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# Clinical and Immunological Insights on Severe, Adverse Neurotropic and Viscerotropic Disease following 17D Yellow Fever Vaccination▽

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**Yellow fever (YF) vaccines (17D-204 and 17DD) are well tolerated and cause very low rates of severe adverse events (YEL-SAE), such as serious allergic reactions, neurotropic adverse diseases (YEL-AND), and viscerotropic diseases (YEL-AVD). Viral and host factors have been postulated to explain the basis of YEL-SAE. However, the mechanisms underlying the occurrence of YEL-SAE remain unknown. The present report provides a detailed immunological analysis of a 23-year-old female patient. The patient developed a suspected case of severe YEL-AVD with encephalitis, as well as with pancreatitis and myositis, following receipt of a 17D-204 YF vaccination. The patient exhibited a decreased level of expression of Fc-γR in monocytes (CD16, CD32, and CD64), along with increased levels of NK T cells (an increased $CD3^+$ $CD16^{+/-}$ $CD56^{+/-}/CD3^+$ ratio), activated T cells ($CD4^+$ and $CD8^+$ cells), and B lymphocytes. Enhanced levels of plasmatic cytokines (interleukin-6 [IL-6], IL-17, IL-4, IL-5, and IL-10) as well as an exacerbated *ex vivo* intracytoplasmic cytokine pattern, mainly observed within NK cells (gamma interferon positive [IFN-$\gamma^+$], tumor necrosis factor alpha positive [TNF-$\alpha^+$], and IL-4 positive [IL-$4^+$]), $CD8^+$ T cells (IL-$4^+$ and IL-$5^+$), and B lymphocytes (TNF-$\alpha^+$, IL-$4^+$, and IL-$10^+$). The analysis of $CD4^+$ T cells revealed a complex profile that consisted of an increased frequency of IL-$12^+$ and IFN-$\gamma^+$ cells and a decreased percentage of TNF-$\alpha^+$, IL-$4^+$, and IL-$5^+$ cells. Depressed cytokine synthesis was observed in monocytes (TNF-$\alpha^+$) following the provision of antigenic stimuli *in vitro*. These results support the hypothesis that a strong adaptive response and abnormalities in the innate immune system may be involved in the establishment of YEL-AND and YEL-AVD.**

Yellow fever (YF) is a mosquito-borne viral hemorrhagic fever and is one of the most lethal viral diseases that remains an important public health problem in the regions of Africa and South America where it remains endemic (17). The easiness of international travel also makes YF a serious health threat to millions of travelers to areas where YF is endemic (8).

Highly effective, live attenuated 17D YF vaccines have been available for decades and are considered to be among the safest vaccines worldwide (24). YF vaccines are produced by inoculation of embryonated chicken eggs with stock virus, harvesting, and freeze-drying with thermostabilizing components (1). The YF vaccines are delivered as a single subcutaneous dose and induce neutralizing antibodies in 90 to 99% of recipients after inoculation (4).

The 17D YF vaccines are well tolerated and cause a very low rate of adverse events postvaccination (21). Less than 25% of vaccinees develop mild systemic symptoms, which may include headache, myalgia, discomfort at the site of vaccination, or low-grade fever, 2 to 6 days after vaccination (17). Despite the strong safety profile of the 17D YF vaccines, reports of rare yellow fever vaccine-associated serious adverse events (YEL-SAEs) have been described in the literature, including severe

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allergic reactions, neurotropic adverse disease (YEL-AND), and viscerotropic disease (YEL-AVD) (5, 12, 13, 16, 22).

The mechanisms underlying the development of these adverse events remain unknown. Distinct hypotheses have been postulated to explain the basis of YEL-SAE, which includes viral and host features (1, 2, 7, 9, 10, 11). The reversion of the 17D YF vaccine virus into a more virulent form and other genetic mutations do not seem to be probable causes of the adverse events, since the YF virus isolated from patients with YEL-SAEs have rarely shown a nucleotide sequence different from that of the original 17D YF vaccine strain (7, 9). It is considered that host factors, mainly host immune responses to the 17D YF vaccine, may represent the major causes of YEL-SAEs (1, 2, 9, 10, 11).

Increasing age, a history of thymus disorder, and immunocompromise are considered risk factors for the development of YEL-SAEs (2, 10, 13). However, several cases with unknown risk factors have been reported, suggesting the involvement of other host immunological features. A recent study with European patients with YEL-SAEs following 17D YF vaccination illustrated the release of sets of proinflammatory cytokines and chemokines different from the sets of cytokines released from vaccinees who did not experience side effects (1). Nevertheless, the specific role of distinct immunological events in the pathogenesis of YEL-SAEs still needs to be clarified.

This report provides a detailed immunological analysis of a 23-year-old female patient who developed combined suspected neurotropic and viscerotropic severe adverse events following 17D-204 YF vaccination.

## CASE REPORT

On 4 February 2006, a 23-year-old female patient presented to an emergency room with 4 days of fever (temperature, up to 102.5°F), chills, nausea, vomiting, fatigue, myalgias, and arthralgia and one episode of loose bowel movements. She took acetaminophen (Tylenol) *pro re nata* for fever. On the morning of presentation, her symptoms had improved, but she noticed a pruritic rash on her trunk and extremities. She reported feeling itchy the night before the onset of the rash. She was given acetaminophen and diphenhydramine (Benadryl), resulting in improvement of the fever and the rash. Her medical history revealed a diagnosis of a partial complement 4 (C4) deficiency; cutaneous lupus erythematous; and allergies to penicillin, cephalosporin, and sulfa drugs.

On 27 January 2006, while she was in the United States, she received 17D-204 vaccine (YF-Vax) in preparation for her travel to Brazil. Upon admission on 4 February, the medical examination revealed an intermittent tinnitus; black spots in visual fields; multiple petechiae on the upper palate; and a maculopapular rash on the trunk, extremities, and face in association with a slightly tender mid-lower abdomen under palpation of the abdomen without rebound or guarding. The patient did not have blurry vision or eye irritation, a sore throat, cough, shortness of breath, neurological deficits, bleeding or bruising, oral ulcers, skin sloughing, lymphadenopathy, or genitourinary complaints.

The physical examination demonstrated a temperature of 98.6°F, a pulse of 88 beats per minute, a blood pressure of 110/55 mm Hg, and a respiration rate of 16 breaths per minute. The patient was alert and was oriented in place, time, and space. Laboratory records showed normal ranges for the white blood cell count ($3.5 \times 10^3/mm^3$) with normal differential counts (neutrophils, 55%, or $1{,}925/mm^3$; eosinophils, 6%, or $210/mm^3$; basophils, 1%, or $35/mm^3$; lymphocytes, 27%, or $945/mm^3$; monocytes, 11%, or $385/mm^3$), the hemoglobin concentration (13 mg/dl), hematocrit (37%), and platelet counts ($173{,}000/mm^3$); and normal electrolyte levels (sodium, 138 mEq/liter; potassium, 3.5 mEq/liter; chloride, 104 mEq/liter; bicarbonate, 26 mEq/liter); as well as a normal range of values for biochemical parameters (glucose concentration, 104 mg/dl; blood urea nitrogen concentration, 7 mg/dl; creatinine concentration, 0.7 mg/dl; aspartate aminotransferase concentration, 25 U/liter; alanine aminotransferase concentration, 25 U/liter; albumin concentration, 3.3 mg/dl). The patient was positive for antinuclear antibody with a speckled pattern and anti-double-stranded DNA negative. Her C3 concentration was 129 mg/dl, her C4 concentration was <1 mg/dl, she was anti-cardiolipin IgG and IgA negative, and her IgM level was 21 mg/dl (an IgM level of 20 to 80 mg/dl is low to medium positive). Because of a suspected allergic reaction, she was treated with acetaminophen and diphenhydramine. She was kept overnight for observation.

