Immune Mediators in Cerebrospinal Fluid during Cryptococcosis Are Influenced by Meningeal Involvement and Human Immunodeficiency Virus Serostatus

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Pro- and anti-inflammatory mediators (tumor necrosis factor [TNF]–α, interleukin [IL]–6, IL-8, IL-10, and soluble TNF receptor II [sTNFR] II) were measured in cerebrospinal fluid (CSF) before treatment (day 0), and after 2 weeks and 3 months of antifungal therapy in 51 human immunodeficiency virus (HIV)–positive and 7 HIV-negative patients with culture-confirmed cryptococcosis. On day 0, all mediator concentrations, except IL-10 in HIV-positive patients, were higher in patients with meningeal, rather than extrameningeal cryptococcosis or in control subjects (P < .05). For meningitis patients, all mediator levels, except sTNFR II, were higher in HIV-negative than HIV-positive patients (P < .05). Day 0 CSF IL-8 levels were higher in HIV-positive patients receiving antiretroviral therapy than in untreated persons (P < .02). Day 0 sTNFR II levels were higher in HIV-positive survivors at 3 months, and elevated levels were sustained in HIV-positive patients with meningitis. Overall, these data support the idea that inflammatory responses are crucial to the eradication of cryptococcal infections in the central nervous system.

Cryptococcus neoformans infection is a potentially life-threatening disseminated disease that usually occurs in persons with major cell-mediated immune deficiencies [1–4]. Cryptococcal meningitis, the most frequent and severe clinical setting, is associated with minor inflammation of cerebrospinal fluid (CSF) [5], which was recently ascribed to the inhibition of neutrophil recruitment by yeast-capsule glucuronoxylomannan (GXM) [6] or binding of C. neoformans to CD18 [7]. In vitro, human mononuclear cells or neutrophils can produce proinflammatory and anti-inflammatory cytokines in the presence of intact C. neoformans or their components [8–14]. Also, human immunodeficiency virus (HIV) infection can either reduce the anticytotoxic activity of human peripheral blood mononuclear cells or their capacity to produce cytokines in the presence of C. neoformans [15–17], and HIV-1 envelope glycoprotein 120 alters the T cell response to these yeasts [18]. The influence of C. neoformans or its antigens on the replication of HIV has also been documented [19–21]. In mice, Th1 cytokines and tumor necrosis factor (TNF)–α have important roles in host defense against C. neoformans [22–28], and we recently demonstrated that plasma TNF-α and interleukin (IL)–10 plasma levels were affected by the severity of the disease in both mice and AIDS patients with cryptococcosis [29].

In contrast to the extensive literature on the role of immune mediators present in the CSF in the pathophysiology of bacterial meningitis, little is known about the local immune response during fungal meningitis [30]. Preliminary studies during cryptococcosis [31–33] enrolled only small numbers of HIV-positive patients, and no information was reported on the outcome. To determine whether an immunologic response is associated with C. neoformans meningeal infection, we measured CSF concentrations of proinflammatory and anti-inflammatory cytokines and mediators in adults with culture-confirmed cryptococcosis. We then evaluated the influence of HIV infection on this local immune response and whether the presence of these mediators reflected the severity of cryptococcal disease.
Patients and Methods

Study design and patients. The present study is part of an ongoing prospective multicenter investigation initiated at the French National Reference Center for Mycoses (NRCM) to examine the clinical and biologic aspects of C. neoformans infection in France [34]. Initially the treating physician or the microbiologist notified the NRCM of a positive India ink test or antigen detection. Patients were enrolled if they were >18 years old and if informed consent was given for lumbar puncture, for sampling of urine and blood for culture, and for storage of these specimens before antifungal therapy was started or within 2 days after the onset of treatment on day 0. The patient also agreed to have all body sites initially infected with C. neoformans resampled, to assess sterilization after 2 weeks and 3 months of antifungal treatment, according to the most recent therapeutic recommendations [35], and storage of the biologic specimens. All routine diagnostic procedures (direct examination, antigen detection, biochemical analyses, and cell counts in blood, CSF, and urine) as well as radiographs or other routine tests were performed in each institution at the discretion of the treating physician. Treatment was also chosen locally, although the optimal antifungal regimen established for AIDS patients [36] was included in the specific recommendations for the protocol. Patients were included in the analysis if ≥1 culture yielded C. neoformans and if it was the first episode of cryptococcosis. All information (questionnaire, isolates, and specimens) were labeled only with the code number assigned to the patient.

