Immunoblot Profile as Predictor of Toxoplasmic Encephalitis in Patients Infected with Human Immunodeficiency Virus

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Received 31 July 2000/Returned for modification 16 November 2000/Accepted 16 February 2001

In order to define more accurately human immunodeficiency virus-infected patients at risk of developing toxoplasmic encephalitis (TE), we assessed the prognostic significance of the anti-Toxoplasma gondii immunoglobulin G (IgG) immunoblot profile, in addition to AIDS stage, a CD4+ cell count <50/mm³, and an antibody titer ≥150 IU/ml in patients with CD4 cell counts <200/mm³ and seropositive for T. gondii. Baseline serum samples from 152 patients included in the placebo arm of the ANRS 005-ACTG 154 trial (pyrimethamine versus placebo) were used. The IgG immunoblot profile was determined using a Toxoplasma lysate and read using the Kodak Digital Science 1D image analysis software. Mean follow-up was 15.1 months, and the 1-year incidence of TE was 15.9%. The cumulative probability of TE varied according to the type and number of anti-T. gondii IgG bands and reached 65% at 12 months for patients with IgG bands of 25 and 22 kDa. In a Cox model adjusted for age, gender, Centers for Disease Control and Prevention (CDC) clinical stage, and CD4 and CD8 cell counts, the incidence of TE was higher when the IgG 22-kDa band (hazard ratio [HR] = 5.4; P < 0.001), the IgG 25-kDa band (HR = 4.7; P < 0.001), or the IgG 69-kDa band (HR = 3.4; P < 0.001) was present and was higher for patients at CDC stage C (HR = 4.9; P < 0.001). T. gondii antibody titer and CD4 cell count were not predictive of TE. Thus, detection of IgG bands of 25, 22, and/or 69 kDa may be helpful for deciding when primary prophylaxis for TE should be started or discontinued, especially in the era of highly active antiretroviral therapy.

Before the era of highly active antiretroviral therapy (HAART), toxoplasmic encephalitis (TE) was the second-most-common AIDS-related opportunistic infection after pneumocystosis, and the most common cause of central nervous system disease in human immunodeficiency virus (HIV)-infected patients because of a high seroprevalence (60 to 70%) of the parasite in France and Europe (9, 11). Cotrimoxazole to prevent the occurrence of TE in at-risk patients has been widely recommended (14). Primary prophylaxis is proposed to patients with CD4+ cell counts lower than 100/mm³ who are seropositive for Toxoplasma gondii. The risk of developing TE at 1 year is estimated to be 10 to 20% in this population (2, 6, 13, 16). The ANRS 005–ACTG 154 trial was a double-blind randomized trial designed to assess efficacy and tolerance of comparing pyrimethamine, 50 mg three times weekly with folinic acid, to the placebo. It recruited 554 patients in three countries, France, the United States, and Spain; 274 were assigned to the pyrimethamine arm, and 280 were assigned to the placebo arm. Eligible patients had a CD4+ cell count lower than 200/mm³ and were seropositive for T. gondii. Informed consent was obtained from all patients, and human experimentation guidelines of the authors’ institutions were greater than 150 IU/ml (4, 8). With the advent of HAART, the incidence of TE has markedly decreased, concomitantly with the decrease in the incidence of other opportunistic infections (10, 11). Although the issues concerning primary prophylaxis of TE have become less urgent, there are still debates concerning the onset of primary prophylaxis in patients with failure of HAART and new debates concerning the appropriate criteria for discontinuing prophylaxis when immune restoration occurs.

The profile of anti-T. gondii antibodies reacting with antigens of the parasite has already been studied in various clinical situations (5, 15). The present study aimed at determining whether a specific immunoblot profile of anti-T. gondii immunoglobulin G (IgG) antibodies is associated with the occurrence of TE, in addition to previously recognized risk factors. This should allow a definition of the patients who would benefit from a primary prophylaxis of TE that was as accurate as possible.

