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Yellow Fever Vaccination and Increased Relapse Rate in Travelers With Multiple Sclerosis

Mauricio F. Farez, MD, MPH; Jorge Correale, MD

Objective: To investigate the effect of yellow fever (YF) immunization on the subsequent multiple sclerosis (MS) relapse risk.

Design: Self-controlled case series study.

Setting: An MS outpatient clinic.

Patients: Seven patients with clinical relapsing-remitting MS traveling to endemic YF areas who received the YF 17D-204 vaccine were studied.

Intervention: The YF 17D-204 vaccine.

Main Outcome Measure: Number of relapses. Secondary outcomes included the number of new lesions on magnetic resonance imaging and peripheral mononuclear cell cytokine and chemokine production.

Results: The annual exacerbation rate during risk periods following immunization was 8.57, while the relapse rate outside the risk period was only 0.67 (rate ratio = 12.778; $P < .001$). Three months after immunization,

patients showed a significant increase in new or enlarging T2-weighted lesions and gadolinium-enhancing lesions compared with 12 months prior to vaccination and 9 months after immunization (both $P < .001$). Moreover, blood myelin basic protein and myelin oligodendrocyte glycoprotein responses showed significant increases in interferon γ -induced protein 10 kDa-, interferon γ -, interleukin 1 α -, interleukin 1 β -, and tumor necrosis factor-secreting cell numbers as well as complement component C1qB production after YF vaccination in patients with MS compared with unvaccinated patients with MS, patients with MS vaccinated against influenza, and healthy control subjects ($P = .01$ and $P < .001$, respectively).

Conclusion: For patients with MS traveling to endemic YF areas, vaccination should be recommended on the basis of carefully weighing the risk of exacerbation against the likelihood of exposure to the YF virus.

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YELLOW FEVER (YF) IS A TYPE of hemorrhagic fever occurring predominantly in tropical areas of Africa and South America, produced by a virus of the Flaviviridae family.¹ The disease is a zoonotic infection in which nonhuman primates and mosquitoes act as reservoirs. Humans contract the infection when bitten by infected mosquitoes. Symptoms range from a mild febrile syndrome to severe liver and kidney dysfunction with jaundice and bleeding diathesis, which can lead to death in up to 50% of subjects.¹ Although no specific treatment for YF exists, the disease can be prevented by vaccination with live attenuated 17D strain virus.² Vaccination can generate protective antibodies in 99% of immunized subjects within the first month and is recommended for people who live in or plan to visit endemic or epidemic YF areas.²

Although the precise mechanisms underlying MS relapses are poorly understood, several infections have been confirmed as exerting a role in triggering them.^{3,4} However, risk of relapse linked to immunizations has not been studied thoroughly.

We describe 7 patients with relapsing-remitting multiple sclerosis (MS) who received the YF 17D vaccine prior to traveling to an endemic YF region and subsequently developed clinical, immunological, and radiological MS exacerbation.

METHODS

PATIENTS AND STUDY DESIGN

Seven patients diagnosed as having clinical relapsing-remitting MS according to the Poser criteria and receiving YF immunization prior to traveling to endemic YF areas or to countries requiring an international certificate of vaccination were studied. All patients received a

Author Affiliations:
Department of Neurology,
Institute for Neurological
Research Dr Raúl Carrea,
Fundación para la Lucha contra
las Enfermedades Neurológicas
de la Infancia, Buenos Aires,
Argentina.

Table 1. Patient Demographic and Clinical Characteristics

Patient No./ Sex/Age, y	Disease Duration, mo	Treatment	EDSS Score		During Follow-up, No.			After Vaccination	
			Study Entry	End of Follow-up	Relapses	New or Enlarging T2 Lesions	Gadolinium- Enhancing Lesions	Time to First Exacerbation, d	Clinical Picture at 1-5 wk
1/F/33	36	Interferon beta-1a	1.0	4.0	3	4	2	15	Optic neuritis
2/F/35	44	Interferon beta-1a	2.0	4.0	2	4	4	22	Myelitis
3/F/28	42	Interferon beta-1a	1.5	1.5	4	2	2	21	Internuclear ophthalmoplegia
4/M/32	38	Glatiramer acetate	1.5	3.5	3	5	3	32	Diplopia, myelitis
5/M/40	50	Interferon beta-1a	2.0	4.0	2	6	4	28	Myelitis
6/F/38	48	Glatiramer acetate	1.5	1.5	0	0	0	0	
7/F/39	62	Glatiramer acetate	2.0	2.0	0	0	0	0	

Abbreviation: EDSS, Expanded Disability Status Scale.