On 5 February 2006, she remained afebrile; however, a maculopapular rash was observed, predominantly on her chest and arms. An allergist defined the rash as a likely case of viral exanthema. The rash was noted to be 80% improved after treatment with diphenhydramine. She was then discharged with a prescription for diphenhydramine. Her laboratory data were within the normal ranges.

On 10 February 2006, she traveled to Rio de Janeiro, Brazil, and developed a headache on the overnight flight. On 12 February 2006, which was day 16 following the 17D-204 YF vaccination, she woke up with an excruciating headache but no fever. She went to the Universidade Federal do Rio de Janeiro Hospital for a medical consultation, where she presented with a generalized tonic-clonic seizure. Upon admission she was alert; however, she developed aphasia. Her body temperature was 97.5°F, her pulse was 99 beats per minute, and her respiratory rate was 20 breaths per minute. The patient's blood pressure was 90/60 mm Hg. At first, the patient appeared to be only slightly ill. No rash, petechiae, or lymphadenopathy was detected; and her mouth and oropharynx were normal. The neck was not rigid, and Kernig's sign was absent. The lungs, heart, abdomen, arms, and legs were normal. A neurological examination revealed no abnormalities. Her urine was normal. Hematological laboratory values showed leukocytosis with a normal differential count, and the other values and electrolyte values were normal. Lumbar puncture revealed a protein concentration of 40 mg/dl and a glucose concentration of 49 mg/dl, and staining of a specimen of cerebrospinal fluid (CSF) disclosed a leukocyte count of $150/mm^3$ with 77% neutrophils (concentration, $11.5/mm^3$). A computed tomographic (CT) scan of the brain with the intravenous injection of contrast material showed brain edema. An electroencephalographic examination revealed generalized low-amplitude slowing; no epileptiform activity or organized electroencephalographic seizures were seen. Specimens of blood were obtained for culture; and the administration of meropenem, acyclovir, steroids, phe-

nytoin, and acetaminophen was initiated. Shortly after admission, the fever subsided.

On the morning of the second day after admission, no rash or lymphadenopathy was detected. Her speech had returned but remained poor. Laboratory values were normal, despite C4 levels close to the lower limit of the normal range. A magnetic resonance imaging (MRI) study of the brain, performed before and after the intravenous administration of contrast, showed a subtle hyperintensity on fluid-attenuated inversion-recovery images in the head of the left *corpus caudatus* and the frontal cortex. The sizes of the ventricles, sulci, and cisterns were appropriate for the patient's age. There was no abnormal fluid collection, intracranial bleeding, midline shift, mass effect, or focal infarction; and the flow voids of the major vessels were patent. Subsequently, the treatments with meropenem and steroids were discontinued.

On the third day of admission, her condition evolved with a fever (maximal axillary temperature, 99.7°F), headache, agitation, mental confusion, poor speech, and no motor deficits associated with the muscle ache and abdominal pain. The laboratory results, however, showed increased levels of amylase (1,231 U/liter), lipase (8,633 U/liter), lactate dehydrogenase (LDH; 1,006 U/liter), creatine phosphokinase (CPK; 6,437 U/liter), and myoglobin (707 mg/dl). An abdominal CT scan (without contrast) showed a sizeable increase in the size of the pancreas, mainly the body and tail, with discrete tissue infiltration and free fluid in the abdominal cavity that could not be ascribed to fluid overload. The results of laboratory studies of the patient's serum showed nonreactive IgM antibodies to dengue virus and leptospires (as determined by enzyme-linked immunosorbent assay [ELISA]); nonreactive IgM antibodies but reactive IgG antibodies to Epstein-Barr virus, rubella virus, and hepatitis A virus and nonreactive IgG antibodies to hepatitis B virus and hepatitis C virus (as determined by microparticle enzyme immunoassay; Abbott); a reactive Monotest result (as determined by agglutination); nonreactive IgM antibodies to cytomegalovirus (as determined by ELISA); and negative PCR and nested PCR results by the use of oligonucleotides specific for the genomic LA and MIE regions of members of the *Herpesviridae* family. In light of the clinical signs and symptoms, the MRI and CT images, and serological findings, acyclovir was discontinued.

On the fourth day after admission, her body temperature became normal and the patient was still complaining of muscle pain, headache, and abdominal pain. There was mental confusion that alternated with drowsiness, accompanied by a new focal neurological manifestation characterized by discrete central facial and upper limb paresis. MRI revealed no abnormalities except a mild contrast enhancement in the meninges. The laboratory tests showed that the pancreatic enzyme, CPK, and LDH levels had further increased; and there was a mild increase in the aminotransferase levels dissociated from the levels of the other liver enzymes.

On the fifth day after admission, the patient became more responsive to commands, although she could not distinguish her right hand from her left hand. Motor deficits were no longer evident. She reported improvement in her headache and epigastric pain, and she recognized her location. All laboratory tests demonstrated a decrease in the levels of the enzymes mentioned above. Serum serology showed negative results for neurotropic viruses, including eastern equine encephalitis virus, western equine encephalitis virus, Venezuelan equine encephalitis virus, St. Louis encephalitis virus, and Rocio virus. The patient was negative for mumps virus neutralizing antibodies (as determined by the plaque reduction neutralization test [PRNT] titer, $<1:5$). Serology for yellow fever virus showed a negative CSF IgM result but a positive serum IgM result (as determined by M antibody capture ELISA). Yellow fever virus neutralizing antibodies in serum (PRNT) were positive at 3,335 mIU/ml. The yellow fever virus genome was not detected in CSF or serum samples (by reverse transcription-PCR [RT-PCR]). Yellow fever virus was not isolated following the inoculation of serum into Vero cell cultures, C6/36 cell cultures, or suckling mice.

On the sixth day after admission, the patient improved clinically, was fully oriented, and talked normally; but she still complained of headache and muscle aches but not epigastric pain. The clinical examination was completely normal. The pancreatic enzyme levels were very close to the upper limit of the normal range, and a liquid diet was started. An MRI study of the abdomen performed before and after intravenous administration of contrast material showed a mild increase in the size of the pancreas, mainly in the body and the tail, without necrosis or other abnormalities. There was a slight fluctuation in the serum lipase levels.

On the seventh day after admission, she continued to improve clinically. She tolerated the liquid diet without nausea or pain. However, the discrete increase in the serum lipase level remained. An MRI study of the abdomen performed before and after the intravenous administration of contrast material showed a mild increase in the size of the pancreas, mainly in the body and the tail, without necrosis or other abnormalities.

On the eighth day after admission, the patient was fully oriented and continued to be asymptomatic, showing normal serum amylase levels and only a slight increase in serum lipase levels. Cultured specimens of blood and cerebrospinal fluid were sterile.

On the ninth day after admission, the patient was alert and lucid. A new neurological sign was observed and consisted of a prominent action and postural tremor in the tongue and palate; this was more evident when the tongue was stretched out.