MATERIALS AND METHODS

Study population. The design and results of ANRS 005–ACTG 154 have already been reported (8). Briefly, this was a double-blind randomized study comparing pyrimethamine, 50 mg three times weekly with folic acid, to the placebo. It recruited 554 patients in three countries, France, the United States, and Spain; 274 were assigned to the pyrimethamine arm, and 280 were assigned to the placebo arm. Eligible patients had a CD4+ cell count lower than 200/mm³ and were seropositive for T. gondii. Informed consent was obtained from all patients, and human experimentation guidelines of the authors’ institutions were
followed in the conduct of clinical research. Baseline serum samples, stored at 
-20°C and available for 152 of the 280 patients of the placebo arm, were used. The 152 patients included in the present study did not differ from the other 128 patients of the placebo arm of the ANRS 005–ACTG 154 study, who could not be included in the present substudy, with regard to the following baseline characteristics (means ± standard deviations): age, 37.8 ± 10.1 years versus 39.2 ± 10.5 years (P = 0.24); proportion of men, 86 versus 85% (P = 0.81); proportion of patients with CDC clinical stage C of HIV infection, 28 versus 30% (P = 0.80); median CD4+ cell count (interquartile range), 121 (50 to 171/mm³) versus 92/mm³ (36 to 153/mm³) (P = 0.11); probability of TE at 1 year (95% CI) 15.9 (10.8 to 23.2%) versus 9.7% (5.2 to 17.5%) (P = 0.42). Median follow-up (95% CI) was nevertheless 13.9 (9.8 to 20.1 months) versus 12.0 months (8.7 to 17.1 months) (P = 0.04).

Study design. Determination of the IgG immunoblot profile was performed using a crude extract of T. gondii tachyzoites as previously reported (5). An antigenic extract was prepared from tachyzoites of the RH strain of T. gondii obtained from mouse peritoneal exudates. Tachyzoites were washed three times in phosphate-buffered saline buffer containing 66 mM Tris buffer (pH 6.8), 5 mM EDTA, 1 M sucrose, 0.001% bromophenol blue, and 5% sodium dodecyl sulfate (SDS) and then denatured by heating at 100°C for 5 min. After centrifugation at 15,000 × g for 10 min, the protein concentration was determined using the bicinchoninic acid method (Pierce, Oud-Biejerland, The Netherlands). Electrophoresis was performed on an SDS–12% polyacrylamide gel with 200 µg of antigenic extract proteins per slab, as described by Laemmli (7). Proteins were then electrotransferred onto a nitrocellulose membrane; simultaneously, rainbow-colored protein molecular weight markers were loaded onto each gel. Strips of immunoblots were incubated with serum samples diluted 1:100 and then with alkaline phosphatase-labeled anti-human IgG (Jackson ImmunoResearch, West Grove, Pa.). Bands were visualized with a chromogenic substrate. Each profile was read using Kodak Digital Science 1D image analysis software. 1D generates a molecular weight curve, and each band is plotted against the standard curve to determine its weight (Fig. 1).

Statistical analysis. The entry date of our study sample was the date of randomization into the trial. Follow-up extended to the end of the trial. Patients still alive or TE free on that date were right censored on the date of their last assessment. Time to TE was calculated as the delay between the date of randomization (considered the baseline time) and the date of a first episode of TE, the date of death, or the date of the last follow-up. Survival curves were plotted using the Kaplan-Meier product limit method. A proportional-hazards regression model was used to estimate the independent effect of the immunoblot profile on the risk of TE adjusted for the following clinical and laboratory variables measured at baseline: age, gender, CDC clinical stage, absolute CD4+ and CD8+ cell counts, and IgG antibody titer (<150 versus ≥150 IU/ml). A reduced model was produced by backward elimination. Results are expressed in terms of the hazard ratio (HR), which estimates how each independent variable affects the baseline instantaneous hazard of TE, considered the dependent variable. The proportional-hazards assumption was checked using graphical methods by examining plots of log [−log (survival probability)] versus log (time) for each covariate in the final model. STATA software, version 5.1. (STATA Corp., College Station, Tex.), was used for statistical analysis.