single dose of the 17D-204 strain vaccine (Stamaril; Sanofi Pasteur MSD, Lyon, France). Patients were prospectively recruited from a cohort regularly followed up at 3-month intervals at our outpatient clinic between March 1, 2007, and March 31, 2009. Demographic and clinical characteristics are summarized in **Table 1**. No patients received steroids, immunosuppressants, or other vaccines for at least 3 months prior to study onset or reported any history or symptoms of other relevant medical conditions. During the study, clinical evidence of concurrent infections was ruled out by laboratory and radiology workup. No patients had previously lived in or traveled to endemic YF areas. After vaccination, patients were prospectively evaluated every 3 months. When a new relapse was identified, patients were asked to return within 72 hours. Evaluations consisted of a comprehensive neurological examination, including physical assessment of disease activity and Expanded Disability Status Scale scoring on all visits.

Exacerbation was defined as development of a new symptom or worsening of preexisting symptoms confirmed on neurological examination, lasting at least 48 hours, and preceded by stability or improvement lasting at least 30 days. Neurological deterioration temporarily associated with fever exclusively was not considered an exacerbation.

Brain magnetic resonance imaging was performed at 3- or 6-month follow-up visits on a 1.5-T Signa unit (General Electric, Milwaukee, Wisconsin). Axial 5-mm-thick slices were obtained with T2-weighted, proton density, fast spin-echo, fluid-attenuated inversion recovery, and T1-weighted sequences before and after administration of gadolinium diethylenetriamine pentaacetic acid (0.1 mmol/kg). At-risk periods (ARPs) following immunization have been defined as lasting 6 to 11 days by some authors³ or up to 2 months by others⁶ depending on the type of immunization and the event studied. The YF vaccine contains live virus; therefore, for this study we established an ARP starting 1 week after immunization based on the time needed for the virus to replicate and generate an immune response⁷ and ending 5 weeks later in an attempt to limit the influence of other factors on the subsequent risk of relapse. Total follow-up lasted 24 months.

Seven age- and sex-matched controls were recruited from among healthy individuals and subjected to thorough clinical and neurological examination as well standard clinical and hematological examination to rule out other potential medical conditions. In addition, 7 age- and sex-matched unvaccinated patients with MS and 7 influenza-vaccinated patients with MS were included as additional control groups. All control subjects were selected contemporaneously with YF-vaccinated patients with MS. Influenza-vaccinated subjects with MS received vaccines according to World Health Organization recommendations for the

southern hemisphere during the 2007, 2008, and 2009 seasonal influenza vaccine campaigns. No differences in demographic or disease characteristics (mean disease duration, annual relapse rate before study entry, disability score, or magnetic resonance imaging activity) were observed between control populations and YF-vaccinated patients with MS. Similarly, no differences in disease characteristics were found between patients who did and did not relapse after YF vaccination. In all cases, data were collected prospectively and results were compared with the clinical MS course in the year prior to vaccination.

The YF-neutralizing antibodies were determined in serum samples from vaccinated patients with MS 15 to 21 days after vaccination by plaque reduction neutralization test,⁸ and the results are expressed as reciprocal to antibody titer levels. Results on all serum tests for other endemic flavivirus infections, namely St Louis encephalitis and dengue virus serotypes 1 to 4, were negative.

Study approval was obtained from the institutional ethics committee. All patients as well as controls signed a written informed consent form.

IMMUNOLOGICAL EVALUATION

To characterize immune responses after YF vaccination, peripheral blood mononuclear cells (PBMCs) were collected at the time of vaccination and at 2, 5, 8, 12, 24, and 48 weeks. Result readers were blind to all patient clinical information. Two hundred thousand PBMCs were stimulated with 10 µg/mL of myelin basic protein 83-102 (MBP₈₃₋₁₀₂), myelin oligodendrocyte glycoprotein 63-87 (MOG₆₃₋₈₇), or the control antigen streptolysin O (Sigma-Aldrich, St Louis, Missouri). Peptide-specific cytokine- and chemokine-secreting cell numbers were measured using commercially available kits or development modules for enzyme-linked immunosorbent spot assays as previously described.⁹ Interleukin 1α (IL-1α) and IL-1β development modules were purchased from eBioscience (San Diego, California), and IL-4, IL-6, IL-10, interferon γ (IFN-γ), tumor necrosis factor (TNF), transforming growth factor β, IFN-γ-induced protein 10 kDa (IP-10), and RANTES (regulated upon activation, normal T-cell expressed, and secreted) enzyme-linked immunosorbent spot assay detection kits were purchased from R&D Systems (Minneapolis, Minnesota). Complement component C1qB was measured in culture supernatants from lipopolysaccharide-stimulated (100 ng/ml; Sigma-Aldrich) PBMCs using a capture enzyme-linked immunosorbent assay developed in our laboratory, with capture and detection monoclonal antibodies purchased from Abnova (Walnut, California). Concentrations of C3a, C4d, C5a, and C5b-9 were