On the tenth hospital day the patient ate well and no longer requested acetaminophen for headache. The tongue action tremor persisted but was better than it was on the day before. She was discharged on the following day.

**Tentative diagnosis.** Considering the combined clinical and laboratory data, the diagnosis for this patient was YEL-SAEs with prominent encephalitis and a residual tongue tremor associated with an important case of pancreatitis and rhabdomyolysis.

## MATERIALS AND METHODS

With the objective of characterizing the immune events underlying the unusual presentation of a combined neurotropic and viscerotropic adverse event following 17D-204 YF vaccination, the immunological status of the patient described here was investigated by detailed immunophenotypic analysis by flow cytometry with a blood sample collected on day 16 following the 17D YF vaccination. The immunological parameters were compared with the reference values (RVs) for peripheral blood leukocyte subsets taken at day 15 after 17DD vaccination from healthy volunteers without adverse events that presented with mean positive PRNT values of 3,699 ± 400 mIU/ml.

**Biological samples.** A 10-ml blood sample was collected from the patient and placed into Vacutainer tubes containing sodium heparin (Becton Dickinson, San Jose, CA) 16 days after YF 17D vaccination.

Peripheral blood samples (10 ml each) from 10 healthy volunteers were collected and placed into Vacutainer tubes containing sodium heparin (Becton Dickinson) 15 days after YF 17DD vaccination, and the values for these blood samples were considered the RVs.

Fresh whole-blood aliquots were used for the *ex vivo* analysis of leukocyte subsets and activation status, as well as the analysis of the intracytoplasmic cytokine pattern following short-term whole-blood culture *in vitro*. Plasma samples were obtained by centrifugation of fresh whole-blood aliquots at 900 × *g* for 10 min at 4°C, and the samples were stored at −70°C for analysis of plasmatic chemokines and cytokines.

***Ex vivo* analysis of leukocyte subsets: frequency, activation status, and chemokine receptor expression.** Immunophenotypic analysis of peripheral blood leukocytes was performed by flow cytometry, as described previously (14, 15, 23). Briefly, 100-μl aliquots of heparin-anticoagulated blood were dispensed into 5-ml polystyrene tubes (Falcon; BD Pharmingen, San Diego, CA) and stained with 5 μl of undiluted monoclonal antibodies (MAbs) conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or tricolor (TC) specific for cell surface markers, including CD3, CD4, CD5, CD8, CD14, CD16, CD18, CD19, CD23, CD25, CD28, CD32, CD38, CD54, CD62L, CD64, and CD69 and HLA-DR, CXCR3, CXCR4, CCR2, CCR3, and CCR5, all of which were from BD Pharmingen or Caltag (Burlingame, CA). The samples were treated by gentle vortexing and they were incubated in the dark for 30 min at room temperature. Following incubation, the samples were treated by gentle vortexing with 2 ml of fluorescence-activated cell sorting (FACS) lysing solution (BD Pharmingen) and were reincubated for an additional 10 min at room temperature in the dark. After erythrocyte lysis was completed, the samples were centrifuged at 600 × *g* for 7 min at room temperature, the supernatant was discarded, and the cell pellet was washed twice with 2 ml of phosphate-buffered saline (PBS) containing 0.01% sodium azide. Cell preparations were fixed in 200 μl of FACS fix solution (10 g/liter paraformaldehyde, 1% sodium cacodylate, 6.65 g/liter sodium chloride, 0.01% sodium azide) and stored at 4°C in the dark prior to flow cytometry analysis, which was performed within 24 h. For data acquisition and analyses, a total of 10,000 events per tube were acquired with a FACSCalibur flow cytometer and CELLQuest software (Becton Dickinson) properly set up to measure forward light scatter (FSC) and side light scatter (SSC) as well as FITC (FL-1), PE (FL-2), and TC (FL-3) fluorescence. Distinct gating strategies were used to analyze the frequency of each leukocyte subset, as described elsewhere (14, 15, 23).

**Analysis of plasmatic chemokines and cytokines.** A cytometric beads array (CBA) immunoassay kit (BD Biosciences) was used for the semiquantitative analysis of plasmatic chemokines and cytokines, including CXCL-8, CCL-2, CCL-5, CXCL-10, interleukin-1β (IL-1β), IL-6, IL-12, tumor necrosis factor alpha (TNF-α), and IL-10, as recommended by the manufacturer and as described previously (19). A sandwich ELISA was used for quantitative analysis of plasmatic IL-17 (Biosource, Nivelles, Belgium) as well as IL-23 and transforming growth factor β (TGF-β; Bender Medsystems, Vienna, Austria), as recommended by the manufacturers.

**Analysis of intracytoplasmic cytokine pattern following short-term whole-blood culture *in vitro*.** The analysis of intracytoplasmic cytokines in peripheral blood leukocyte subsets was performed as described by Peruhype-Magalhães et al. (19). The cytokine profile was analyzed after short-term incubation *in vitro* in the absence (control cultures) or the presence of the 17DD YF vaccine antigen as an exogenous stimulus. Briefly, 500-μl aliquots of heparinized peripheral blood were dispensed into individual 14-ml polypropylene tubes (Falcon; BD Pharmingen) and incubated for 6 h at 37°C in a 5% $CO_2$ humidified atmosphere in the presence of 550 μl of RPMI 1640 (Gibco, Grand Island, NY) as the control culture or in the presence of 500 μl of RPMI 1640 (Gibco) plus 50 μl live attenuated 17DD YF vaccine (lot 02UVFB005Z; BioManguinhos-FIOCRUZ) at a final concentration of $10^6$ viral particles/ml. Following incubation, 10 μg/ml of brefeldin A (Sigma Chemical Company, St. Louis, MO) was added and the samples were reincubated for 4 h at 37°C in a 5% $CO_2$ humidified atmosphere. After incubation, the cultures were treated with 2 mM EDTA (Sigma) and were kept at room temperature for 15 min. The culture samples were washed twice with 6 ml of FACS buffer (0.015 M PBS, 0.5% bovine serum albumin, 0.1% sodium azide) by centrifugation at 600 × *g* for 7 min at room temperature. After resuspension in 1 ml of FACS buffer, 200-μl aliquots were dispensed into 5-ml polystyrene tubes (Falcon; BD Pharmingen) and stained with anti-surface molecule MAbs, including antibodies to the surface molecules CD4, CD8, CD14, CD16, and CD19. After 30 min of incubation in the dark at room temperature, the samples were treated by gentle vortexing with 2 ml of FACS lysing solution (BD Pharmingen) and were reincubated for 10 min at room temperature in the dark. After erythrocyte lysis was complete, the samples were centrifuged at 600 × *g* for 7 min at room temperature, the supernatant was discarded, and the cell pellet was resuspended and kept for 10 min at room temperature in the dark with 2 ml of FACS permeabilizing solution containing FACS buffer supplemented with 0.5% saponin. Following incubation, the samples were centrifuged at 600 × *g* for 7 min at room temperature, the supernatant was gently decanted, and the cell pellet was washed with 3 ml of FACS buffer. After centrifugation, the cells were resuspended in 200 μl of FACS buffer and were distributed in 30-μl aliquots over 96-well U-bottom microtiter plates. The cells were then stained with 20 μl of PE-labeled anti-cytokine MAbs, including MAbs to gamma interferon (IFN-γ), TNF-α, IL-4, IL-5, IL-10, and IL-12, at a final concentration of 25 μg/ml. The MAbs had previously been diluted in sterile FACS permeabilizing solution by incubation for 30 min at room temperature in the dark. The cells were then washed twice, first with 150 μl of FACS permeabilizing solution and then with 200 μl of FACS buffer. The cell preparation was fixed in 200 μl of FACS fix solution and stored at 4°C in the dark prior to flow cytometry analysis, which was performed within 24 h. A total of 30,000 events per tube were acquired with a FACSCalibur flow cytometer (Becton Dickinson) properly set up to detect FSC and SSC as well as FITC (FL-1), PE (FL-2), and TC (FL-3) fluorescence. The CELLQuest software provided by the manufacturer was used for data acquisition and analysis. Distinct gating strategies were used to analyze the cytokine profiles of the specific leukocyte subpopulations (19). Selective analysis of neutrophils was performed by establishing a specific scatter gate by using the combination of anti-cell surface antigens and laser SSC for discrimination of the neutrophils, and the neutrophils were gated as $SSC^{High}$ $CD16^{+High}$ (high granularity and high CD16 expression). Analysis of the monocytes was performed by staining immunophenotyping with SSC versus FL-1 and anti-CD14-TC dot plots to select the monocytes as $SSC^{Low}$ $CD14^{+High}$ cells. Identification of the natural killer (NK) cells, T $CD4^+$ or T $CD8^+$ lymphocytes, and B cells was initially performed by using a lymphocyte scatter gate setup and FSC versus SSC dot plots, followed by a phenotype gating strategy with anti-CD16-FITC, anti-CD19-TC, anti-CD4-TC, and anti-CD8-FITC MAbs to select a given cell subpopulation. The frequency of cytokine-expressing cells was determined by using quadrant statistics over FL-2 and anti-cytokine-PE versus FL-1 and anti-cell surface marker-FITC or FL-3/anti-cell surface marker-TC dot plots. All results for the different gated leukocyte subpopulations were expressed as a percentage of the cytokine-positive cells.

