Immune Response during Adverse Events after 17D-Derived Yellow Fever Vaccination in Europe

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Background. In 1999–2000, reports of fatalities after vaccination with 17D-derived yellow fever vaccine (YEL) focused mainly on cases of YEL-associated adverse events (YEL-AEs) and YEL-associated viscerotropic disease (YEL-AVD). Here, we investigated 6 recent European cases to provide insight regarding immune response involvement and to identify potential risk factors.

Methods. Clinical, microbiological, molecular biological, and immunological assays were performed on serum from 6 patients with YEL-AEs, including 5 with YEL-AVD and 1 with YEL-associated neurotropic disease (YEL-AND).

Results. The levels of 3 liver enzymes associated with infection were clearly increased in all patients with YEL-AVD, but no elevations were observed in the patient with YEL-AND. In the patients with severe YEL-AVD, platelet counts were markedly reduced (<100,000 cells/µL). The only patient with fatal YEL-AVD exhibited a cytokine profile comparable to that seen in YF: high levels of interleukin (IL)–6, IL–8, monocyte chemotactic protein (MCP)–1, monokine induced by interferon–γ, and growth-related oncogene (GRO). The other patients with YEL-AVD exhibited similar but less severe cytokine profiles. The patient with YEL-AND exhibited a cytokine profile similar to that found in vaccinees without YEL-AEs: elevated levels of RANTES and low levels of GRO, MCP–1, transforming growth factor–β, and tumor necrosis factor–β.

Conclusions. On the basis of these results, we conclude that elevations in cytokine levels and reductions in platelet counts are suitable surrogate markers for patients likely to experience severe adverse reactions to YEL.

Yellow fever virus (YFV) is a member of the Flavividae genus in the Flaviviridae family. YFV infections in humans are characterized by acute and often fatal systemic involvement [1]. Symptoms of yellow fever (YF) can range from mild, nonspecific complaints to hemorrhagic fever involving all organs, especially the liver, in a viscerotropic manifestation. The incidence of YF varies significantly, with estimates ranging from 18 to 50 cases/100,000 persons, and the case fatality rate varies from 20% to 80%. According to the World Health Organization, ~200,000 new cases of YF occur each year, with ~30,000 deaths [1–3]. To address this health concern, a YF vaccine is available. Since 1990, >500 million doses have been administrated worldwide, and ~25–30 million doses are produced annually [4].

The live attenuated YF vaccine (YEL) is one of the safest and most efficacious vaccines ever made. Seroconversion occurs in >95% of recipients, and long-lasting immunity is conferred [1, 5, 6]. YEL is produced by seeding embryonated chicken eggs with stock virus, harvesting, and freeze-drying with thermostabilizing components [7]. The vaccine is heterogeneous; it consists of quasispecies distinguishable by plaque size, neurovirulent potency in mice, and growth capacity in human monocytic cells [8, 9]. After 1941, when the propagation of a pathogenic 17D strain caused 199 nonfatal encephalitis cases in 55,000 YEL vaccinees, a stringent, reliable seed-lot system was introduced [10].
In healthy persons, viremia usually occurs between 2 and 7 days after vaccination and then subsides [11]. Mild side effects occur in 15%–20% of vaccinees 3–10 days after vaccination and include headaches, myalgia, and low-grade fever. Anaphylaxis has an estimated incidence between 1/130,000 and 1/250,000 and occurs primarily in persons with allergies to egg protein or gelatin [12]. Encephalitis has been reported in 27 subjects after YEL vaccination, mainly in infants <9 months old, and 20 of these subjects recovered without sequelae [4]. The first fatality due to YEL vaccination occurred in 1965 with a case of acute encephalitis in a 3-year-old girl. This occurrence was followed by occasional reports of other isolated fatalities [13–16].

In 2001, 6 of 7 cases of vaccine-induced multiorgan failure resulted in death [17–19]. In the same year, 3 more case reports were published, describing similar symptoms with no fatalities [20–22]. This prompted a review by the Advisory Committee on Immunization Practices, and 2 new conditions were acknowledged: YEL-associated neurotropic disease (YEL-AND) and YEL-associated viscerotropic disease (YEL-AVD) [23].