Table 2. Exacerbation Rates During and Outside the At-Risk Period

Period	Time, Patient-Years	Exacerbations, No.	Annual Exacerbation Rate
At risk	0.58	5	8.57 ^a
Nonrisk	13.42	9	0.67

^aRate ratio = 12.778 (95% confidence interval, 4.28-38.13; $P < .001$).

assessed after lipopolysaccharide stimulation using commercially available enzyme-linked immunosorbent assay kits (Quidel Corp, San Diego, California) and following the manufacturer's instructions.

STATISTICAL ANALYSIS

We selected the self-controlled case series method¹⁰ for data analysis. Originally developed in the 1990s, the method has been extensively used to evaluate the relationship between immunization and adverse effects,¹¹ including MS relapses,¹² and only requires use of cases for same-patient comparison. Briefly, relapse rates were calculated by dividing the total number of exacerbations by the time contributed by each individual during the 2 different risk periods, namely the ARP and the remaining 23 months of follow-up. A Poisson regression model was then used to estimate the incidence rate ratio of MS exacerbation adjusted for age, sex, disease duration (years), and treatment. All statistical analyses were performed using Stata version 11.1 statistical software (StataCorp LP, College Station, Texas).

RESULTS

RISK OF EXACERBATION AFTER VACCINATION

During the 2-year follow-up, the annual exacerbation rate was 0.99. When follow-up was divided into the ARP and the nonrisk period, the annual exacerbation rate was 8.57 during the ARP vs 0.67 during the nonrisk period. The exacerbation rate ratio of the ARP over the nonrisk period was 12.778 (95% confidence interval, 4.28-38.13; $P < .001$) (**Table 2** and **Figure 1**). Interestingly, immunization against influenza in the same patients and during the same follow-up period showed no effect on the annual exacerbation rate ($P > .05$).

Clinical findings in 5 of 7 patients with MS who had exacerbations at 1 to 5 weeks following vaccination are summarized in Table 1. It is important to note that 4 of these 5 patients had a significant and persistent Expanded Disability Status Scale score increase (≥ 2 points) on neurological evaluations conducted 12 months following the exacerbation (Table 1). In the remaining patient, exacerbations were transient, without further sequelae.

MAGNETIC RESONANCE IMAGING CHANGES AFTER VACCINATION

Three months after YF immunization, patients had a mean (SEM) of 2.6 (0.7) new or enlarging T2 lesions and 2.14 (0.6) gadolinium-enhancing lesions compared with 0.1

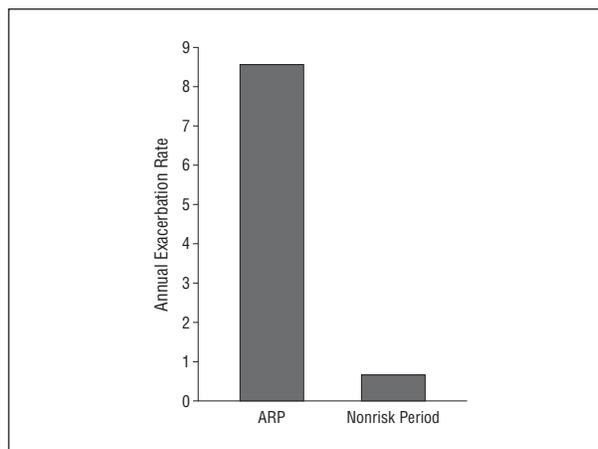


Figure 1. Annual exacerbation rate during the at-risk period (ARP) and nonrisk period.

(0.1) new or enlarging T2 lesions and 0 gadolinium-enhancing lesions during the remaining follow-up (both $P < .001$). Six months after immunization, the average number of new or enlarging lesions on T2-weighted images and the mean number of gadolinium-enhancing lesions was significantly greater compared with those observed 12 months prior to and 9 months following vaccination (both $P < .001$). Immunization against influenza did not change imaging parameters in the 3 months after immunization ($P = .11$ for new or enlarging T2 lesions and $P = .42$ for gadolinium-enhancing lesions).