**Statistical analysis.** Data analysis was performed, and the data were considered to be statistically significant when the values for the patient were outside of the mean ± 2 standard deviations (SDs) of the RV range. Statistical significance was also considered when the values for the patient were outside the range of 0.5 to 2.0 times the mean RV.

## RESULTS

***Ex vivo* analysis of leukocyte subsets: frequency, activation status, and chemokine receptor expression.** The *ex vivo* analysis of the phenotypic features of the circulating leukocytes demonstrated that the patient's monocytes displayed lower levels of expression of Fc-γR (CD16, CD32, and CD64) compared to the RVs (Table 1). Additionally, a higher frequency of NK T cells ($CD3^+$ $CD16^{+/-}$ $CD56^{+/-}$/$CD3^+$ ratio) was the hallmark of the innate immunity of the patient.

An increased frequency of activated $CD4^+$ T cells ($CD69^+$ and HLA-DR positive [$HLA\text{-}DR^+$]), in addition to increased levels of $CD54^+$ and $CD38^+$ cells, decreased levels of $CD62L^+$ events within the $CD4^+$ T cells, as well as the lower levels of expression of CD18 by $CD4^+$ T cells compared to the RVs, was observed in the case patient. A reduced frequency of regulatory T cells ($CD4^+$ $CD25^{High}$) along with a lower level of expression of IL-10 receptor (IL-10R) by $CD4^+$ T cells, was also observed (Table 1). Moreover, analysis of $CD8^+$ T cells also demonstrated increased levels of activated cells and a high percentage of $CD54^+$ cells, a lower frequency of $CD62L^+$ cells, and a reduced level of expression of CD18 compared to the RVs (Table 1).

An increased frequency of activated B cells ($CD19^+$

TABLE 1. *Ex vivo* analysis of peripheral blood leukocyte subsets and activation status in the patient with combined neurotropic and viscerotropic disease following 17D YF vaccination

| Cell type | Cell phenotype | Value[a] | |
|---|---|---|---|
| | | RV | Patient |
| Monocytes | $CD14^+$ $CD16^+$ $DR^{++}/CD14^+$ $CD16^+$ | 71.0 ± 14.8 | 54.0 |
| | CD16 (MFI) in $CD14^+$ | 101.0 ± 48.0 | ↓ 20.0* |
| | CD32 (MFI) in $CD14^+$ | 215.0 ± 47.0 | ↓ 39.0** |
| | CD64 (MFI) in $CD14^+$ | 91.0 ± 25.0 | ↓ 28.0** |
| NK cells | $CD3^-$ $CD16^{-/+}$ $CD56^{-/+}$ | 15.0 ± 7.5 | 12.0 |
| | $CD3^-$ $CD16^+$ $CD56^-$/NK cells | 11.0 ± 7.1 | 7.0 |
| | $CD3^-$ $CD16^+$ $CD56^+$/NK cells | 78.0 ± 9.6 | 93.0 |
| NK T cells | $CD3^+$ $CD16^{+/-}$ $CD56^{+/-}$/$CD3^+$ | 12.0 ± 3.9 | ↑ 40.0** |
| T cells | $CD3^+$ | 67.0 ± 7.2 | 66.0 |
| | $CD4^+$ | 39.0 ± 6.2 | 38.0 |
| | CD18 (MFI) in $CD4^+$ | 40.0 ± 9.9 | ↓ 9.0** |
| | $CD4^+$ $CD54^+/CD4^+$ | 4.0 ± 2.2 | ↑ 24.0** |
| | $CD4^+CD62L^+/CD4^+$ | 68.0 ± 8.9 | ↓ 23.0** |
| | $CD4^+CD69^+/CD4^+$ | 3.0 ± 1.8 | ↑ 10.0** |
| | $CD4^+CD38^+/CD4^+$ | 41.0 ± 9.8 | ↓ 10.0** |
| | $CD4^+CD28^+/CD4^+$ | 96.0 ± 1.8 | 95.0 |
| | $CD4^+$HLA-$DR^+/CD4^+$ | 4.0 ± 2.0 | ↑ 11.0** |
| | $CD4^+CD25^{High}$ | 1.9 ± 0.9 | ↓ 0.8* |
| | IL-10R (MFI) in $CD4^+$ | 10.7 ± 5.5 | ↓ 3.0* |
| | $CD8^+$ | 28.0 ± 5.8 | 28.0 |
| | CD18 (MFI) in $CD8^+$ | 68.0 ± 20.4 | ↓ 10.0** |
| | $CD8^+$ $CD54^+/CD8^+$ | 6.0 ± 4.0 | ↑ 18.0** |
| | $CD8^+$ $CD62L^+/CD8^+$ | 48.0 ± 6.4 | ↓ 16.0** |
| | $CD8^+$ $CD69^+/CD8^+$ | 11.0 ± 2.8 | 10.0 |
| | $CD8^+$ $CD38^+/CD8^+$ | 54.0 ± 21.2 | 64.0 |
| | $CD8^+$ HLA-$DR^+/CD8^+$ | 5.0 ± 2.4 | 6.0 |
| | $CD8^+$ $CD28^+/CD8^+$ | 59.0 ± 12.1 | 40.0 |
| | IL-10R (MFI) in $CD8^+$ | 17.0 ± 6.6 | 11.0 |
| B cells | $CD19^+$ | 13.0 ± 4.7 | 17.0 |
| | $CD19^+$ $CD5^+/CD19^+$ | 24.0 ± 5.5 | 22.0 |
| | $CD19^+$ $CD5^-/CD19^+$ | 75.0 ± 8.2 | 78.0 |
| | $CD19^+$ $CD23^+/CD19^+$ | 49.0 ± 15.3 | 53.0 |
| | $CD19^+$ $CD69^+/CD19^+$ | 12.0 ± 3.6 | ↑ 54.0** |
| | CD32 (MFI) in $CD19^+$ | 160.0 ± 32.0 | ↓ 11.0** |

