications were described. Although SAE have been identified among older persons and breastfeeding mothers, no change in the current understanding of the risk of 17D SAE could be provided by this comprehensive review.[12]

Several inconclusive reports described mutations in 17D that may be associated with SAE.[13–16] However, a particular strain related to the lack of AE in an immunosuppressed individual has not been described. Host genetic susceptibilities may also have an important role in the development of YFV AE. In previous reports, cases of YFV AE have been associated with high and prolonged 17D viral load, abnormalities in innate immune response and genetic polymorphisms in chemokine receptor CCR5, and its ligand RANTES.[17,18]

Populations of immunosuppressed individuals are likely to increase worldwide, raising concern about the use of live attenuated vaccines and their possible AE. YFV zones have been expanding in recent years in several regions of South America, in order to prevent potential urban outbreaks of the disease.[3] Understanding viral and immune response dynamics following YFV in healthy and immunosuppressed persons is paramount to elucidate the mechanisms for YFV-associated neurotropic and viscerotropic diseases. Moreover, for immunosuppressed patients

**Table 1.** 17D viral load and NA levels following YFV

| *Post-vaccine day* | *17DD viral load (RNA copies/mL)* | *Neutralizing antibodies*[a] *(mUI/mL)* |
|---|---|---|
| 7[b] | 103372 | <210 |
| 8 | NP | 288.4 |
| 9 | 23822 | <210 |
| 10 | 10664 | <210 |
| 12 | 7508 | <210 |
| 13 | 4343 | 281.1 |
| 14 | 4343 | <210 |
| 15 | 2941 | <210 |
| 16 | <1000 | 465.4 |
| 17 | <1000 | <210 |
| 19 | <1000 | <210 |
| 28 | NP | 3102.9 |

Abbreviations: NP = not performed, YFV = yellow fever vaccination. [a]NA levels are considered positive if 794 mUI/mL and higher. [b]Post-vaccine day 7 was the day of chemotherapy start.

who live or travel to YF endemic zones, it is important to establish safe vaccination guidelines, which would also grant protection against this highly lethal but preventable disease.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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