Definitions. For all information specific to HIV-positive patients, we based our classifications on those established by the Centers for Disease Control and Prevention in 1993. HIV infection was diagnosed according to the recommendations of the French Ministry of Health, on the basis of the results of 2 independent tests (ELISA and Western blot). Cases were classified as cryptococcal meningitis when an isolate of C. neoformans was recovered from the CSF and as extrameningeal cryptococcosis when the CSF culture was negative. Dissemination was arbitrarily defined as the isolation of C. neoformans from ≥2 noncontiguous sites (e.g., bone marrow and blood or CSF and brain). Sterilization was assessed by culture of specimens from all infected sites on the subsequent monitoring date (week 2 or month 3). Meningitis was considered to carry a poor prognosis, on the basis of previously identified clinical symptoms (abnormal mental status, palsy, or seizures) [37–39]. Overall mortality at 3 months was considered.

Sample processing and cytokine immunoassays. In each participating laboratory, fluid samples were collected, were directly examined, were cultured, and were subjected to other routine tests before storage as frozen supernatants within hours after sampling (coded with the patient’s protocol number). Samples were mailed to the NRCM (within 1 month when stored at −20°C or after the last sampling when stored at −70°C). In our laboratory, samples were stored at −70°C until assayed. After thawing, the samples were immediately used for TNF-α and IL-10 measurements and then were aliquoted and refrozen at −70°C. All samples were tested by one of us who was unaware of clinical and biologic information.

Isolates were identified as C. neoformans in each institution on the basis of urease activity and assimilation patterns shown with commercial strips. At least 1 isolate was mailed to the NRCM for serotyping [40]. CSF cryptococcal capsule polysaccharide antigen titers were determined at the NRCM by ELISA (Premier; Meridian Diagnostics), according to the manufacturer’s recommendations.

Control subjects were 4 HIV-positive and 4 HIV-negative patients for whom blood, CSF, and urine cultures were negative for C. neoformans and CSF and serum samples were negative for cryptococcal antigen. All the CSF samples were considered to be normal. None of the controls had an active infection or a central nervous system disease at the time of sampling.

Proinflammatory cytokine (TNF-α, IL-6, and IL-8) and anti-inflammatory marker (IL-10 and soluble TNF receptor [sTNFR] II) concentrations were assessed by commercial ELISA kits (R&D Systems). All samples from a given patient (day 0, week 2, and month 3) were tested simultaneously. If there were limited volumes, selected markers were measured (exact numbers of patients tested for a given parameter are provided in Results). Furthermore, half the volumes recommended by the manufacturer were used for all cytokine dosages, except for sTNFR II. We previously verified with spiked samples and standard curves that this modification did not alter the results (data not shown). Concentrations were determined by comparison with standard curves. When we considered the distribution of the sample (1:2 for all dosages except sTNFR II tested at 1:10) and the thresholds established by manufacturer, the minimum detectable levels (all in pg/mL) were 8.8 for TNF-α, 1.4 for IL-6, 20 for IL-8, 3 for IL-10, and 10 for sTNFR II.

Data management. Administrative data (date of birth, sex, ethnicity, occupation, and travel history), clinical information (underlying disease, including HIV and immune status, signs, and symptoms and their duration before diagnosis, evolution, treatment dose, and duration), and results of biochemical and radiologic analyses were coded by the treating clinician on the questionnaire. Missing information was systematically recovered by the study medical supervisor. Anonymity was respected, as requested by the National Commission on Informatics and Freedom.

Statistical analysis. Statistical analyses were performed using Statview software, version 4.5 (Abacus Concepts). Mediator levels were compared by the Mann-Whitney U test or the Kruskal-Wallis test, depending on the number of groups. We used Spearman’s test to establish correlations between cytokines and other parameters. A correlation was considered to be $r > .80$; significance was defined as $P < .05$.