[a] RV, RVs for peripheral blood leukocyte subset frequencies observed 15 days after 17DD vaccination in the absence of adverse reactions ($n$ = 10); Patient, peripheral blood leukocyte subset frequencies observed in the patient with combined neurotropic and viscerotropic disease following 17D YF vaccination. The results are expressed as the mean percentage ± SD of positive cells unless MFI is indicated, in which case the results are expressed as the mean MFI ± SD for the expression of a given cell surface marker within gated lymphocytes. *, statistical significance was considered when the values for the patient were outside of the RV range (mean ± 2 SDs); **, additional statistical significance was considered when the values for the patient were outside the range of 0.5 to 2.0 times the mean RVs. ↑ and ↓, increase and decrease in the values, respectively, in comparison to the RVs.

$CD69^+$), in addition to a reduced level of expression of CD32 by B cells, was the major phenotypic feature suggesting the high level of activation of B cells, as determined by comparison of the levels of activation with the RVs (Table 1).

The results of the analysis of chemokine receptor expression by monocytes and T lymphocytes are presented in Table 2. The data analysis demonstrated that the T cells from the patient presented a decreased level of expression of

TABLE 2. *Ex vivo* expression of chemokine receptors by peripheral blood leukocyte subsets in the patient with combined neurotropic and viscerotropic disease following 17D YF vaccination

| Cell type | Chemokine receptor | Value[a] | |
|---|---|---|---|
| | | RV | Patient |
| Monocytes | CXCR4 | 8.34 ± 2.33 | 8.7 |
| | CXCR3 | 10.70 ± 2.54 | 9.25 |
| | CCR5 | 8.91 ± 3.17 | 9.80 |
| | CCR2 | 9.93 ± 4.12 | 10.29 |
| $CD4^+$ T cells | CXCR4 | 4.50 ± 1.02 | 4.70 |
| | CXCR3 | 26.68 ± 4.67 | ↓ 4.33** |
| | CCR5 | 3.34 ± 1.71 | 3.75 |
| | CCR2 | 2.80 ± 0.60 | 3.26 |
| | CCR3 | 2.89 ± 0.81 | 3.46 |
| $CD8^+$ T cells | CXCR4 | 7.99 ± 1.99 | 5.06 |
| | CXCR3 | 32.73 ± 6.50 | ↓ 5.59** |
| | CCR5 | 5.38 ± 1.74 | 3.84 |
| | CCR2 | 4.65 ± 1.71 | 3.53 |
| | CCR3 | 3.93 ± 0.73 | 3.93 |

[a] RV, RVs for chemokine receptor expression by peripheral blood leukocyte subsets observed 15 days after 17DD vaccination in the absence of adverse reactions ($n$ = 10); Patient, values for chemokine receptor expression by peripheral blood leukocyte subset frequencies observed in the patient with combined neurotropic and viscerotropic adverse events temporally associated with 17D vaccination YF. The results are expressed as the mean percentage ± SD of positive cells unless MFI is indicated, in which case the results are expressed as the mean MFI ± SD for the expression of a given cell surface marker within gated lymphocytes. *, statistical significance was considered when the values for the patient were outside of the RV range (mean ± 2 SDs); **, additional statistical significance was considered when the values for the patient were outside the range of 0.5 to 2.0 times the mean RVs. ↑ and ↓, increase and decrease in the values, respectively, in comparison to the RVs.

CXCR3 by both $CD4^+$ and $CD8^+$ T cells in comparison with the RVs (Table 2).

**Analysis of plasmatic chemokines and cytokines.** Semiquantitative analysis demonstrated that the patient's plasma exhibited a chemokine profile with values comparable to the RVs: high levels of CXCL-8 (IL-8) and CCL-5 (RANTES) and low levels of CCL-2 (monocyte chemoattractant protein 1 [MCP-1]), and CXCL-10 (interferon-inducible protein 10) (Fig. 1A). Additional analysis demonstrated that YEL-AND resulted in a general exacerbated cytokine pattern, with higher levels of plasmatic IL-6, IL-17, IL-4, IL-5, and IL-10, despite the lower levels of TNF-α, in comparison to the RVs (Fig. 1B).

**Analysis of intracytoplasmic cytokine pattern following short-term whole-blood culture *in vitro*.** Analysis of the *ex vivo* leukocyte intracytoplasmic cytokine pattern of control cultures revealed increased levels of IFN-γ-positive (IFN-$\gamma^+$), TNF-α-positive (TNF-$\alpha^+$), and IL-4-positive (IL-$4^+$) NK cells (Fig. 2A). On the other hand, in the presence of the 17DD YF vaccine antigenic stimulus, the patient's leukocytes exhibited decreased levels of TNF-$\alpha^+$ monocytes compared to the RVs. Additional analysis also revealed increased levels of IFN-$\gamma^+$ in NK cells in comparison to the RVs (Fig. 2B).

Analysis of lymphocytes from the patient showed increased levels of IL-$12^+$ $CD4^+$ and IFN-$\gamma^+$ $CD4^+$ cells and lower levels of TNF-$\alpha^+$ $CD4^+$, IL-$4^+$ $CD4^+$, and IL-$5^+$ $CD4^+$ T cells in comparison to the RVs. Analysis of $CD8^+$ T-cells revealed increased levels of IL-$4^+$ and IL-$5^+$ cells. The B-cell cytokine profile demonstrated increased levels of TNF-$\alpha^+$, IL-$4^+$, and IL-$10^+$ cells (Fig. 2C). Analysis in the presence of or after the