RESULTS

Descriptive analysis. The immunoblot profile showed that variable proportions of the 152 patients had antibodies to numerous T. gondii antigens with molecular masses ranging
from 14,000 to 150,000 Da (Fig. 2). The most frequent bands were the 27-, 30-, 32-, 35-, 38-, 55-, 80-, 95-, and 150-kDa bands, detected in more than 50% of the patients. The median number of bands (interquartile range) significantly increased according to antibody titer, from 7 bands (6 to 9 bands) in patients with antibody titers of $\geq 34$ IU/ml to 9 bands (7 to 10 bands) in patients with antibody titers of 35 to 149 IU/ml, to 10 bands (9 to 11 bands) in patients with antibody titers of 150 to 399 IU/ml, and to 12 bands (10 to 13 bands) in patients with antibody titers of $\geq 400$ IU/ml (Kruskal-Wallis test; $P = 0.0001$). The presence of the 17-, 20-, 25-, 27-, 32-, 55-, 87-, 95-, or 150-kDa band was associated with significantly higher anti-\textit{T. gondii} antibody titers (Wilcoxon rank sum test; $P < 0.05$ for each band) (Table 1). The antibody titer was not significantly different according to the presence of the 14-, 22-, 30-, 35-, 38-, 40-, 42-, 46-, 50-, 60-, 69-, 73-, or 80-kDa band. The 27-, 30-, 32-, 35-, 38-, 55-, 60-, 80-, 95-, and 150-kDa bands were present in more than 50% of the 82 patients with IgG titers of $\geq 150$ IU/ml.

**TABLE 1. Incidence of TE in 152 HIV-infected patients seropositive for \textit{T. gondii} with CD4$^+$ cell counts $<200$/mm$^3$ according to the immunoblot profile of anti-\textit{T. gondii} IgG antibodies (ANRS 005–ACTG 154 substudy)**

| Band(s) kDa$^a$ | No. of patients | Median IgG antibody titer (IU/ml) | No. of patients with TE | Cumulative probability of onset of TE (%) at: | $P$ |
|-----------------|-----------------|---------------------------------|-------------------------|-----------------------------------------------|-----|
|                 |                 |                                 |                         | 3 mo | 6 mo | 12 mo |                   |
| 22 Present      | 68              | 192                             | 19                      | 4.4  | 10.6 | 20.5 | <0.01             |
| Absent          | 84              | 172                             | 10                      | 1.2  | 2.4  | 12.2 |                   |
| 25 Present      | 36              | 722                             | 13                      | 5.6  | 14.7 | 42.5 | <0.001            |
| Absent          | 116             | 123                             | 16                      | 1.7  | 3.5  | 8.3  |                   |
| 69 Present      | 63              | 221                             | 16                      | 1.6  | 9.8  | 20.6 | 0.12              |
| Absent          | 89              | 141                             | 13                      | 3.4  | 3.4  | 12.3 |                   |
| 80 Present      | 81              | 221                             | 21                      | 3.7  | 9.9  | 21.3 | 0.04              |
| Absent          | 71              | 116                             | 8                       | 1.4  | 2.9  | 9.9  |                   |
| 22, 25 Both present | 16          | 126                             | 9                       | 2.5  | 26.1 | 64.5 | <0.001            |
| 22 absent, 25 present | 20       | 240                             | 4                       | 0.0  | 5.6  | 24.8 |                   |
| 22 present, 25 absent | 52          | 132                             | 10                      | 1.9  | 5.9  | 8.0  |                   |
| Both absent     | 64              | 90                              | 6                       | 1.6  | 1.6  | 8.4  |                   |
| 25, 69 Both present | 18          | 544                             | 7                       | 5.6  | 23.0 | 43.1 | 0.01              |
| 25 absent, 69 present | 45       | 170                             | 9                       | 0.0  | 4.6  | 12.2 |                   |
| 25 present, 69 absent | 18       | 787                             | 6                       | 5.6  | 5.6  | 43.7 |                   |
| Both absent     | 71              | 79                              | 7                       | 2.8  | 2.8  | 5.9  |                   |
| 22, 69 Both present | 21          | 144                             | 8                       | 4.8  | 24.6 | 24.6 | 0.03              |
| 22 absent, 69 present | 42       | 233                             | 8                       | 0.0  | 2.5  | 18.2 |                   |
| 22 present, 69 absent | 47       | 199                             | 11                      | 4.3  | 6.5  | 18.1 |                   |
| Both absent     | 42              | 37                              | 2                       | 2.4  | 2.4  | 5.0  |                   |
| 22, 25, 69 All present | 4           | 1,273                           | 3                       | 25.0 | 75.0 | 75.0 | NR$^b$            |
| All absent      | 36              | 34                              | 2                       | 2.8  | 2.8  | 5.8  |                   |

$^a$ Band(s) associated with TE by univariate analysis ($P < 0.25$).

