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Yellow fever vaccination of the elderly: The humoral immune response lagging behind

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Abstract

Background

Yellow fever vaccine (YF-17D) can cause, although rarely, serious adverse events (SAEs). The mechanism of these SAEs is poorly understood. Older age has been identified as one of the risk factors for developing such events. We investigated the adaptive immune response against YF-17D in elderly subjects, to elucidate the mechanism of SAEs.

Methods

Young volunteers (age range 18-28 yrs, N=30) and elderly travellers (age range 60-81 yrs, N=28) were vaccinated with YF-17D from the same vaccine batch. Neutralising antibody titres and plasma YF-17D RNA copy numbers were measured at day 5 after vaccination. Following vaccination, adverse events were documented in a diary during 3 weeks.

Results

Ten days after vaccination seroprotection (80% virus neutralisation in plaque assay by minimally diluted serum) was attained by 77% (23/30) of the young participants and by 50% (14/28) of the elderly ($p = 0.03$, Chi-square test). At day 10, the younger participants had a Geometrical Mean Titres (GMT) of 0.18 IU/ml, ten-fold higher than the GMT in the elderly (0.017 IU/ml) ($p = 0.004$). At day 14 the GMT also differed (respectively 4.8 IU/ml and 2.7 IU/ml, $p = 0.035$). Seroprotection was attained by all participants (young and elderly) by day 14. At day 5, viraemia was more common in the elderly (18%) than in the younger participants (3%). Viraemia was associated with fever but not with the time to seroprotection. The elderly participants reported fewer mild adverse events.

Conclusion

We found that elderly subjects (age ≥60 yrs) had a slower antibody response against yellow fever vaccine after primovaccination. We hypothesize that this allows attenuated virus to cause higher viraemia that may result in severe disease.

Introduction

The live attenuated 17D yellow fever vaccine is regarded as one of the safest and most effective vaccines [1]. However, it can cause fatal adverse events in immunocompromised individuals [2]. A hampered immune response allows the vaccine virus to replicate unrestrictedly, leading to vaccine-associated disease that resembles wild type yellow fever (yellow fever vaccine associated viscerotropic disease, YEL-AVD). YEL-AVD is fatal in 50% of cases [3]. In the last decade, a series of these serious and sometimes fatal adverse events following yellow fever vaccination appeared in the literature [4-10]. The risk of YEL-AVD increases with a history of thymectomy [11], male gender [12] and old age. For vaccinees of 60-69 years, this risk is estimated to be 1.1:100.000 doses and for vaccinees of ≥70 years it is 3.2:100.000, a 4.4 and 13.4 fold higher risk than for young adults [13].

The higher risk of YEL-AVD in elderly travellers has led to a more restrictive policy towards vaccinating travellers older than 60 years [14,15]. In this group the risk of serious adverse events following vaccination is weighed against the risk of infection, using disease surveillance data of the WHO and reports of yellow fever outbreaks.

The biological mechanism for the association between adverse events and older age has not yet been elucidated [3]. The innate and adaptive immune response wanes with age [16]. However, yellow fever neutralising antibody (NA) levels are equal in elderly and young vaccinees at 30 days following vaccination [17]. Although the immune response eventually leads to equal NA levels, it may be that the response develops more slowly in elderly subjects. This would allow the vaccine virus more time to replicate and cause adverse events.

Methods

Objectives

This study was conducted to determine whether the adaptive immune response to yellow fever vaccine is slower to develop in persons of 60 years or older compared with younger persons (18-40 years). The humoral response to yellow fever vaccination was measured by Plaque Reduction Neutralisation Test (PRNT). Yellow Fever 17D (YF-17D) viraemia after vaccination was quantified by real time PCR (qRT-PCR). In addition, baseline naïve and memory T cells ($CD4^+$ and $CD8^+$) were quantified.

Study design and Participants

In this prospective controlled observational trial, participants were recruited at the Travel Clinic of the Leiden University Medical Centre, and Municipal Health Centres of Leiden and The Hague. Healthy volunteers aged between 18 and 40 years were eligible for inclusion into the control group. Participants in the control group did not need to have an indication for yellow fever vaccination. Healthy travellers aged 60 years or above, who had an indication for yellow fever vaccination based on their travel destination (National Coordination Centre for Travelers' Health, LCR) [18], were eligible for the study group.

Those who had previously received yellow fever vaccine, those who had a compromised immunity due to underlying illness or immunosuppressive medication and –in the young– those who were pregnant were excluded. The study was carried out between April 2008 and April 2009. Vaccinations were administered at the Travel Clinic of the LUMC.