IMMUNOLOGICAL CHANGES AFTER VACCINATION

The MBP- and MOG-peptide-specific IL-1 α -, IL-1 β -, IFN- γ -, TNF-, and IP-10-secreting cell numbers were significantly higher in samples collected from YF-vaccinated patients with MS compared with unvaccinated patients with MS, influenza-vaccinated patients with MS, or controls ($P = .01$ to $P < .001$), with maximum levels between 2 and 5 weeks following vaccination (**Figure 2A-E**). Similar changes were observed for C1qB levels ($P < .001$) (Figure 2F) from lipopolysaccharide-stimulated PBMCs. In contrast, no significant differences in the number of MBP- and MOG-peptide-specific IL-4-, IL-6-, IL-10-, transforming growth factor β -, and RANTES-secreting cells were observed between groups over the same period. Likewise, secretion of complement components C3a, C4d, C5a, and C5b-9 was not influenced by YF vaccination. Similar results were obtained after PBMC stimulation with either MBP₈₃₋₁₀₂ or MOG₆₃₋₈₇. Although neutralizing antibody titers varied considerably among all 7 YF-vaccinated patients with MS (range, 160-1280), positive results were observed in all subjects. Antibody titers did not predict disease outcome.

COMMENT

We describe a group of patients with relapsing-remitting MS whose disease worsened a short time after YF 17D-204 immunization. Relapsing-remitting MS and