By March 2007, a total of 36 YEL-AVD cases had been reported to manufacturers worldwide. Two to 8 days after vaccination, persons with YEL-AVD develop symptoms similar to those observed in wild-type YFV infections [24–26]. To date, no factors common to either the vaccines or the vaccinees have been linked with certainty to YEL-AVD. The genetic stability and consistency of the YEL virus was confirmed in phenotypic analyses over a manufacturing period of 12 years [9]. Furthermore, the 17D YFV strains isolated from patients with YEL-AVD did not reveal any mutations that could explain the unusual clinical outcome [26, 27]. Only 2 potential risk factors have been suggested: advanced age and thymus disorders [28–31]. However, both the severity and the time of onset of YEL-associated adverse events (YEL-AEs) suggest that interference with the immune response is a contributing factor. In this report, we provide insight regarding the involvement of the immune response in 6 cases of YEL-AEs, including one in which the outcome was fatal.

**METHODS**

We describe the immune response in 6 European patients with cases of YEL-AEs, 5 of which were reported through the European Network for Diagnostics of Imported Viral Diseases. One case was identified as definite YEL-AVD with a fatal outcome, 3 were considered to be probable YEL-related cases, and 2 were considered to be suspected YEL-related cases. For one of the probable cases, the patient presented with symptoms of YEL-AND; the other 5 patients presented with symptoms of YEL-AVD. The criteria for classification of YEL-AND and YEL-AVD were based on the recommendations of the Yellow Fever Vaccine Safety Clinical Working Group of the Centers for Disease Control and Prevention (CDC) [23, 32].

**Patient material.** Patient serum and tissue samples were obtained from the corresponding hospitals after routine diagnostic investigations. In all cases, virological studies were performed on serum samples obtained several days after vaccination. For the YEL-AVD case with a fatal outcome, whole blood, liver, and kidney tissue samples were also available. Specimens used for virus isolation and serology were stored at −70°C until use. Tissue samples for histological studies were preserved at room temperature in 10% buffered formaldehyde.

The samples for YEL-AEs were obtained from J. Morris, Health Protection Agency, Aylesbury, United Kingdom (patient GB-03-06); S. Milas, Centre Hospitalier Regional Mons-Warquignies, Mons, Belgium, and M. Van Esbroeck, Institute of Tropical Medicine, Antwerp, Belgium (patient BE-10-05); J. Muñoz and J. Gascón, Hospital Clinic, Barcelona, Spain (patient ES-08-05); P. Weber and B. Lazic, Siloah Krankenhaus, Pforzheim, Germany (patient DE-07-05); A. Doblas, D. Mora, and F. J. Carrasco, Hospital Juan Ramón Jimenez, Huelva, Spain (patient ES-10-04); and M. García, Hospital General Universitario de Valencia, Valencia, Spain (patient ES-09-02). Informed consent was obtained from all patients, and the guidelines for clinical studies of the national ethical committees of the ministries of health of Belgium, Spain, and Germany were followed.

**Serological and clinical analysis.** Serum samples were analyzed for IgM and IgG antibodies against YFV antigen strain 17D by indirect immunofluorescence with commercial slides (EUROIMMUN) [33]. Serum samples from 3 patients (GB-03-06, BE-10-05, and DE-07-05) were analyzed for neutralizing antibodies by a plaque reduction neutralization test (PRNT), as described elsewhere [11]. Serum and cerebrospinal fluid (CSF) samples of 3 patients (ES-08-05, ES-10-04, and ES-09-02) were analyzed by a microneutralization assay, as described elsewhere [26].

Patient samples were tested for common clinical parameters, including 3 liver enzymes—glutamic-oxaloacetate transaminase (GOT), glutamic-pyruvate transaminase (GPT), and γ-glutamyl transferase (GGT)—as well as C-reactive protein (CRP), fibrinogen, alkaline phosphatase, and thrombocytes, in a routine clinical laboratory by means of commercial assays. To confirm YEL-AND, we also examined the suspected samples for CSF protein, CSF white blood cell count, CSF lymphocytes, and CSF neutrophils, according to the case definitions of the CDC.

