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Yellow fever vaccine: Comparison of the neurovirulence of new 17D-204 Stamaril™ seed lots and RK 168-73 strain

Jean-Claude Moulin [a,*], Jérémy Silvano [b], Véronique Barban [a], Patrice Riou [a], Caroline Allain [c]

[a] Sanofi Pasteur, 1541 Avenue Marcel Mérieux, 69280 Marcy l'Etoile, France
[b] Centre International de Toxicologie, B.P. 563, 27005 Evreux, France
[c] Sanofi Pasteur, Parc Industriel d'Incarville, 27101 Val de Reuil, France

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ABSTRACT

The neurovirulence of two new candidate 17D-204 Stamaril™ working seed lots and that of two reference preparations were compared. The Stamaril™ working seed lots have been used for more than twenty years for the manufacturing of vaccines of acceptable safety and efficacy. The preparation designated RK 168-73 and provided by the Robert Koch Institute was used as a reference. It was confirmed that RK 168-73 strain was not a good virus control in our study because it has a very low neurovirulence regarding both the clinical and histopathological scores in comparison with Stamaril™ strain and is not representative of a vaccine known to be satisfactory in use. The results were reinforced by the phenotypic characterization by plaque assay demonstrating that RK 168-73 was very different from the Stamaril™ vaccine, and by sequencing results showing 4 mutations between Stamaril™ and RK 168-73 viruses leading to amino acid differences in the NS4B and envelop proteins.

1. Introduction

Yellow fever (YF) is a viral hemorrhagic fever that is endemic in 33 countries in Africa and 11 countries in South America. The disease is caused by a flavivirus transmitted from human to human by the mosquito *Aedes aegypti*. At the end of the 20th century, this virus infected each year an estimated 200 000 non-immune persons and accounted for an estimated 30 000 deaths a year. The infection causes a severe illness, characterized by high fever, headache and abdominal discomfort and malaise, which resolves spontaneously after 3–4 days. However, some patients do not recover after this first phase and develop a severe hemorrhagic disease, for which there is no specific treatment, with a case-fatality rate varying from 20% to 80% in hospitalized patients. The case-fatality rate is the highest among young children and elderly patients. There are no antiviral drugs for any flavivirus infection including YF, so the availability of vaccines is important for both resident populations and travelers [1].

Two live attenuated yellow fever vaccines were developed in the 1930s: the French neurotropic vaccine (FNV) prepared from human virus passaged in mouse brain and the 17D vaccine prepared from the 17D strain of human virus passaged in embryonated chicken eggs. As the use of FNV was associated with a high incidence of encephalitic reactions in children, the only type of YF vaccine produced today is the 17D vaccine developed by Theiler and Smith in 1937 [2]. This live attenuated vaccine was widely used and proved safe and effective, considering that over 500 million doses have been delivered since 1945. However, some cases of serious reactions associated with the administration of the 17D vaccine were reported: hypersensitivity and more rarely, Yellow Fever Vaccine-Associated Neurotropic Disease (YEL-AND) or Yellow Fever Vaccine-Associated Viscerotropic Disease (YEL-AVD). The hypersensitivity reactions are believed to be associated with egg protein because the vaccine is prepared in embryonated chicken eggs. As the few cases of YEL-AND were observed mostly in children immunized at 4 months of age or younger, a panel of experts

Abbreviations: YF, yellow fever; FNV, French neurotropic vaccine; YEL-AND, Yellow Fever Vaccine-Associated Neurotropic Disease; YEL-AVD, Yellow Fever Vaccine-Associated Viscerotropic Disease; Eur. Ph, European Pharmacopoeia; WHO, World Health Organization; WSL, working seed lot; IABS, International Association for Biologicals symposium; CIT, Centre International de Toxicologie; TRS, Technical report series.

* Corresponding author. Tel.: +33 (0)4 37 37 35 93; fax: +33 (0)4 37 37 32 50.
E-mail addresses: Jean-Claude.Moulin@sanofipasteur.com, jc_moulin27@yahoo.fr (J.-C. Moulin).

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recommended that the YF vaccine should not be routinely given before 6 months of age [2], and this minimum age may be changed to 9 months by the national control authorities. The mechanism of YEL-AVD remains unexplained.

Even if YEL-AND and YEL-AVD occur rarely, it is important to ensure that new master and working seed lots (WSLs) of the YF vaccine have levels of neurotropism and viscerotropism within safe limits. The guidelines of both World Health Organization (WHO) [1,2] and European Pharmacopoeia (Eur. Ph.) [3,4] require the testing of attenuation of each new seed lot by evaluation of its neurovirulence in monkeys. New seed lots are to be tested in rhesus monkeys or cynomolgus monkeys for viscerotropism (viremia is monitored as a surrogate of viscerotropism), immunogenicity and neurotropism in order to allow quantitative assessment of the effects of the virus, and are to be compared with a reference virus approved by the national control authority and injected in a similar manner.