$^b$ NR, not relevant.

Univariate analysis of prognostic factors. In our sample, the following previously recognized risk factors for TE (4, 8) were also found to be associated with a higher incidence of TE: CDC clinical stage B versus A (HR = 2.6; 95% CI, 0.7 to 9.5; $P = 0.14$), CDC clinical stage C versus A (HR = 5.4; 95% CI, 1.5 to 19.5; $P = 0.01$), CD4$^+$ cell count of $<50$ mm$^{-3}$ versus $\geq 50$ mm$^{-3}$ (HR = 2.4; 95% CI, 1.1 to 5.6; $P = 0.04$), IgG antibody titer of $\geq 150$ IU/ml versus $<150$ IU/ml (HR = 3.1; 95% CI, 1.3 to 7.5; $P = 0.01$). In addition, in our univariate analysis, the cumulative probability of TE was significantly higher in the presence of a 22-, a 25-, or an 80-kDa band on the immunoblot profile (Table 2). As an example, the probability of occurrence of TE according to the presence or absence of the 25-kDa band is shown on Fig. 3.

Multivariate analysis of prognostic factors. Multivariate analysis, including all previously known risk factors for TE and the results of the immunoblot profile, found that only clinical CDC stage C versus A and specific IgG bands of 22, 25, and 69 kDa remained significantly and independently associated with a higher risk of TE (Table 2). Interactions between different bands were not statistically significant, although there was a
higher risk of TE in the presence of both the 22- and 25-kDa bands ($P < 0.0001$) or in the presence of both the 25- and 69-kDa bands ($P < 0.01$) or in the presence of both the 22- and 69-kDa bands ($P = 0.03$).

**DISCUSSION**

In the present study, specific bands of 22-, 25-, and 69-kDa IgG antibodies were associated with a higher risk of TE: the risk increased by factors of 5.4, 4.7, and 3.4, respectively, for patients with the band compared with that for patients without the band. The only risk factor which remained independently associated with further occurrence of TE was CDC clinical stage C of HIV infection. Thus, determination of the immunoblot profile of anti-*T. gondii* antibodies in the serum of HIV-infected patients at risk for TE with CD4 $\leq 200$/mm$^3$ and seropositive for *T. gondii*, especially those with IgG titers $\geq 150$ IU/ml, can allow a more accurate prediction of the risk of developing TE. Baseline characteristics of the group of patients studied were not significantly different from those of the other patients without available sera from the placebo arm. Thus, our results may be considered as representative of

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the whole placebo arm and can therefore be extrapolated to all HIV-infected patients seropositive for T. gondii with CD4 cell counts <200/mm³.

Analysis of the data from the placebo arm of the ANRS 005–ACTG 154 primary prophylaxis trial has already found that CDC clinical stage and CD4⁺ cell count, reflecting the intensity of the immunosuppression, were risk factors for TE (8). Our previous analysis showed that, in addition to host factors, the titer of humoral response, possibly reflecting the reactivation of the microorganism, was also a prognostic factor for the development of TE (4). In the SEROCO and HEMOCO cohorts monitored from 1988 to 1995, Bellanger et al. have confirmed that both the progression of immunosuppression, as assessed on the basis of a decrease of CD4⁺ cell count to <200/mm³, and the titer of the humoral response were independent predictors of TE (3). One could argue that, in our study, the presence of the 22-, 25-, and 69-kDa bands may result from higher antibody titers. While this could be true for the 25-kDa band, it is unlikely for the 22- and 69-kDa bands, as shown in Table 1.

In the strategy to assess the risk of developing TE in a patient, determination of the immunoblot profile remains useful. In order to optimize the strategy, determination of the total IgG antibody titer first is proposed. Those patients with a titer greater than 150 IU/ml are candidates for prophylaxis. In patients with a titer lower than 150 IU/ml, an immunoblot profile may be useful to detect those who should also be given prophylaxis (the prognostic significance of the bands remained unchanged in the subgroup of patients with IgG titers <150 IU/ml [data not shown]).

Bellanger et al. have shown that the increase in antibody titer may occur early in the course of HIV infection, when patients have CD4⁺ cell counts of approximately 400/mm³ (3). Our data suggest that the qualitative pattern of anti-T. gondii Ig antibodies might be more important for predicting the occurrence of TE than several of those previously identified predictors, especially the quantitative level of these antibodies.