The expression of cytokines was determined using the RayBio Human Cytokine Antibody Array I (Ray Biotech). The simultaneous detection of multiple cytokines in serum samples was performed according to the manufacturer’s instructions; cytokines included granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth-related onconeogene (GRO), GRO-α, interleukin (IL)–1α, IL-2, IL-3, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, interferon (IFN)–γ, monocyte chemotactic protein (MCP)–1, MCP-2, MCP-3, monokine induced by IFN–γ (MIG), RANTES, trans-
forming growth factor (TGF)–β1, tumor necrosis factor (TNF)–α, and TNF-β.

Cell culture. For virus isolation, Vero E6 cells were inoculated with 100 μL of serum. In addition, tissue fragments (~125 mg) were homogenized in 0.3 mL of modified Eagle medium and clarified by centrifugation (1000 g, 10 min, 4°C). The supernatant, plasma, or serum from the tissue homogenates (0.2 mL) was used to inoculate Vero E6 monolayers. Cell culture supernatants were harvested 4 days after inoculation for the detection of viral genome by polymerase chain reaction (PCR). Viral cell cultures were maintained through 6 passages every 7 days.

Viral RNA isolation, reverse-transcription PCR, and TaqMan PCR for the determination of virus load in tissue. Viral RNA was obtained from serum and cell culture supernatants, using the QIAamp Viral RNA Mini Kit (Qiagen) in accordance with the manufacturer’s instructions. RNA was obtained from tissue homogenates (liver and kidney), whole blood, and plasma by guanidine-thiocyanate purification, as described elsewhere [34]. Total RNA was used for cDNA synthesis, as described elsewhere [35].

Virus load was determined by quantitative real-time PCR performed by use of the PerkinElmer 7000 Sequence Detection System (Applied Biosystems), with TaqMan Universal PCR Master Mix used as reaction buffer (Applied Biosystems) [35]. All samples were analyzed in duplicate.

RESULTS

We investigated the immune responses of 6 European patients with YEL-AEs between 2002 and 2006. All 6 patients, 5 with YEL-AVD and 1 with YEL-AND, were hospitalized with severe clinical signs and symptoms. One patient with YEL-AVD died, and another required intensive care and 3 weeks of hospitalization before recovering completely. To gain insight into the involvement of the patients’ immune responses, we analyzed clinical, molecular biological, microbiological, and immunological parameters (table 1).

Within 1 week after vaccination, all of the patients with YEL-AVD displayed elevated levels of liver enzymes; the highest values were observed 8 and 16 days after vaccination. In contrast, no elevated liver-enzyme values (GPT, GOT, or GGT) were found in the patient with YEL-AND (table 1). All vaccinees developed a protective immune response, demonstrated by neutralizing antibody titers >1:10, as determined by PRNT. Shortly after vaccination, serum neutralizing-antibody titers for patients ES-08-05 (up to 1:1024), ES-09-02 (1:724), and ES-10-04 (1:512) were higher than those found in vaccines without side effects (1:100). In patients with severe nonfatal YEL-AVD, thrombocyte counts decreased to pathological levels (<150,000 cells/μL) by 7–12 days after vaccination and then increased rapidly to normal levels after day 15, when the patients recovered. In contrast, platelet counts in the patient with YEL-AND were always within the reference range. In 3 of the 5 patients with YEL-AVD, YFV genome copies were detected 8, 10, or 18 days after vaccination and ranged from 1.0 × 10³ to 9.0 × 10⁷ genome equivalents/mL.

Patient DE-07-05 had pathological elevations of CRP, lactate dehydrogenase (LDH), and urea levels; CRP levels reached 26.3 mg/dL (reference range, 0–0.5 mg/dL); LDH levels reached 527 U/L (reference range, 135–225 U/L); and urea levels reached 131 mg/dL (reference range, 0–50 mg/dL). Patient ES-08-05 also displayed elevated CRP, LDH, and urea levels (data not shown).