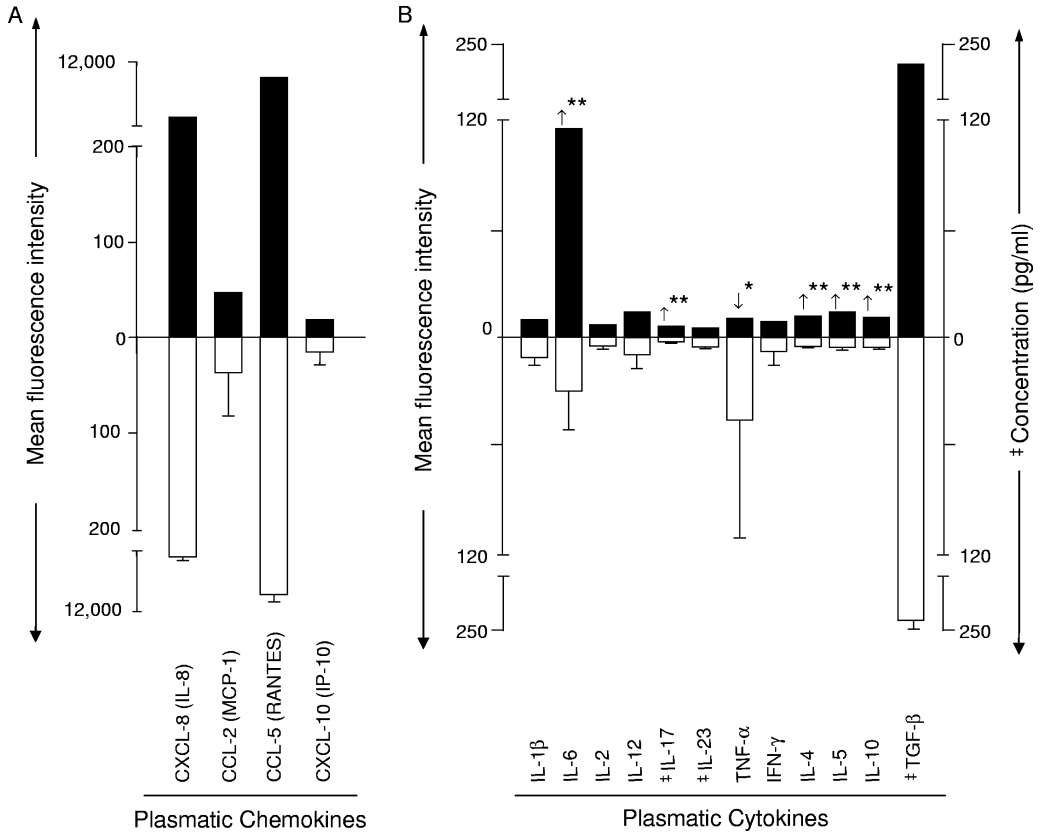


FIG. 1. Plasmatic chemokine (A) and cytokine (B) patterns observed in the patient with combined neurotropic and viscerotropic disease following 17D YF vaccination (■) compared to the reference values observed 15 days after 17DD YF vaccination in the absence of adverse reactions (□). The results are expressed as the mean fluorescence intensities ± SDs for CXCL-8, CCL-2, CCL-5, CXCL-10, IL-1β, IL-6, IL-12, TNF-α, and IL-10 or the mean concentrations (pg/ml) ± SDs for IL-17, IL-23, and TGF-β. **, statistical significance was considered when the values for the patient were outside of the reference value range (mean ± 2 SDs); *, additional statistical significance was considered when the values for the patient were outside the range of 0.5 to 2.0 times the mean RV; ↑, increase in comparison to the reference value.

17DD YF vaccine antigenic stimulus demonstrated that lymphocytes from the patient showed increased percentages of IL-12$^+$ CD4$^+$ and IL-5$^+$ CD4$^+$ T cells. Analysis of the CD8$^+$ T cells revealed increased levels of IL-12$^+$ and TNF-α$^+$ cells, in addition to an increase in the levels of IL-4$^+$ and IL-5$^+$ cells. Analysis of the B-cell cytokine profile demonstrated increased levels of TNF-α$^+$ cells (Fig. 2D).

Together, these data demonstrate that the patient exhibited an exacerbated cytokine pattern with an enhanced frequency of cytokine-positive cells, mainly observed within NK cells, CD8$^+$ T cells, and B lymphocytes, regardless of the experimental conditions under which they were evaluated (under the control condition or with the 17DD YF vaccine stimulus). The analysis of CD4$^+$ T cells revealed the most complex profile, with assorted changes being detected when the control and 17DD YF vaccine-stimulated cultures were considered. On the other hand, a depressed level of TNF-α synthesis was observed in monocytes after antigenic stimulation.

## DISCUSSION

The 17D YF vaccines (17D-204 and 17DD) are well tolerated, and the rate of serious adverse events is very low. Cases of encephalitis and other neurotropic adverse events (YEL-AND) following YF vaccination are estimated to occur in 0.4 per 100,000 vaccinees (16).

The clinical presentation of YEL-AND includes a high-grade fever (temperature, ≥100.5°F) and headache for more than 24 h. It may also be accompanied by focal neurological dysfunction, including, but not limited to, ataxia, aphasia, and paresis. More severe cases could show mental status changes, confusion, lethargy, personality changes, the new onset of seizures, or a recurrence of previously controlled seizures (16).

The clinical course of the adverse events for the patient described here is unusual in comparison to the profiles of the cases reported so far. Indeed, the patient presented with overt pancreatitis and moderate liver involvement, clinical signs not yet described in the cases reported to date. In addition, whereas the neurological signs and symptoms were presented by day 14, the viscerotropic signs and symptoms of pancreatitis and rhabdomyolysis came into the clinical picture only belatedly, on day 22. (It is unclear on how the mild mid-lower abdominal pain that occurred 9 days following vaccination should be interpreted.) On the basis of the clinical and laboratory data, however, a diagnosis of a suspected combined neurotropic and viscerotropic syndrome may be made.

Following 17D YF vaccination, most primary vaccinees cause low level of and transient viremia. It is noteworthy that