Results

Study population characteristics. Of the persons evaluated, 51 were HIV positive and 7 were HIV negative (table 1). All had low CD4 T lymphocyte counts. Risk factors for HIV infection were mostly sexual behavior (24 homosexual or bisexual and 18 heterosexual). Cryptococcosis led to the detection of HIV infection in 22 patients and was an AIDS-defining illness in another 19. For 7 patients, cryptococcosis was associated with another opportunistic infection (Pneumocystis carinii pneumonia in 6 and cerebral toxoplasmosis in 1). At the time of diagnosis, 16 HIV-positive patients were receiving antiretroviral therapy, including a protease inhibitor for 12, for a median duration of 6.5 months (range, 1–13 months). Antiretroviral therapy was associated with a significant reduction of virus load.
Table 1. Baseline characteristics of human immunodeficiency virus (HIV)-positive and HIV-negative patients with cryptococcosis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-positive patients</th>
<th>HIV-negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>51</td>
<td>7</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>50:1</td>
<td>4:3</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>37 (26–58)</td>
<td>59 (28–71)</td>
</tr>
<tr>
<td>Percentage white</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median no. CD4 lymphocytes/mm³ (range)</td>
<td>28 (1–480)</td>
<td>244 (22–464)</td>
</tr>
<tr>
<td>Median log10 plasma virus load (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients receiving antiretroviral treatment</td>
<td>4.2 (1.7–5.7)</td>
<td>—</td>
</tr>
<tr>
<td>Patients without antiretroviral treatment</td>
<td>5.3 (3.4–6.2)</td>
<td>—</td>
</tr>
</tbody>
</table>

* P < .02 vs. patients receiving antiretroviral treatment (Kruskal-Wallis test).

Table 2. Characteristics of the yeast infection in the study population: 51 human immunodeficiency virus (HIV)-positive and 7 HIV-negative patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-positive patients</th>
<th>HIV-negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever (°C) ≥38 °C, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissemination, %</td>
<td>78</td>
<td>86</td>
</tr>
<tr>
<td>Cryptococcal meningitis, %</td>
<td>71</td>
<td>57</td>
</tr>
<tr>
<td>Signs of poor prognosis in meningitis patients, %</td>
<td>86</td>
<td>100</td>
</tr>
<tr>
<td>Median weeks between first symptom and diagnosis of meningitis (range)</td>
<td>2 (0.3–12)</td>
<td>4 (0.2–62)</td>
</tr>
<tr>
<td>Median CSF characteristics in meningitis patients (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/mm³</td>
<td>16 (1–530)</td>
<td>110 (1–175)</td>
</tr>
<tr>
<td>Protein, mg/mL</td>
<td>0.7 (0.2–3.8)</td>
<td>1.0 (0.7–3.5)</td>
</tr>
<tr>
<td>CSF:plasma glucose ratio</td>
<td>0.5 (0.1–0.9)</td>
<td>0.4 (0.2–0.5)</td>
</tr>
<tr>
<td>Antigen titer, reciprocal log₁₀</td>
<td>11.2 (2.5–18.1)</td>
<td>8.8 (4.6–13.0)</td>
</tr>
<tr>
<td>Median CSF characteristics in patients without meningitis (range)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells/mm³</td>
<td>1 (1–9)</td>
<td>—</td>
</tr>
<tr>
<td>Protein, mg/mL</td>
<td>0.3 (0.3–0.4)</td>
<td>—</td>
</tr>
<tr>
<td>CSF:plasma glucose ratio</td>
<td>0.5 (0.4–0.7)</td>
<td>—</td>
</tr>
</tbody>
</table>

*NOTE. CSF, cerebrospinal fluid.
Figure 1. Cytokine and mediator concentrations (pg/mL) on day 0 in cerebrospinal fluid (CSF) of human immunodeficiency virus (HIV)–negative patients with cryptococcal meningitis (CM HIV–), HIV-negative control subjects without cryptococcosis (C HIV–), HIV-positive patients with CM (CM+ HIV+), HIV-positive patients with extrameningeal cryptococcosis (CM+ HIV–), and HIV-positive control subjects (C HIV+). Data are shown as boxes: internal horizontal lines, medians; tops and bottoms of boxes, 25th and 75th percentiles, respectively. Upper and lower bars, tenth and 90th percentiles, respectively. Values 10%–90% range. Tumor necrosis factor (TNF)–α median and 25th percentile for CM HIV– patients are superimposed (P < .05 for CM+ within HIV-positive and HIV-negative groups, except for IL-10 in HIV-positive patients, Kruskal-Wallis test). IL, interleukin; sTNFR II, soluble TNF receptor II.

unaffected by the results of the CSF cultures at week 2, whereas IL-6, IL-8, and IL-10 levels tended to be higher in the CSF of the 2 HIV-negative patients whose cultures remained positive, compared with those measured in negative CSF samples (table 3). For HIV-positive patients whose CSF was available for testing at day 0, week 2, and month 3, all mediator concentrations were significantly lower at week 2, compared with day 0, whereas only TNF-α and sTNFR II differed significantly between week 2 and month 3 (figure 4). In these patients, all month 3 CSF cultures were negative.