Table 1. Cases of yellow fever vaccine (YEL)–associated viscerotropic disease (YEL-AVD) and YEL-associated neurotropic disease (YEL-AND) between 2002 and 2006.

<table>
<thead>
<tr>
<th>Classification of YEL-AEs</th>
<th>Sex/age</th>
<th>Onseta</th>
<th>ge/mLc,d</th>
<th>Virus isolation</th>
<th>Titerd</th>
<th>Maximum liver-enzyme values, IU/La</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEL-AVD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PRNT</td>
<td>IF</td>
</tr>
<tr>
<td>Suspected GB-03-06</td>
<td>M/47</td>
<td>5/1</td>
<td>–</td>
<td>–</td>
<td>1.80 (11)</td>
<td>1.500 (11)</td>
</tr>
<tr>
<td>Suspected BE-10-05</td>
<td>M/61</td>
<td>5/9</td>
<td>–</td>
<td>–</td>
<td>1.74 (13)</td>
<td>1.100 (13)</td>
</tr>
<tr>
<td>Probable ES-08-05</td>
<td>M/64</td>
<td>4/8</td>
<td>1.0 × 10⁸ (10)</td>
<td>–</td>
<td>1.1024 (15)</td>
<td>1.128 (15)</td>
</tr>
<tr>
<td>Probable DE-07-05</td>
<td>M/67</td>
<td>9/26</td>
<td>1.0 × 10⁸ (18)</td>
<td>–</td>
<td>1.10240 (18)</td>
<td>–</td>
</tr>
<tr>
<td>Definite (fatal) ES-10-04</td>
<td>F/26</td>
<td>3/4</td>
<td>9.0 × 10⁶ (8)</td>
<td>+</td>
<td>1.512 (8)</td>
<td>IgM+</td>
</tr>
<tr>
<td>YEL-AND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PRNT</td>
<td>IF</td>
</tr>
<tr>
<td>Probable ES-09-02</td>
<td>F/34</td>
<td>6/15</td>
<td>–</td>
<td>–</td>
<td>1.1024 (12)</td>
<td>&gt;1.80, 1:128*</td>
</tr>
</tbody>
</table>

NOTE. –, negative or within reference range; +, clear positive reaction; F, female; ge, genome equivalents; GGT, γ-glutamyl transferase (reference range, 5–80 IU/L); GOT, glutamic-oxaloacetate transaminase (reference range, 5–43 IU/L); GPT, glutamic-pyruvate transaminase (reference range, 5–60 IU/L); IF, immunofluorescence test; M, male; PRNT, plaque reduction neutralization test; YEL-AEs, YEL-associated adverse events.

a Patient nos. signify country code (BE, Belgium; DE, Germany; ES, Spain) and date of vaccination (month-year).
b No. of days after vaccination that onset of symptoms occurred/no. of days of hospitalization.
c Determined by polymerase chain reaction.
d Data in parentheses are the no. of days after vaccination that the measurement was obtained.

* Ligase chain reaction.
The cytokine profiles of all 6 patients were analyzed by an antibody array that enabled the detection of 23 cytokines (figure 1 and table 2). The patient with fatal YEL-AVD (ES-10-04) had the highest levels of IL-6, IL-8, GRO, MIG, and MCP-1, along with slight increases in TGF-β, TNF-β, RANTES, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-2, IL-3, IL-5, IL-7, IL-10, IL-13, IL-15, and TNF-α [26]. The remaining 4 patients with YEL-AVD (GB-03-06, BE-10-05, ES-08-05, and DE-07-05) exhibited moderate...