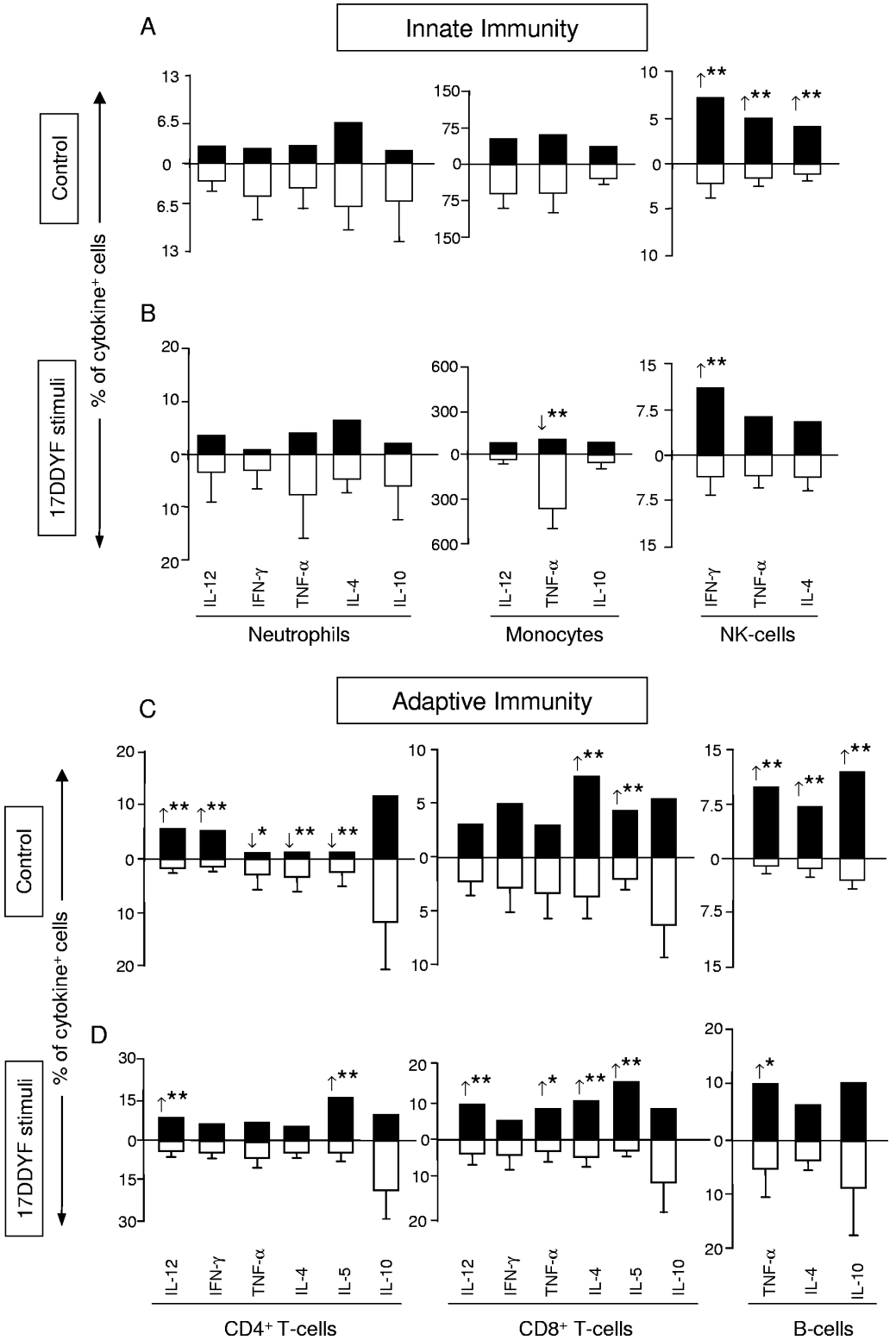


FIG. 2. Intracytoplasmic cytokine pattern of peripheral blood leukocytes associated with innate immunity (A and B) and adaptive immunity (C and D) observed in the patient with combined neurotropic and viscerotropic disease following 17D YF vaccination (■) compared to the reference values observed 15 days after 17DD vaccination in the absence of adverse reactions (□) after short-term *in vitro* incubation in the absence of exogenous stimuli (A and C) or in the presence of 17DD antigen stimulation (B and D). For each leukocyte subset (neutrophils, monocytes, NK cells, $CD4^+$ T cells, $CD8^+$ T cells, and B cells), the results are expressed as the mean percentage of cytokine-positive cells within 1,000 gated events ($^0/_{00}$) ± SD. **, statistical significance was considered when the values for the patient were outside of the reference value range (mean ± 2 SDs); *, additional statistical significance was considered when the values for the patient were outside the range of 0.5 to 2.0 times the mean RV. ↑ and ↓, increase and decrease in the values, respectively, compared to the reference values.

the viral load in the blood from 17D YF-immunized persons is far below the infection threshold for blood-feeding mosquitoes, reflecting the restricted replication of the attenuated virus. On the other hand, YF virus has been detected in the blood, serum, plasma, heart, liver, spleen, kidney, lung, brain, spinal cord, and skin of patients with YEL-SAEs (1, 3, 6, 18). Occasionally, 17D YF virus has been detected in CSF, suggesting its involvement with the YEL-SAE, but it has recently been suggested that the neurological manifestations are due to the immune response to the 17D YF virus infection rather than the direct action of the virus through the central nervous system.

In this study, the 17D YF virus genome was not detected in either CSF or serum samples (as determined by RT-PCR), nor was the 17D YF virus isolated following the inoculation serum into Vero cell cultures, C6/36 cell cultures, or suckling mice. Therefore, the case reported here may be considered a suspected case of combined neurotropic and viscerotropic adverse events following 17D YF vaccination.

It has previously been reported that the titers of neutralizing antibodies against 17D YF virus in patients with YEL-SAEs were considerably higher than those observed in healthy patients (3, 6, 18). However, the titers of neutralizing antibodies identified in the patient described here resembled the titers observed in healthy vaccinees who did not experience any side effects (4).

The occurrence of YEL-SAEs has been associated with increasing age, a history of thymus disorder, and immunocompromise; however, no apparent risk factor could be identified for several cases. The patient described here exhibited some abnormalities in her cellular immune response compared to the immunological profile exhibited by time-matched first-time vaccinees who did not experience any adverse effects. Our data demonstrated that the patient had impaired FC-γR expression in monocytes, despite a robust increase in the level of NK T cells, activated T cells ($CD4^+$ and $CD8^+$), and B lymphocytes. Additionally, enhanced levels of plasmatic cytokines together with exacerbated intracytoplasmic cytokines were mainly observed within NK cells, $CD8^+$ T cells, and B lymphocytes. Analysis of the $CD4^+$ T cells revealed a complex cytokine profile in control and 17DD YF-stimulated cultures. On the other hand, depressed TNF-α synthesis was observed in monocytes following antigenic stimulation. It is important to note that since the two vaccine substrains do not have identical sequences, it is possible that the immune response to routine immunization may not be identical for the two vaccines.

It has recently been reported that YEL-SAEs are accompanied by robust antigen-specific T- and B-cell responses, so the adverse event is unlikely to be caused by impairment of the magnitude of adaptive immunity. On the contrary, the magnitude of this adaptive response might have been a result of the abnormalities in the innate immune system and a possible disruption of the CCR5-RANTES axis (20). Our data did not demonstrate changes in the levels of CCR5 expression on monocytes. However, we found an unexpected decrease in the levels of CXCR3 expression by $CD4^+$ and $CD8^+$ T cells, which may suggest the internalization of CXCR3 following hyperactivation. CXCR3 is usually expressed in type 1 T cells, and it is speculated that the lower level of expression of CXCR3 is associated with the massive activation of T cells, since the CXCR3-ligand axis play an important role in T-lymphocyte activation and recruitment and the subsequent amplification of inflammation in human diseases.

Although cytokines and chemokines may act as immune modulators in YEL-SAEs, their role is still not fully understood. It was shown that growth-related oncogene (GRO), MCP-1, TGF-β, and RANTES levels were moderately increased in one patient with YEL-AND, whereas healthy vaccinees who had no any adverse effects exhibited increases only in RANTES levels and had lower GRO, MCP-1, TGF-β, and TNF-β levels (1). Nevertheless, the specific roles of various cytokines and chemokines in the pathogenesis of YFV infection need to be further clarified.

Together, our findings support the hypothesis that a robust adaptive response and abnormalities in the innate immune system may be involved in the establishment of severe adverse events following primary 17D YF vaccination. Further research on the virus-host immune response is required to obtain a better understanding of the basis of this process.