The immunoblot profile of specific antibodies in the course of T. gondii infection in AIDS has already been assessed in several studies (1, 12, 15). All these studies concerned the diagnostic value of the test for patients with AIDS-related TE, and to our knowledge, none addressed the issue of prediction of TE. The usefulness of the immunoblot profile of T. gondii antibodies for diagnosis of TE has been controversial. The 22-kDa band was found in 5% of patients who were HIV seronegative and who had a positive T. gondii serology and in 15% of HIV-infected patients who had positive T. gondii serology (5). This frequency reached 54 and 66% in HIV-infected patients with TE and toxoplasmic primary infection, respectively. The 69-kDa band was present in 45% of patients who were HIV seronegative with toxoplasmic primary infection and in 100% of HIV-infected patients with TE. In the recent BIOTOXO study, IgG bands of 27 and 32 kDa were strongly associated with a final diagnosis of TE among HIV-infected patients undergoing empirical antitoxoplasmic therapy (12). In the present study the three bands significantly and independently associated with the risk of occurrence of TE were the 22-, 25-, and 69-kDa bands (the 80-kDa band, significant in univariate analysis, was no longer predictive of TE in the multivariate analysis). It is interesting that a common IgG band of 25 to 27 kDa is associated with both diagnosis and prediction of occurrence of TE, while some other bands appear more specific for acute TE, such as the 32-kDa band, or of reactivation of T. gondii, such as the 22- and 69-kDa bands. This 25- to 27-kDa band is probably the same as the 26- to 28-kDa double band recognized by sera of patients with TE reported by Weiss et al. in 1988 (15). Since there was a trend for a significant interaction between the 22- and 25-kDa bands or between the 25- and 69-kDa bands, resulting in an increase in the risk of TE, the recommendation to maintain TE primary prophylaxis is especially important when these bands are combined.

Since the antibody profile may reflect activation of the parasite and since previous data have suggested that, in the majority of cases, TE in HIV-infected patients is the result of reactivation of the endogenous parasite, better characterization of the humoral response to T. gondii may contribute to a definition of the appropriate time for TE prophylaxis (2). However, drug-based prevention is not devoid of serious adverse events which may require discontinuation of the preventive treatment (8). Thus, the practical implication of our results is that determination of the immunoblot profile can be used to define as accurately as possible the risk of reactivation of T. gondii leading to TE in patients immunosuppressed due to HIV. This will allow proposing preventive chemotherapy to a targeted population of patients for whom it may be the most useful approach and to discontinue the treatment when the risk is minor, especially in the present era of immune restoration after HAART.

ACKNOWLEDGMENTS

This work was supported by a grant of the French Agency for Research on AIDS (Agence Nationale de Recherche sur le SIDA).

We thank the patients and investigators who participated in the study in the different centers as members of the ANRS 005–ACTG 154 Trial Group. Members of the Scientific Committee were J.-P. Abouelker, J. Aubertin, A. Certain, G. Chêne, F. Derouin, J. Dormont, R. Hafner, C. Leport, B. Luft, J. Miro, P. Morlat, S. Pueyo, F. Rousseau, R. Salamon, M.-C. Saux, D. Schwartz, and J.-L. Vildé. Members of the Data Safety Monitoring Board were M. Amouretti, I. Charreau, J. Dormont, J.-F. Dartigues, D. Hémon, and R. Pollard. Investigators at the clinical sites participating to the trial were Blanc (Aix en Provence); Schmitt (Amiens); Arlaud (Avignon); Estavoyer (Besanc¸on); Dellamonica (Nice); Duret, Frottier, Herson, Seligmann, Sérényi, and Vildé (Paris); Beqc Giraudon (Poitiers); Dienlevin and Belfond (Paris); Auribert, Beylot, and Lacut (Bordeaux); Granier (Bourg-en-Bresse); Cosnard (Bris-sur-Forges); Bazin (Caen); Dormont (Clamart); Rey (Clermont-Ferrand); Delaunay (Dort-de-France); Troisvallet (Gonesse); Picard (Grenoble); Weinbrenck (Limoges); Trepo (Lyon); Gallais and Gastaut (Marseille); Allard (Meaux); Janbon (Montpellier); Canton (Nancy); Grolleau (Nantes); Cassuto and Dellamonica (Nice); Duret, Frottier, Herson, Seligmann, Sérényi, and Vildé (Paris); Beqc Giraudon (Poitiers); Dien (Saint-Brieuc); Ruel (Senlis); Storck (Strasbourg); Jaubert and Lefaucheux (Toulon); Mouton (Tours); Chouvet (Tours); and Lafaix (Villeneuve Saint Georges) in France; Gatell and Guelar (Barcelona) in Spain; and Hewitt (Buffalo), Van Der Horst (Chapel Hill); Phair (Chicago); Skahan (Cincinnati); Bartlett and Waskin (Durham); Nicholas (Elmhurst); Glatt (East Meadow); Balfour (Minneapolis); Connor (Newark); Armstrong, Gricco, Mildvan, Sacks, Soiero, Steigbigel, and Valentine (New York); Ho (Pittsburgh); Fessyl (San Francisco); Powdersley (St. Louis); Steigbigel (Stony Brook); Blair (Syracuse); MacArthur (Toledo); and Cheeseman (Worcester) in the United States. We also thank Jack S. Remington, who contributed to the initiation of ANRS 005–ACTG 154 and made helpful and detailed comments on the manuscript, J.-L. Vildé, R. Salamon, and R. Hafner, who drove the ANRS 005–ACTG 154 study and made helpful comments, Christiane
REFERENCES