Yellow fever vaccine

The live, attenuated, 17D vaccine used in this study was manufactured on embryonated chicken eggs according to WHO regulations and stored according to manufacturer's guidelines. All administered vaccines originated from the same vaccine lot (Stamaril, Lot no B5355, Sanofi Pasteur, France). The vaccine was administered subcutaneously in the deltoid region of the right arm.

Data collection

At the time of inclusion, data on demographic characteristics of the participants were obtained. Blood samples for the determination of neutralising antibodies (NA) and YF-17D viraemia were collected before (day 0), and 3, 5, 10, 14 and 28 days after vaccination. Peripheral Blood Mononuclear Cells (PBMC) for determination of cellular immunity were collected at day 0 and 14.

Participants were asked to document local and systemic adverse events after vaccination in a three-week diary. Solicited symptoms were: erythema, pain and swelling at the site of injection, fever and myalgia.

Constant virus – varying serum dilution Plaque Reduction Neutralisation Test (PRNT)

The tests were carried out in 6-well plates (Corning Inc., USA) using a slightly modified technique described originally by De Madrid and Porterfield [19]. Briefly, approximately 6×10^5 Vero cells/mL were seeded per well in 6–well plates and cultured to obtain a

confluent monolayer. Sera were complement inactivated at 56°C for 1 hour. Prevaccination sera were tested in 1:16 dilution, to which 100 plaque forming units (PFU) of 17D-YF were added. Postvaccination sera were tested in two-fold dilutions starting from 1:4 to 1:1024. One hundred PFU of YF–17D virus were added to each serum dilution. All test sera were assayed in duplicate. After 1 hour incubation on ice, the mixtures of virus and serum were added to the Vero cell monolayers and incubated for 1 hour at 37°C. An overlay of 2 x DMEM and 2% agarose was added. After 5 days of incubation at 37°C, the overlay was discarded and cell monolayers were stained with crystal violet. Plaques were counted by eye. Virus neutralisation (VN) was calculated for each serum dilution (i) with the following formula: VN(i) = 100 x (number of PFU in diluted postvaccination serum / number of PFU in pre-vaccination serum (in a 1:16 dilution)). The serum dilution at which $\log_{10}$ neutralisation index 0·7 (80% VN) occurred was taken as endpoint, as this corresponds to the generally accepted definition of protection [20]. A reference serum, obtained from the National Institute for Biological Standards and Control (http://www.nibsc.ac.uk/) was used for quantification of International Units per milliliter (IU/mL). In our hands a 0.7 log10 plaque reduction in 1:10 diluted serum corresponds to a titre of 0.5 IU/mL [95%CI: 0.3–0.8 IU/mL] [21]. Similar values have been found by others [22]. Geometrical Mean Titres (GMT) were compared between the two groups.

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Flow cytometry

PBMC samples were thawed and allowed to rest overnight in RPMI supplemented with 10% fetal calf serum (Gibco, Breda, The Netherlands). After washing, cells were incubated with ViVid Live/Dead stain (Invitrogen, Breda, The Netherlands) for 10 minutes at 4°C before staining with the following antibodies: CD3-APC-Cy7, CD4-Alexa 700, CD8-Am Cyan, CD45RA-PE-Cy5, CCR7-PE-Cy7 (all BD Biosciences, Breda, the Netherlands) and CD19-Pacific Blue and CD14-Pacific Blue (Biolegend, ITK Diagnostics, Uithoorn, the Netherlands) for 30 minutes at 4°C. Cells were acquired on a LSRII flowcytometer using FACSDiva Software (BD Biosciences) and analysed using FlowJo Software (TreeStar, Ashland, Oregon, USA). Analysis was performed by gating on lymphocytes, followed by selection of live cells that were not CD14 or CD19 positive. Subsequently, $CD3^+$ T cells were selected and samples were gated for $CD4^+CD8^-$ cells and $CD8^+CD4^-$ cells before analyzing memory subpopulations.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Viral RNA was isolated from 200 μl plasma using a MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche Molecular Diagnostics, Penzberg, Germany). cDNA was

synthesized with 10 µl elute (200µl total) in a Tprofessional ThermoCycler (Biometra, Germany), and quantitative reverse transcription-PCR (qRT-PCR) of YFV RNA was performed in a BioRad i-cycler IQ™ real-time PCR detection system (BioRad, Veenendaal, The Netherlands). The following YFV specific primers and probe were used [23]:

YFV-1 (forward)	AATCGAGTTGCTAGGCAATAAACAC
YFV-2 (reverse)	TCCCTGAGCTTTACGACCAGA
YFV-P (probe)	FAM-ATCGTTGAGCGATTAGCAG-BHQ

FAM (6–carboxyfluorescein) was used as 5'-reporter dye and BHQ (Black Hole Quencher) as 3'-quencher dye. In order to quantify YFV RNA, $\log_{10}$ dilutions of in vitro transcribed RNA standards were included as standard curves. RNA copy numbers were calculated with the standard curves from Cycle threshold (Ct) values to compare viraemia in both groups quantitatively.