Table 2. Cytokine profiles in patients with yellow fever vaccine-associated adverse events and in control vaccinees.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sample analysis, days after vaccination</th>
<th>IL-6</th>
<th>IL-8</th>
<th>GRO</th>
<th>MIG</th>
<th>MCP-1</th>
<th>MCP-2</th>
<th>TGF-β1</th>
<th>TNF-β</th>
<th>RANTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB-03-06</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>BE-10-05</td>
<td>6</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>ES-08-05</td>
<td>10</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DE-07-05</td>
<td>18</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>ES-10-04</td>
<td>8</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ES-09-02</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Control vaccinees</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vac1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>Vac5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**NOTE.** Reactions are coded as follows: −, negative reaction; +, weak reaction; ++, moderate reaction; ++++, strong reaction. For details regarding the individual patients, see table 1. IL, interleukin; GRO, growth-related oncogene; MCP, monocyte chemotactic protein; MIG, monokine induced by interferon-γ; TGF, transforming growth factor; TNF, tumor necrosis factor.
 increases in IL-8, GRO, MIG, and MCP-1, as well as increases in TGF-β1 and TNF-β. In addition, GRO, MCP-1, TGF-β1, and RANTES were moderately increased in the patient with YEL-AND (ES-09-02). Healthy vaccinees without any side effects (Vac1 and Vac5), who served as control subjects, exhibited increases only in RANTES, with minor elevations in GRO, MCP-1, TGF-β1, and TNF-β (table 2).

**DISCUSSION**

As of March 2007, 36 YEL-AVD cases had been reported worldwide, but only some case reports were published [17–21, 23]. Because several similar cases involved YF vaccines from different manufacturers, the most reasonable cause of these very rare events is a host-related factor. There are currently several initiatives led by expert committees in the United States and Europe to analyze these AEs more closely [4, 23, 31, 36].

A few potential risk factors have already been suggested. First, persons >60 years old have a higher incidence of serious AEs [28–30]. Although there are several findings that the humoral immune response in older persons is reduced relative to that in younger persons, it is unclear how this might play a role. Second, a history of thymus disease is considered to be a risk factor for YEL-AEs. Four of 29 patients with YEL-AVD had a history of thymus disease [19, 21, 31]. It is noteworthy that, to our knowledge, all patients with YEL-AEs who had a history of thymoma had YEL-AVD exclusively. The patient with YEL-AND analyzed here (ES-09-02) is of interest because, during follow-up investigations, a thymoma was diagnosed and surgically removed.

The onset of the signs and symptoms of YEL-AEs occurred between 3 and 9 days after vaccination, which coincides with the timing of the viremia found in healthy vaccinees [11]. The viral genome was detected successfully in only half of the patients; however, it is remarkable that, in these 3 patient, viremia was detected between 8 and 18 days after vaccination. This is delayed compared with findings in healthy vaccinees, who exhibit viremia between 4 and 6 days after vaccination [11]. The copy number was very low in patient DE-07-05; this finding may be related to the duration and severity of the AE. Virus reisolation was possible in only 1 patient (ES-10-04), who had an extremely high viral load because of a general YFV infection in many organs. The reisolated YFV was identical to the attenuated YFV vaccine strain 17D, demonstrating that viral mutations were not responsible for the outcomes [26]. The inability to detect the viral genome or reisolate the virus from some patients may be related to potential suboptimal conditions, including sampling at the wrong time point to detect virus and/or incorrect storage or transportation conditions that impeded the preservation of the YFV.

All YEL-AVD cases were accompanied by elevated liver-enzyme levels within 1 week after vaccination; the highest levels were observed between 8 and 16 days after vaccination. In 3 of the patients with nonfatal cases of YEL-AEs (BE-10-05, ES-08-05, and DE-07-05), GOT, GPT, and GGT values were lower than those previously found in a patient with wild-type infection (table 1 and figure 2A) [23, 24].

The significant increase in liver-enzyme levels found in the patient with fatal YEL-AVD is assumed to have resulted from the destruction of the liver; YFV could be detected in liver tissue by PCR and histological analysis [26]. These pathological findings suggest that the liver is one of the first organs targeted by the attenuated YFV, a sequela similar to that observed in wild-type YFV infections. Thus, the attenuated YFV retains some of its viscerotropic potential, and restriction of the vaccine’s pathogenicity and replication potential requires a healthy host immune response. This also suggests a reason for the sex specificity observed in the strength of the immune response. A higher humoral immune response in men may be due to a higher viremia accompanied by a higher rate of AEs in men than in women. It is unclear whether the higher viremia in men is caused by a delayed immune response, which may put them at greater risk for YEL-AEs [33, 37].