Discussion

Our data showed that, when cryptococcosis was diagnosed, the presence of yeast in the CSF was associated with elevated levels of several immune mediators that remained almost undetectable in the CSF of patients with extrameningeal cryptococcosis. This finding suggests that these molecules are produced locally in response to the presence of cryptococci. The contrast between the “high” CSF IL-6 levels and the “low” TNF-α levels parallels the low TNF-α levels and the higher IL-6 levels measured in the brains of infected mice [29, 41] and with preliminary results obtained by Chaka et al. in humans [31].

Primary cultures of human fetal astrocytes stimulated with IL-1β and interferon (IFN–γ) can inhibit C. neoformans growth [42]. Human microglial cells can phagocytize opsonized cryptococci without killing them [43, 44], and the anticryptococcal activities of human polymorphonuclear leukocytes and monocytes are enhanced on endothelial monolayers, compared with plastic surfaces [45]. Although human microglial cells, cerebral endothelial cells, and astrocytes produce diverse cytokines [30, 46], to the best of our knowledge, no study has been published on their ability to synthesize cytokines in the presence of C. neoformans, whereas murine microglial cells have been reported to do so [47]. Because of the lack of correlation between the mediators and CSF cellularity, our data suggest that cytokine production is mediated by the resident cells and not by mononuclear cells attracted to the infection site.

In contrast with the low levels of proinflammatory and anti-inflammatory mediators measured here, local synthesis is intense during the acute phase of bacterial meningitis [30, 48–50].
Figure 2. Interleukin (IL)-8 concentrations (pg/mL) on day 0 in cerebrospinal fluid (CSF) of patients with cryptococcal meningitis: human immunodeficiency virus (HIV)-positive (HIV\(^1\)) patients receiving antiretroviral treatments on day 0 (Rx) or not (no Rx) and HIV-negative (HIV\(^2\)) subjects (\(P < .02\), HIV\(^1\) Rx vs. no Rx; Mann-Whitney U test). Data are shown as boxes: internal horizontal lines, medians; tops and bottoms of boxes, 25th and 75th percentiles, respectively. Upper and lower bars, tenth and 90th percentiles, respectively. ○, Values >10%–90% range.

However, our data are similar to those obtained during other subacute infections (e.g., Mycobacterium tuberculosis and Coccioides immitis meningitides) [51, 52]. Such low concentrations, although not deleterious for the host, as during bacterial meningitis [53], may contribute to the delayed clearance of *C. neoformans* from the CSF by nonactivated effector cells and thus might explain the persistence of the infection for weeks, despite adequate antifungal therapy.

Of note, HIV-positive patients with cryptococcosis-associated clinical factors of poor prognosis at diagnosis had significantly higher CSF levels of the different mediators than those without. Although we were unable to measure CSF pressure in the present study, others recently showed that it was higher in such cases [54]. Whether the pathophysiology of the cranial hypertension can be attributed to a particular cytokine response or to GXM itself [55] remains to be determined.

We wondered whether HIV infection influenced the synthesis of cytokines in the CSF, because Lee et al. [56] found free yeasts only in the brain parenchyma of HIV-positive patients, whereas granulomas were observed only in the brains of HIV-negative patients. Our results confirm a difference between HIV-positive and HIV-negative patients, in terms of local immune responses, as evaluated by CSF cellularity and all mediators but sTNFR II. Two mechanisms might explain the lower cytokine production in the CSF during *C. neoformans* and HIV coinfection. First, resident cells, especially macrophages, could be infected by HIV [57, 58] and therefore lose at least part of their functions. This

Figure 3. Comparison of cerebrospinal fluid (CSF) cytokine and mediator concentrations (pg/mL) on day 0 in human immunodeficiency virus (HIV)-positive patients who were alive after 3 months of antifungal therapy and in those who died within 90 days after the diagnosis of cryptococcosis. Data are shown as boxes: internal horizontal lines, medians; tops and bottoms of boxes, 25th and 75th percentiles, respectively. Upper and lower bars, tenth and 90th percentiles, respectively. ○, Values >10%–90% range. IL, interleukin; sTNFR II, soluble tumor necrosis factor receptor II; TNF-α, tumor necrosis factor–α.
possibility was demonstrated for monocytes and lymphocytes [16] and was ascribed to defects of both oxidative and nonoxidative effector pathways after the internalization of the yeast [17] and for alveolar macrophages at least in the late stage of AIDS [59]. Another, more likely, explanation is the extreme CD4 lymphopenia, which could also hamper the activity of mononuclear cells, as shown in the simian AIDS model [60].