Ethics

The protocol and consent forms were approved by the Dutch Central Committee of Human Research (CCMO) and by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC) in the Netherlands. The trial was registered under NTR1040 and ISRCTN42180653, (http://irsctn.org). Written informed consent was obtained from each participant prior to inclusion.

Statistical methods

Power calculations were based on an expected 80% virus neutralisation of 95% in the control group and 66% in the elderly group at day 14, based on previous observations at the Travel Clinic (unpublished data). With an α of 0·05 and a β of 0·2, 26 participants per group were needed to confirm a significant difference under these assumed conditions. To take into account a possible lost to follow up of 15%, 30 participants were included per group.

The Student's t-test was used for comparison of the Geometrical Mean Titres (GMT) at which 80% virus was neutralised between the control group and elderly vaccinees. Where appropriate, Chi-square tests were used, and Wilcoxon's test for non-parametrical distributed numerical data. Statistical analysis was performed using a computer-assisted software package (SPSS version 16.0, SPSS Inc., Chicago, IL).

Results

Population

We enrolled 60 volunteers. None of the participants withdrew prematurely. In 2 elderly participants, virus neutralisation already occurred at day 0, meaning that these persons had been vaccinated against yellow fever previously (Figure 1). These two were excluded from further analysis. Baseline characteristics of the study population are given in table 1. Gender and possible previous exposure to flaviviruses did not differ between the groups (Table 1).

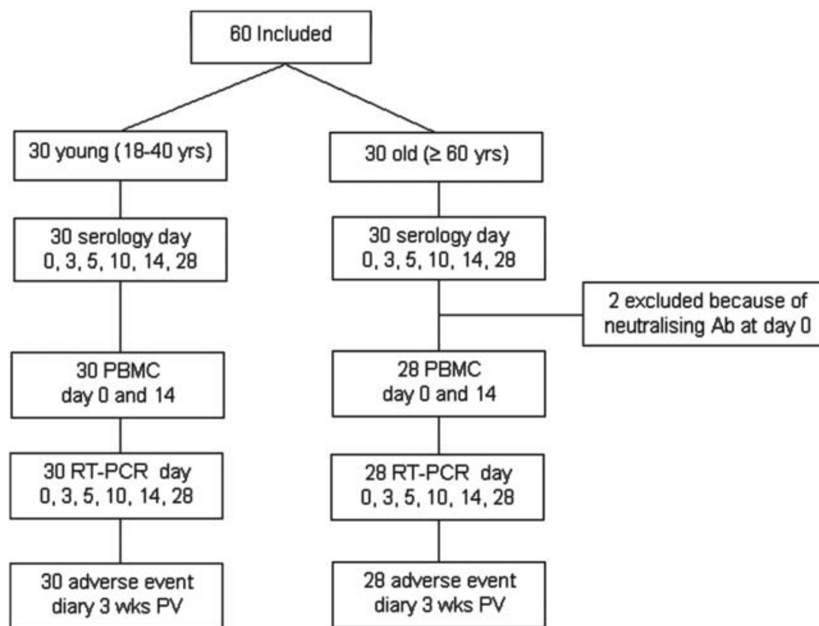

Figure 1 Inclusion of study population. Two participants in the group of ≥ 60 yrs had neutralising antibodies before vaccination. These were excluded from analysis. Ab = Antibodies, PBMC = Peripheral Blood Mononuclear Cells, RT-PCT = Real Time - Polymerase Chain Reaction, PV = Post-vaccination.