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## REFERENCES

1. **Bae, H. G., C. Domingo, A. Tenorio, F. de Ory, J. Muñoz, P. Weber, D. E. Teuwen, and M. Niedrig.** 2008. Immune response during adverse events after 17D-derived yellow fever vaccination in Europe. J. Infect. Dis. **197:**1577–1584.
2. **Barwick, R.** 2004. History of thymoma and yellow fever vaccination. Lancet **364:**936.
3. **Belsher, J. L., P. Gay, M. Brinton, J. DellaValla, R. Ridenour, R. Lanciotti, A. Perelygin, S. Zaki, C. Paddock, T. Querec, T. Zhu, B. Pulendran, R. B. Eidex, and E. Hayes.** 2007. Fatal multiorgan failure due to yellow fever vaccine-associated viscerotropic disease. Vaccine **25:**8480–8485.
4. **Camacho, L. A., S. G. de Aguiar, M. D. S. Freire, M. D. L. Leal, J. P. do Nascimento, T. Iguchi, J. A. Lozana, and R. H. Farias.** 2005. Reactogenicity of yellow fever vaccines in a randomized, placebo-controlled trial. Collaborative Group for the Study of Yellow Fever Vaccines. Rev. Saude Publica **39:**413–420.
5. **Chan, R. C., D. J. Penney, D. Little, I. W. Carter, J. A. Roberts, and W. D. Rawlinson.** 2001. Hepatitis and death following vaccination with 17D-204 yellow fever vaccine. Lancet **358:**121–122.
6. **Doblas, A., C. Domingo, H. G. Bae, C. L. Bohorquez, F. de Ory, M. Niedrig, D. Mora, F. J. Carrasco, and A. Tenorio.** 2006. Yellow fever vaccine-associated viscerotropic disease and death in Spain. J. Clin. Virol. **36:**156–158.
7. **Engel, A. R., P. F. Vasconcelos, M. A. McArthur, and A. D. Barrett.** 2006. Characterization of a viscerotropic yellow fever vaccine variant from a patient in Brazil. Vaccine **24:**2803–2809.
8. **Expanded Programme on Immunization.** 1996. Inclusion of yellow fever vaccine in the EPI. Wkly. Epidemiol. Rec. **71:**181–185.
9. **Jennings, A. D., C. A. Gibson, B. R. Miller, J. H. Mathews, C. J. Mitchell, J. T. Roehrig, D. J. Wood, F. Taffs, B. K. Sil, S. N. Whitby, P. D. Minor, T. P. Monath, and A. D. T. Barrett.** 1994. Analysis of a yellow fever virus isolated from a fatal case of vaccine-associated human encephalitis. J. Infect. Dis. **169:**512–518.
10. **Khromava, A. Y., R. B. Eidex, L. H. Weld, K. S. Kohl, R. D. Bradshaw, R. T. Chen, M. S. Cetron, and the Yellow Fever Vaccine Safety Working Group Vaccine.** 2005. Yellow fever vaccine: an updated assessment of advanced age as a risk factor for serious adverse events. Vaccine **23:**3256–3263.
11. **Lawrence, G. L., M. A. Burgess, and R. B. Kass.** 2004. Age-related risk of adverse events following yellow fever vaccination in Australia. Commun. Dis. Intell. **28:**244–248.
12. **Lindsey, N. P., B. A. Schroeder, E. R. Miller, M. M. Braun, A. F. Hinckley, N. Marano, B. A. Slade, E. D. Barnett, G. W. Brunette, K. Horan, J. E. Staples, P. E. Kozarsky, and E. B. Hayes.** 2008. Adverse event reports following yellow fever vaccination. Vaccine **26:**6077–6082.
13. **Martin, M., T. F. Tsai, B. Cropp, G. J. Chang, D. A. Holmes, J. Tseng, W. J. Shieh, S. R. Zaki, I. A. Sanouri, A. F. Cutrona, G. Ray, L. H. Weld, and M. S. Cetron.** 2001. Fever and multisystem organ failure associated with 17D-204 yellow fever vaccination: a report of four cases. Lancet **358:**98–104.

14. **Martins, M. A., M. L. Silva, A. P. Marciano, V. Peruhype-Magalhães, S. M. Eloi-Santos, G. L. Ribeiro, R. Correa-Oliveira, A. Homma, E. G. Kroon, A. Teixeira-Carvalho, and O. A. Martins-Filho.** 2007. Activation/modulation of adaptive immunity emerges simultaneously after 17DD yellow fever first-time vaccination: is this the key to prevent severe adverse reactions following immunization? Clin. Exp. Immunol. **148:**90–100.
15. **Martins, M. A., M. L. Silva, S. M. Elói-Santos, J. G. Ribeiro, V. Peruhype-Magalhães, A. P. Marciano, A. Homma, E. G. Kroon, A. Teixeira-Carvalho, and O. A. Martins-Filho.** 2008. Innate immunity phenotypic features point toward simultaneous raise of activation and modulation events following 17DD live attenuated yellow fever first-time vaccination. Vaccine **26:**1173–1184.
16. **McMahon, A. W., R. B. Eidex, A. A. Marfin, M. Russell, J. J. Sejvar, L. Markoff, E. B. Hayes, R. T. Chen, R. Ball, M. M. Braun, M. Cetron, and Yellow Fever Working Group.** 2007. Neurologic disease associated with 17D-204 yellow fever vaccination: a report of 15 cases. Vaccine **25:**1727–1734.
17. **Monath, T. P.** 2001. Yellow fever: an update. Lancet Infect. Dis. **1:**11–20.
18. **Munoz, J., A. Vilella, C. Domingo, J. M. Nicolas, F. de Ory, M. Corachan, A. Tenorio, and J. Gascon.** 2008. Yellow fever-associated viscerotropic disease in Barcelona, Spain. J. Travel Med. **15:**202–205.
19. **Peruhype-Magalhães, V., O. A. Martins-Filho, A. Prata, L. D. A. Silva, A. Rabello, A. Teixeira-Carvalho, R. M. Figueiredo, S. F. Guimarães-Carvalho, T. C. Ferrari, J. Van Weyenbergh, and R. Correa-Oliveira.** 2006. Mixed inflammatory/regulatory cytokine profile marked by simultaneous raise of interferon-gamma and interleukin-10 and low frequency of tumour necrosis factor-alpha(+) monocytes are hallmarks of active human visceral leishmaniasis due to *Leishmania chagasi* infection. Clin. Exp. Immunol. **146:**124–132.
20. **Pulendran, B., J. Miller, T. D. Querec, R. Akondy, N. Moseley, O. Laur, J. Glidewell, N. Monson, T. Zhu, H. Zhu, S. Staprans, D. Lee, M. A. Brinton, A. A. Perelygin, C. Vellozzi, P. Brachman, Jr., S. Lalor, D. Teuwen, R. B. Eidex, M. Cetron, F. Priddy, C. del Rio, J. Altman, and R. Ahmed.** 2008. Case of yellow fever vaccine-associated viscerotropic disease with prolonged viremia, robust adaptive immune responses, and polymorphisms in CCR5 and RANTES genes. J. Infect. Dis. **198:**500–507.
21. **Silveira-Lemos, D., M. A. Martins, M. L. Silva, A. Homma, A. Teixeira-Carvalho, and O. A. Martins-Filho.** 2008. Insights on yellow fever immune response following natural infection and vaccination. Curr. Trends Immunol. **9:**1–14.
22. **Vasconcelos, P. F., E. J. Luna, R. Galler, L. J. Silva, T. L. Coimbra, V. L. Barros, T. P. Monath, S. G. Rodigues, C. Laval, Z. G. Costa, M. F. G. Vilela, C. L. S. Santos, C. M. O. Papaiordanou, V. A. F. Alves, L. D. Andrade, H. K. Sato, E. S. T. Rosa, G. B. Froguas, E. Lacava, L. M. R. Almeida, A. C. R. Cruz, I. M. Rocco, R. T. M. Santos, O. F. P. Oliva, and Brazilian Yellow Fever Vaccine Evaluation Group.** 2001. Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases. Lancet **358:**91–97.
23. **Vitelli-Avelar, D. M., R. Sathler-Avelar, J. C. Dias, V. P. Pascoal, A. Teixeira-Carvalho, P. S. Lage, S. M. Elói-Santos, R. Corrêa-Oliveira, and O. A. Martins-Filho.** 2005. Chagasic patients with indeterminate clinical form of the disease have high frequencies of circulating $CD3^{+}CD16\text{-}CD56^{+}$ natural killer T cells and $CD4^{+}CD25^{High}$ regulatory T lymphocytes. Scand. J. Immunol. **62:**297–308.
24. **Wilson, M. E., L. H. Chen, and E. D. Barnett.** 2004. Yellow fever immunizations: indications and risks. Curr. Infect. Dis. Rep. **1:**34–42.