The work presented in this report was initiated in 2010 to establish the suitability of two candidate WSLs (designated A and B) intended to replace the previous WSL PV26/S706 (YF 17D-204 strain at passage 234) that has been in continuous use for more than 20 years for the manufacturing, after one passage in embryonated eggs, of the YF vaccine licensed under the name of Stamaril™ (Sanofi Pasteur, Val de Reuil, France). To prevent the shortage of the WSL PV26/S706, the two candidate WSLs were manufactured from the IP/F1 master seed lot or primary seed lot (YF 17D-204 strain at passage 233) derived from the 17D/AB 237 strain (YF 17D-204 strain at passage 232). The passage history of the YF vaccine is shown Fig. 1. The neurovirulence of the 2 candidate WSLs was compared with that of the current WSL PV26/S706 according to the WHO [2] and Eur. Ph. [3] regulatory requirements. The WHO reference preparation RK 168-73 was also included in the study. RK 168-73, like Stamaril™, was derived from the 17D/AB 237 virus, but after 5 additional passages (passage 237) of the virus by the Robert Koch Institute, Berlin, Germany [5]. At that time, this preparation was intended to be a potency reference for standardizing the mouse protection assay, but has been used as a

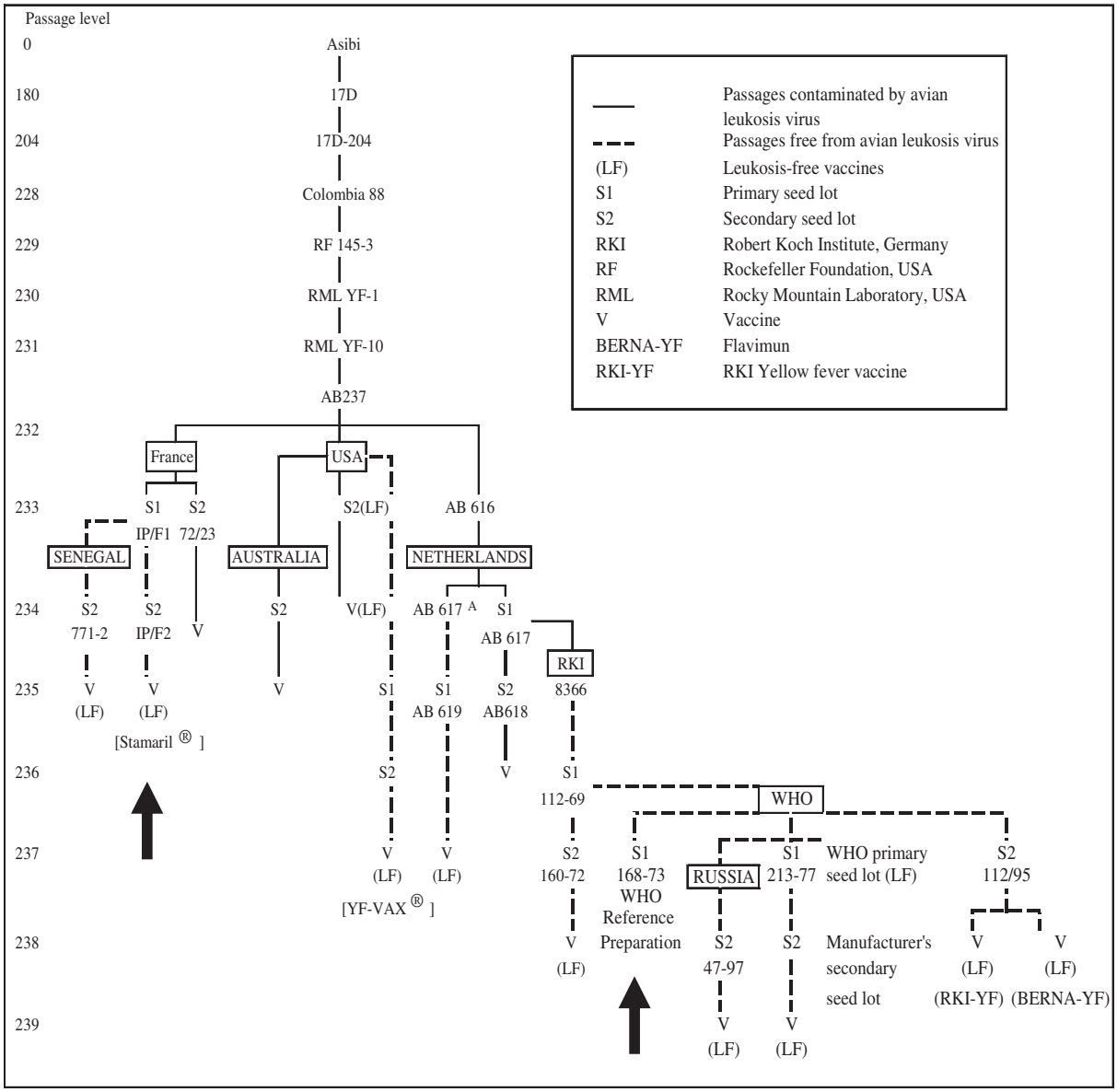

Fig. 1. Genealogy of 17D-204 Stamaril™ and RK 168-73. This simplified table is extracted from the complete table describing the genealogy of yellow fever vaccine strains derived from the 17D strain published in Refs. [1] and [6].