Table 1 Demographic characteristics of the study population

Demographic characteristics		Participants		p-value
		Young (18-40 years) N = 30	Elderly ($\geq$ 60 years) N = 28	
Females (%)		22 (73)	20 (71)	0.9
	Median	21	66	-
Age (years)	IQL range	20-22.5	65-69	-
	Range	18-28	60-81	-
Flavivirus [$] N yes (%)		8 (27)	8 (29)	0.9

[$] Flavivirus = possible flavivirus encounter in past five years defined as travelled to flavivirus endemic destination

Neutralising antibody response

At day 3 and 5 after vaccination, no neutralising antibodies were found (data not shown). Ten days after vaccination seroprotection was attained by 77% (23/30) of the young participants and by 50% (14/28) of the elderly group ($p = 0.03$, Chi-square test). This result is also reflected in the height of the antibody concentration (Figure 2). At day 10, the younger participants had a GMT of 0.18 IU/ml, ten-fold higher than the GMT in the elderly participants (0.017 IU/ml) ($p = 0.004$). At day 14, seroprotection was attained by all participants (young and elderly). The GMT at day 14 still differed significantly between the young and the old (respectively 4.8 IU/ml and 2.7 IU/ml, $p = 0.03$). At 28 days after vaccination there was no longer a significant difference in the GMT between the younger and older group (resp. 13.3 IU/ml and 9.0 IU/ml, $p = 0.1$). Female gender or recent travel to flavivirus endemic countries did not result in a significantly different virus neutralisation titre in either group (data not shown).

Vaccine safety

Participants reported the occurrence and duration of adverse events after yellow fever vaccination in a 3–week diary. In younger vaccinated participants vaccination evoked redness and swelling at the site of inoculation more frequently and for a longer period than in the elderly participants. Although not significant (with $\alpha = 0.05$), adverse events occurred more frequently and earlier (respectively 1.3 and 5.3 days after vaccination) in the younger participants compared to the elderly group.

Yellow fever vaccine virus RNA

YF-17D viraemia was measured by qRT-PCR at day 5. The detection limit was 1300

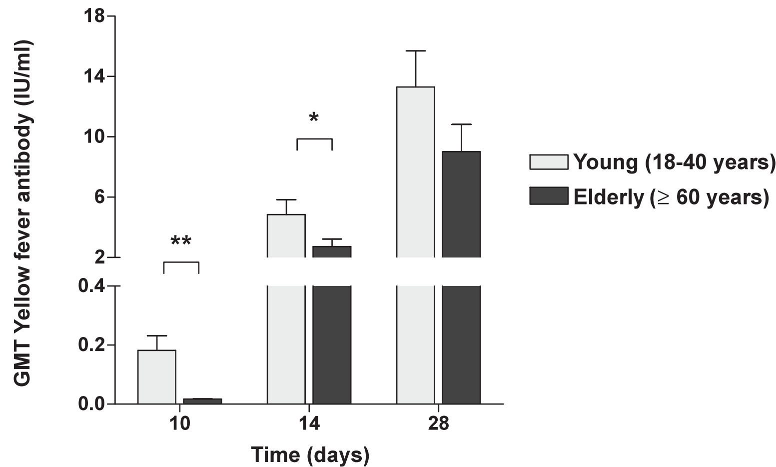

Figure 2 Geometric mean titres (GMT) of yellow fever antibodies in participants with a measurable antibody response at 10, 14 and 28 days after vaccination. No antibodies were measured at day 3 and 5 after vaccination. * p=0.03, ** p=0.004. GMT were analysed with non-parametric test (day 10) and Student's t-test (day 14 and 28). Error bars represent 95%CI.

copies/ml. At day 5 viraemia was detected in more elderly (18%) than young participants (3%) (p = 0.05, Fisher's Exact Test). The one young participant with viraemia did have a protective antibody titre at day 10. Of the six elderly people with viraemia, two had attained seroprotection by day 10 (33%) compared with 12 of 22 (55%) of those without detectable viraemia. In the seven participants with detectable viraemia at day 5, the GMT was 0.3 IU/ml (95% CI 0.1 to 0.8) at day 10, 8.6 IU/ml (95% CI 5.6 to 13.1) at day 14 and 16.5 IU/ml (95% CI 10.9 to 24.8) at day 28. In those without detectable viraemia the GMT was 0.05 IU/ml (95% CI 0.03 to 0.07) at day 10, 3.3 IU/ml (95% CI 2.8 to 3.7) at day 14 and 10.5 IU/ml (95% CI 9.1 to 12.0) at day 28. P-values for the difference in GMT between those with and those without viraemia were 0.14 for the comparison at day 10, 0.06 at day 14 and 0.33 at day 28.