Low platelet counts (<150,000 cells/μL) were observed in patients with severe AEs (figure 2B). Abnormal blood clotting (hemostasis) can be caused by many diseases. If a reduction in platelet count is caused by excessive internal bleeding consistent with viral pathogenesis, then this parameter could serve as a prognostic or surrogate marker indicating the severity of YEL-AVD cases. This observation warrants further analysis in a larger cohort. In 4 patients with YEL-AVD (DE-07-05, BE-10-05, GB-03-06, and ES-08-05) elevations in CRP, LDH, and urea levels were also reported, indicating an acute infection accompanied by liver damage and renal failure (data not shown).

In this study, we investigated whether the cytokine profile in the serum of YF vaccinees could characterize the quality of the immune response. We also investigated whether any particular cytokines could serve as markers for indicating the severity of YEL-AEs.

Immunologically active cytokines and chemokines function as immune modulators in regulating a YFV infection, but their role is still incompletely understood. After YF vaccination, the viremia (3–7 days after vaccination) in first-time vaccinees stimulates a release of nonspecific infection markers, including IFN-α, TNF-α, neopterin, and β2-microglobulin [11, 38, 39]. Our analysis of the cytokine profile included a representative selection of 23 cytokines. As shown in figure 1 and table 2, different selections of proinflammatory cytokines and chemokines were released in the patients with YEL-AVD and YEL-AND.

We observed elevations in levels of RANTES, a regulator of the activation of normal T cell expression and secretion, in all patients. This finding is similar to the up-regulation of RANTES gene expression and the increased release of RANTES observed in Japanese encephalitis virus (JEV) infections [40, 41]. RANTES may affect platelet function by activating the T cell release of
arachidonic acid, which selectively attracts Th1 lymphocytes to the site of infection. RANTES may also noncompetitively inhibit activation of platelets and thus may play a regulatory role in the platelet response to inflammation [42]. In addition, both RANTES and MCP-1 can affect vascular permeability by inducing the release of superoxide radicals and histamine. A differential cellular response may result from the susceptibility of endothelial cells to YFV [43]. In our findings, RANTES expression was detectable in all patients with YEL-AEs as well as in primary vaccinees without AEs. RANTES release may be a normal immune reaction to the YF vaccine virus.

MCP-1 and MCP-2 are monocyte chemoattractant proteins produced by macrophages, monocytes, and endothelial cells. MCP-1 and MCP-2 are involved in the recruitment of macrophages and T cells to sites of infection. A direct correlation between viral replication and MCP-1 levels has been suggested in patients with HIV infection [44]. Similarly, our findings of high levels of MCP-1 in the patient with fatal YEL-AVD may be related to a high rate of YFV replication. These findings have also been reported in wild-type YFV infections [45]. YFV replication can occur in macrophages; it is conceivable that YFV may exploit macrophages by control-

![Graph A: Liver-enzyme levels in 3 patients with nonfatal yellow fever vaccine (YEL)-associated viscerotropic disease (YEL-AVD). Diamonds represent glutamic-oxaloacetate transaminase measurements, triangles represent glutamic-pyruvate transaminase measurements, and circles represent γ-glutamyl transferase measurements. Dark solid lines indicate values for patient DE-07-05, light solid lines indicate values for patient BE-10-05, and dotted lines indicate values for patient ES-08-05. The straight dashed line indicates reference values.](image1)

![Graph B: Thrombocyte counts in 3 patients with nonfatal YEL-AVD. The straight dashed lines represent maximum and minimum reference values (450,000 and 150,000/µL, respectively). dpv, days postvaccination.](image2)
ling the release of MCP-1 and other cytokines to promote its own propagation within the host [46].