IL-8 is detectable in the CSF of HIV-positive patients with cryptococcal meningitis [31], and GXM can stimulate the IL-8 synthesis by human microglial cells [6]. Also, it was recently demonstrated that neutrophils from persons with late-stage HIV infection (CD4 lymphocyte count <200/mm³) had significantly impaired IL-8 production in response to C. neoformans [61]. We showed that antiretroviral treatments at least partially

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**Table 3.** Cerebrospinal fluid (CSF) mediator concentrations (pg/mL) at week 2 in human immunodeficiency virus (HIV)-positive and HIV-negative patients with cryptococcal meningitis, according to the presence of live Cryptococcus neoformans in the CSF.

<table>
<thead>
<tr>
<th>Mediator</th>
<th>HIV-positive patients</th>
<th>HIV-negative patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF positive</td>
<td>CSF negative</td>
</tr>
<tr>
<td>TNF-α</td>
<td>8.8 (8.8–34)</td>
<td>&gt;8.8 (8.8–29)</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>sTNFR II</td>
<td>2822 (685–4879)</td>
<td>2011 (430–5304)</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>IL-6</td>
<td>14 (2–1106)</td>
<td>12.5 (4.0–1180)</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>IL-8</td>
<td>60 (&lt;20–2008)</td>
<td>54.5 (&lt;20–814)</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>IL-10</td>
<td>6 (&lt;3–38)</td>
<td>6.0 (&lt;3–50)</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>20</td>
</tr>
</tbody>
</table>

*NOTE.* Data are median (range), except where noted. IL, interleukin; sTNFR II, soluble tumor necrosis factor receptor II; TNF-α, tumor necrosis factor-α.

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Figure 4. Kinetics of cerebrospinal fluid cytokine and mediator concentrations from day 0 (D0) to week 2 (W2) and month 3 (M3) of antifungal therapy in human immunodeficiency virus (HIV)-positive patients with cryptococcal meningitis: tumor necrosis factor (TNF)-α, n = 18; soluble TNF receptor II (sTNFR II), n = 17; interleukin (IL)-6, n = 21; IL-8, n = 19; IL-10, n = 20. Data are shown as boxes: internal horizontal lines, medians; tops and bottoms of boxes, 25th and 75th percentiles, respectively. *Upper and lower bars,* tenth and 90th percentiles, respectively. ○, Values >10%–90% range.
restored the ability of the cells to produce IL-8 in the presence of \textit{C. neoformans} at a level similar to that measured in CSF of HIV-negative patients. Others have shown the restoration of IL-12 production by human HIV-positive blood mononuclear cells primed with IFN-\(\gamma\) in the presence of \textit{C. neoformans} \cite{17} and the restoration of anticytotoxic activity of NK cells of HIV-positive persons in the presence of IL-12 \cite{15}. The defective immune response against \textit{C. neoformans} observed during HIV infection may thus be partially reversible. However, the optimal timing of antiretroviral drug administration or even cytokine therapy after the diagnosis of cryptococcal meningitis remains to be determined. Woods et al. \cite{62} reported an aggravation of the signs and symptoms, probably reflecting the restoration of the local inflammatory response with highly active antiretroviral therapy. In any case, the lower cytokine production in the brain in the first 24 h \cite{63} are sometimes associated with a poorer prognosis \cite{64}. However, our results suggest that increased sTNFR II levels could be a marker of better prognosis in HIV-positive patients with cryptococcosis. Vullo et al. \cite{33} reported that, among HIV-infected patients with various neurologic disorders, the highest CSF sTNFR II levels occurred in the subgroup of patients with cryptococcal meningitis \cite{33}. The significance of elevated sTNFR II concentrations in the CSF is unclear. They may neutralize TNF-\(\alpha\) activity and be anti-inflammatory or may stabilize the biologically active forms of TNF-\(\alpha\), thereby prolonging the proinflammatory effect of this cytokine \cite{30}. Our results seem to be in agreement with this latter hypothesis, because there was a trend toward higher concentrations of all proinflammatory cytokines and lower IL-10 concentrations in survivors, compared with those who died during the 3 months after diagnosis. Whether sTNFR II could be used in clinical practice remains to be seen and warrants further studies in a larger sample.