Viraemia was associated with fever. Of the six participants with detectable viraemia, three (50%) had self-reported fever compared with 4 of 47 (9%) who did not have detectable viraemia (p = 0.03, Fisher's Exact Test), The mean number of days between

Table 2 Solicited adverse events after primary and booster YF-17D vaccination. Safety of vaccination expressed in various parameters

Adverse event			Participants		p-value
			Young N=30	Elderly N=28	
Local	Erythema	N yes (%)	8 (27)	2 (7)	0.05
		Mean N days (s.e.m.)	3.4 (±0·8)	2.0 (±1·0)	0.4
	Swelling	N yes (%)	3 (10)	1 (4)	0.3
		Mean N days (s.e.m.)	2·7 (±1·2)	2·0 (-)	0.8
	Pain	N yes (%)	3 (10)	2 (7)	0.7
		Mean N days (s.e.m.)	1·7 (±0·7)	2·0 (±0·0)	0.7
Systemic	Myalgia	N yes (%)	12 (40)	6 (21)	0.1
		Mean N days (s.e.m.)	2·3 (±0·5)	2·3 (±0·2)	0.9
	Fever	N yes (%)	3 (10)	4 (14)	0.6
		Mean N days (s.e.m.)	6·3 (±3.8)	3·0 (±4.0)	0.4
	(N days after vaccination)		1.3 (±1.3)	5.3 (±0.3)	0.09

S.e.m. = standard error of the mean. Fever was defined as self-measured temperature above 38 degrees Celsius.

the vaccination and start of symptoms did not differ between those with and those without viraemia (respectively 3.7 and 3.5 days). Myalgia and injection site reactions were not associated with viraemia (data not shown).

T-cell subsets

We investigated the percentage of naïve, central memory (CM), effector memory (EM) and terminally differentiated effector memory (TEMRA) cells in the $CD4^+$ and $CD8^+$ T cell subsets at baseline (day 0). Previous publications have shown that naïve cells are relatively more numerous in younger persons, and that the percentage of more differentiated T cells (EM and TEMRA) is augmented in elderly persons [24]. This was confirmed in our population (Figure 3). The increased percentage of naïve cells in the younger participants, compared with the elderly, was more pronounced in the $CD8^+$ than in the $CD4^+$ T cell subset. In addition, the elderly group had a higher percentage of $CD8^+$ effector T cells. Irrespective of age, no correlation was found between the percentage of naïve T cells and either anti-YF17D GMT or viraemia.

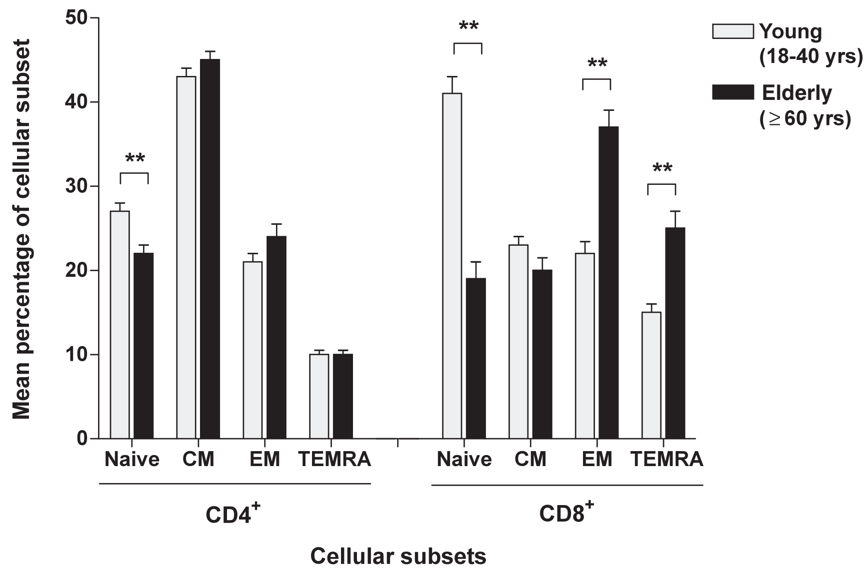

Figure 3 Percentage of T lymphocyte subsets in the study population, divided in CD4+ and CD8+ subsets. Young and elderly participants were compared with respect to their naïve, central memory (CM), effector memory (EM) and terminally differentiated effector memory cells (TEMRA) at day 0 (before vaccination) with Student's t-test. ** $p \le 0.001$.

Discussion

We demonstrated that in elderly persons (≥ 60 years) the initial humoral response to yellow fever vaccine lags behind that of younger vaccinees. GMT of neutralising antibodies were significantly lower at 10 and 14 days after vaccination but not at 28 days. Five days after vaccination viraemia was more common in the elderly. Viraemia was associated with having a fever but was not associated with the time to sero-protection.