Levels of the chemokines IL-8 and GRO, chemoattractants for neutrophils, were elevated in most of the patients with YEL-AEs. The highest elevations were found in the patient with fatal YEL-AVD, consistent with findings in fatal wild-type YFV infections [45]. IL-8 also triggers firm adhesion of monocytes to activated vascular endothelium, suggesting a potential role in monocyte recruitment. Extravasation of activated, infected monocytes is a potential mechanism that could enable YFV to spread from the bloodstream into organ tissues during the infection, as occurs during infection with Ebola virus [47].

In the patient with fatal YEL-AVD, a high level of IL-6 was detected, also consistent with previous findings in patients with wild-type YFV infection in Africa and in some JEV infections [40, 45]. IL-6 is known to stimulate hepatocytes and the release of acute-phase proteins, including fibrinogen, which is involved in coagulation. Thus, IL-6 may contribute to a dysfunction of the coagulation cascade, promoting the hemorrhagic syndrome.

In addition, other proinflammatory stimuli, such as TNF-α, were found in the patient with fatal YEL-AVD and have been found previously in fatal wild-type YFV infections [45]. The patient with fatal YEL-AVD displayed the strongest cytokine response; however, it remains unclear whether strong systemic inflammatory responses contributed to the hemorrhagic manifestations.

The cytokine TGF-β was released in elevated amounts after YEL vaccination regardless of whether AEs were absent or severe. TGF-β is a multifunctional protein that initiates diverse cellular responses by binding and activating specific types I and II serine-threonine kinase receptors. TGF-β can act as a regulator of proliferation, migration, survival, differentiation, and extracellular matrix synthesis in endothelial cells and vascular smooth muscle cells, and it participates in the maintenance of vascular homeostasis. Therefore, it may play a key role in vascular disorders in YFV-infected persons. A rise in serum levels of inflammatory cytokines may be related to the development of plasma leakage and disease severity [48]. The release of cytokines as a result of monocyte and macrophage activation is involved in the development of shock [49].

The findings presented here have provided new insights into cytokine release after YF vaccinations. Cytokines such as IL-6, IL-8, GRO, MIG, MCP-1, TGF-β, TNF-β, and RANTES may play major roles in the development and severity of YEL-AEs. The roles of various cytokines and chemokines in the pathogenesis of YFV infection need to be further clarified, particularly with regard to endothelial cell activation and its relationship to vasculopathy. It would also be interesting to investigate whether cytokines and chemokines can cause endothelial cell damage during YFV infection.

Vascular leakage, liver abnormality, and hemorrhagic diathesis are life-threatening complications that occur in YFV infections and in severe and fatal cases of YEL-AEs [18]. As often occurs with any viral hemorrhagic fever, abnormalities in platelet function and signs of vascular instability were noted in some of our patients with YEL-AEs (data not shown). In severe cases, vascular instability progressed, and death was associated with hemorrhage and vascular shock, as is common in various hemorrhagic fever infections [45].

Further investigation and assessment of cases of YEL-AEs is necessary to improve our understanding and methods of prevention. Collection of adequate data on YEL-AEs requires better surveillance with complete and detailed patient data and, when feasible, patient material suitable for molecular analysis. Because these cases occur very rarely, medical personnel have to be attentive and should be trained to recognize YEL-AEs and encouraged to report them to their respective laboratories.

Nevertheless, YF vaccines are among the safest attenuated viral vaccines ever developed. The European cases included in this survey indicated that elderly men were more likely to experience AEs than younger persons and/or women. The pathophysiology of YEL-AEs in all groups needs to be better understood. Despite the identification of increased severe AEs in elderly subjects, it is inappropriate to make age >60 years an absolute contraindication to YF vaccination [29, 50]. With the current state of knowledge, an individual risk-benefit assessment is more appropriate and is recommended in light of the high fatality rate of YFV infection in unvaccinated persons living in or traveling to areas of endemicity. However, YEL vaccination should be avoided in persons with a history of thrombosis, because they run a relatively high risk of YEL-AVD [31].

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