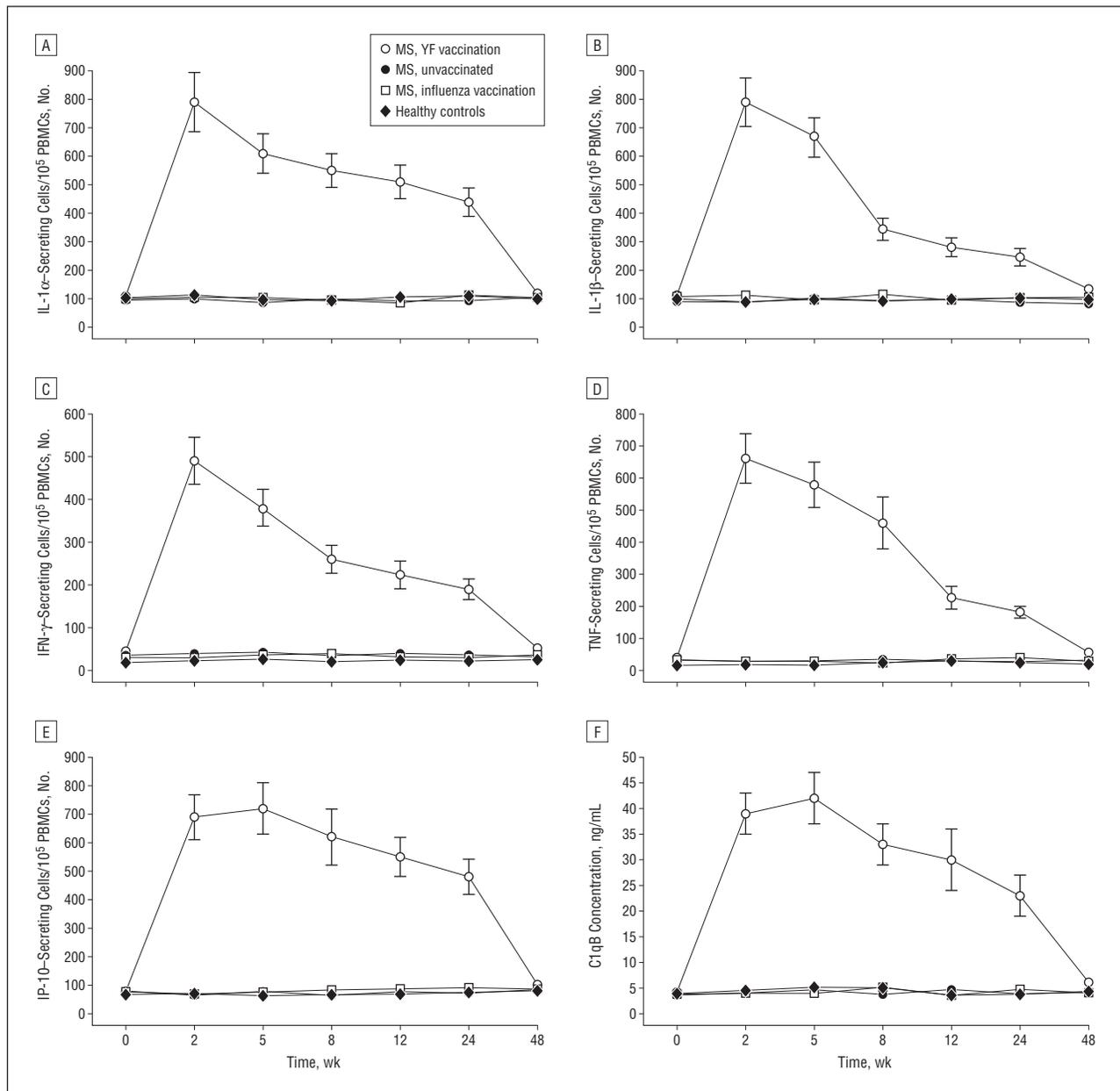


Figure 2. Number of myelin basic protein 83-102-specific peripheral blood mononuclear cells (PBMCs) producing interleukin 1 α (IL-1 α) (A), IL-1 β (B), interferon γ (IFN- γ) (C), tumor necrosis factor (TNF) (D), and IFN- γ -induced protein 10 kDa (IP-10) (E) from yellow fever (YF)-vaccinated patients with multiple sclerosis (MS), unvaccinated patients with MS, influenza-vaccinated patients with MS, and healthy controls. Cytokine- and chemokine-secreting cell numbers were calculated by subtracting the number of spots obtained in control cultures without antigen stimulation from the number of spots obtained in cultures exposed to stimulating antigens. Results are reported as number of spots per 10⁵ PBMCs. F, Production of complement component C1qB by PBMCs after lipopolysaccharide stimulation in the same groups of patients assessed by enzyme-linked immunosorbent assay. Data indicate mean values from 7 subjects studied in each group. Error bars indicate SD, shown for YF-vaccinated patients with MS exclusively. All other group variation comparisons were below 2 SDs and therefore considered to lack statistical significance.

vaccination against YF do not overlap often, which is why only a small number of patients is included. This fact and the unblinded design of clinical and radiological assessments are obvious limitations to this study. Nevertheless, the more than 10-fold increase observed in the relapse rate associated with significant clinical, radiological, and immunological changes suggests that further research is warranted. Yellow fever is a potentially fatal disease affecting approximately 200 000 people annually and causing an estimated 30 000 deaths per year.¹³ Vector-control strategies that once were successful for YF eradication

have faltered in many regions, leading to reemergence of the disease. Consequently, with no specific treatment, vaccination combined with mosquito bite avoidance remains the best strategy for prevention. The YF 17D-204 vaccine has a very good safety record, with only rare cases of serious adverse effects following immunization of healthy individuals (allergic reactions, 1 in 131 000 persons; vaccine-related neurological disease, 1 in 250 000 persons; and vaccine-related viscerotropic disease, 1 in 300 000 persons).^{13,14} In nonendemic areas of Africa, the risk of contracting YF in

unvaccinated subjects has been estimated to be 1 in 2000; of these, 1 in 7 individuals develops clinical illness and 1 in 10 000 dies from the disease. Rates are 10 times lower for South America.^{1,14} There is very little information available on YF and MS. A study by Confavreux et al⁶ addressed the subsequent risk of relapse after immunizations in general, but unfortunately there were only 2 cases of YF vaccination and the risk could not be estimated.

It is not known how YF immunization affects immunological response in MS, and the mechanisms through which autoimmune reactions can be triggered by vaccination are not understood, although they probably vary according to the type of vaccine and individual genetic susceptibility.¹⁵ Proposed mechanisms include molecular mimicry, epitope spreading, bystander activation, and polyclonal activation.¹⁶ In this study, vaccination against YF generated an increase in unrelated MBP- and MOG-autoreactive cells, which may account for the clinical and radiological changes observed in these patients. In agreement with these observations, it has been shown that the 17D vaccine strain activates different dendritic cell populations, resulting in proinflammatory cytokine and chemokine production, with IP-10, IL-1 α , IL-1 β , IFN- γ , IFN- α/β , and TNF induction.¹⁷⁻¹⁹ More recent studies have demonstrated that C1qB and the translation factor EIF2AK4 both correlate with and predict YF 17D CD8⁺ T-cell responses.¹⁸ Whether the more than 10-fold increase in the relapse rate observed in these 7 patients can be confirmed by larger studies remains to be seen. A causal relationship between YF vaccination and MS relapses can be indirectly inferred by the temporal relationship between events, the strength of the exacerbations observed, and the biological plausibility of such a link.²⁰ Doctors should be open to discuss the pros and cons of YF vaccination with patients with MS. Depending on specific patient travel plans, potential local epidemics, and length of stay, the final decision on whether to administer the vaccine should result from a careful balance between the risk of MS exacerbation and the likelihood of exposure to the YF virus.

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Correspondence: Jorge Correale, MD, Institute for Neurological Research Dr Raúl Carrea, Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia, Montañeses 2325, Buenos Aires 1428, Argentina (jcorreale@fleni.org.ar).

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