These results may offer a biological explanation for the increased susceptibility to YEL-AVD in old age. Immunological senescence leading to an impaired ability to clear

the vaccine virus has been put forth as a possible reason for increased risk of YEL-AVD in elderly people [17]. In a retrospective study of two large clinical trials of two YF-17D vaccines from different manufacturers, involving a total of 4,532 subjects, neutralising antibody responses at 30 days after vaccination were equivalent in younger and elderly subjects. Due to the retrospective nature, early responses (i.e. < 30 days after vaccination) could not be compared and were assumed to be equal in both groups. Our results show that this assumption needs to be modified.

We observed a striking resemblance with respect to the occurrence of adverse events between the previous analysis of yellow fever vaccination in elderly subjects and our study (AE) [17]. Overall, the incidence injection site adverse events was lower in elderly than in younger subjects. If injection site reactions are a result of immune activation, observing less adverse events in elderly subjects could reflect a weaker or slower immune response in elderly people. Similarly, this line of argumentation is consistent with the later onset of adverse events in the elderly compared with the younger subjects (5.3 versus 1.3 days).

Neutralising antibodies are the gold standard for monitoring the immune response against yellow fever vaccine. This has in part a biological reason. In vitro, the antibodies inhibit viral replication. In vivo, passively immunised primates are protected against challenge with wild-type yellow fever [25]. Therefore, the delayed humoral response in the first two weeks after vaccination in the elderly could enable augmented virus replication. Whether this hampered antibody response coincides with an impaired cellular immune response remains to be studied.

In the elderly, an impaired innate and acquired immunity [26,27] is generally held responsible for the increased susceptibility of the elderly to infections, and reduced ability to respond to vaccines [28]. Clinical examples of this hampered response against vaccines are the influenza vaccine, with an efficacy between 70% and 90% in those under 65 years of age, but of 30% to 40% for those over 65 years of age [29], the pneumococcal polysaccharide vaccine [30] and hepatitis B vaccines [31]. On a cellular level, it has been shown that the subset of naïve T lymphocytes decreases dramatically with age (possibly due to thymic involution), together with an increase of effector T cells, which we also demonstrated in our study population. This altered distribution of lymphocytes at specific differentiation stages may restrict the diversity of the immune cell repertoire, leading to a diminished response to neoantigens, like yellow fever vaccine [27,28].

Beside the immunosenescence in elderly subjects, other hypotheses on the mechanism of YEL-AVD have been postulated. For example, it is possible that the vaccine virus reverts or mutates to a more virulent form. However, extensive genetic analyses of the viral strains extracted from patients with YEL-AVD do not provide evidence to support this hypothesis [3]. The hypothesis of host genetic susceptibility for developing YEL-AVD seems more plausible. Pulendran and colleagues found a heterozygous CCR5 32 mutation in a patient who suffered from YEL-AVD [32]. Since the prevalence of heterozygosity of the CCR5 32 mutation in the general population is 15% [33] and the occurrence of YEL-AVD among yellow fever vaccinees is significantly less [13], other host factors (e.g. immunosenescense) must also play a role in the development of YEL-AVD [34]. On the other hand, milder forms of YEL-AVD might occur more frequently, but might not be severe enough to be published, thus introducing publication bias. In addition to the hypothesis of genetic susceptibility, recently discovered genetic host factors, including complement protein C1qB and eukaryotic translation initiation factor 2 alpha kinase 4- (an orchestrator of the integrated stress response) predicted YF-17D $CD8^+$ T cell responses with up to 90% accuracy. A B-cell growth factor, TNFRS17, predicted the neutralising antibody response with up to 100% accuracy [35].

Although occurrence of YEL-AVD is very rare, fear of this adverse event could reduce utilisation of yellow fever vaccine. An “International Laboratory Network for Yellow Fever Vaccine-Associated Adverse Events” has been established in 2008, to complement the USA and the European Yellow Fever Vaccine Safety Working Groups [36]. Its goal is to determine the pathogenesis of severe adverse events following yellow fever vaccination through systematic and coordinated laboratory evaluation of reported cases. With this study, we contribute to this goal. A greater understanding of the pathogenesis of YEL-AVD may lead to new approaches to prevent this serious complication. One possibility may be to inject much less vaccine virus in a more immunostimulant manner (eg. intradermally) [37].

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