Finally, in our kinetic study of mediators in the CSF, we observed sustained elevated levels of sTNFR II, which again contrasts with results found during bacterial meningitis \cite{63}. The persistent replication of HIV, as demonstrated in the plasma independently of the presence of \textit{C. neoformans} \cite{65, 66}, leads us to wonder whether dead yeasts and soluble antigens that persist over several weeks and even months in the CSF of patients with cryptococcosis \cite{67} could also be the stimulating agents \cite{19}.

In conclusion, meningeal invasion by \textit{C. neoformans} and HIV serostatus influence the local synthesis of immune mediators. Our data add evidence to previous studies \cite{26, 27} showing that inflammatory responses are crucial to the eradication of \textit{C. neoformans}, even in the central nervous system. Other studies are underway to investigate the cytokine response induced by \textit{C. neoformans} in other clinical sites and the interaction(s) between \textit{C. neoformans} and HIV in these compartments.

**French Cryptococcosis Study Group Members**

These members of the French Cryptococcosis Study Group enrolled patients in the present study and collected biologic samples: B. Couprie, M. Dupon, H. Dutronc, J. Y. Lacut, and J. M. Ragnaud (Hôpital Pellegrin, Bordeaux); J. L. Pellegrin and J. F. Viallard (Hôpital Haut-Lévêque, Bordeaux); A. Desveaux, M. Gavigné, and A. S. Labussière (Bourges); A. Bonnin, M. Duong, and H. Portier (Dijon); D. Bouhour, E. Dannaoui, M. A. Piens, and D. Peyramond (Lyon); Y. Berland, A. Blancard, and J. Sampol Roubècè (Marseille); M. F. Biava, P. Cantion, and C. Rabaud (Nancy); P. Clevenbergh, P. Dellamonica, and M. Gari-Toussaint (Nice); B. Becq-Giraudon, C. Kauffmann-Lacroix, and T. Pasdeloup (Poitiers); J. M. Korach and D. Toubas (Châlon en Champagne et Reims); C. Arvieux, F. Cartier, S. Chevrier, B. Degelih, T. Frouget, C. Guiguenn, and V. Noyon (Rennes); S. Chadapaud, X. Delbeck, and A. Laveullade (Toulon); E. Bonnet, M. D. Linas, and P. Massip (Toulouse); J. Bi. Guiard-Schmid, J. Frottier, B. Lebeau, M. C. Meyohas, J. L. Poïrot, and P. Roux (Hôpital St. Antoine, Paris); F. Botterel, J. F. Delfraissy, Y. Kertaimont, P. Lozeron, K. Rerat, and G. Saïd (Hôpital Bicêtre, Paris); J. P. Bedos, J. Belaisch, O. Benveniste, C. Bouchard, C. Carbon, C. Chocillon, J. P. Coulaud, V. Descamps, O. Launay, C. Leport, P. Longuet, H. Mouas, F. Vachon, and J. L. Vildé (Hôpital Bichat, Paris); C. Bouges-Michel, B. Jarrousse, and L. Guilleven (Bignon); M. E. Bougnoux, X. Montréal, S. Morelon, and E. Rouveix (Boulogne); M. Kazatchkine, V. Lavarde, and C. Pickett (Hôpital Broussais, Paris); Y. Boussouagou, C. Michon, and P. Vincencieux (Colombes); S. Bretagne, A. S. Lascaux, and A. Sobel (Créteil); M. Gauthier and O. Salmon (Evry); A. Bernardou, M. Cornet, and S. Oudard (Hôtel-Dieu, Paris); F. Daniel and J. Gilquin (Hôpital St. Joseph, Paris); C. Aznar, M. Eliašewicz, and H. Poncelet (Hôpital Pasteur, Paris); B. Baril, P. Bossi, F. Bricaire, J. Carrière, A. Datry, S. Heron, M. Jouan, M. Levy-Soussan, and M. M. Thiebaut (Hôpital de La Salpêtrière, Paris); O. Blétry, P. Cahen, and D. Zucman (Suresnes); G. Delzant, G. Kac, and C. Trivalle (Hôpital Tenon, Paris).

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References