1. Ashburn, D., M. M. Davidson, A. W. Joss, T. H. Pennington, and D. O. Ho-Yen. 1998. Improved diagnosis of reactivated toxoplasmosis. J. Clin. Pathol. Mol. Pathol. 51:105–109.

2. Beaman, H. M., B. J. Luft, and J. S. Remington. 1992. Prophylaxis for toxoplasmosis in AIDS. Ann. Intern. Med. 117:163–164.

3. Bellanger, F., F. Derouin, L. Grangeot-Keros, L. Meyer, and the HEMOCO and SEROCO Study Groups. 1999. Incidence and risk factors of toxoplasmosis in a cohort of human immunodeficiency virus-infected patients: 1988–1995. Clin. Infect. Dis. 28:575–581.

4. Derouin, F., C. Leport, S. Pueyo, P. Morlat, B. Letrillart, G. Chêne, J. L. Ecobichon, B. Luft, J. Aubertin, R. Hafner, J. L. Vilde, R. Salamon, and the ANRS 005–ACTG 154 Trial Group. 1996. Predictive value of Toxoplasma gondii antibody titres on the occurrence of toxoplasmic encephalitis in HIV infected patients. AIDS 10:1521–1527.

5. Franck, J., C. Mary, E. Jarroux, H. Dumon, and M. Quilici. 1993. Apport de l’immunoblot au diagnostic et à la surveillance de la toxoplasmose au cours du syndrome d’immunodéficience acquise. Pathol. Biol. 41:865–872.

6. Grant, I. H., J. W. M. Gold, M. Rosenblum, D. Niedzwiecki, and D. Armstrong. 1990. Toxoplasma gondii serology in HIV-infected patients: the development of central nervous system toxoplasmosis in AIDS. AIDS 4:519–521.

7. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

8. Leport, C., G. Chêne, P. Morlat, B. J. Luft, F. Rousseau, S. Pueyo, R. Hafner, J. Miro, J. Aubertin, R. Salamon, J. L. Vilde, and ANRS-005–ACTG 154 Group members. 1996. Pyrimethamine for primary prophylaxis of toxoplasmic encephalitis in patients with human immunodeficiency virus infection: a double-blind, randomized trial. J. Infect. Dis. 173:91–97.

9. Luft, B. J., and J. S. Remington. 1992. Toxoplasmic encephalitis in AIDS. Clin. Infect. Dis. 15:211–222.

10. Palella, F. J., K. M. Delaney, A. C. Moorman, M. O. Loveless, J. Fuhrer, G. A. Satten, D. J. Aschman, S. D. Holmberg, and the HIV Outpatient Study Investigators. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N. Engl. J. Med. 338:853–860.

11. Phillips, A. N., S. Grabar, J. M. Tassie, D. Costagliola, J. Lundgren, M. Egger for the EuroSIDA, the French Hospital Database on HIV, and the Swiss HIV Cohort Study Groups. 1999. Use of observational databases to evaluate the effectiveness of antiretroviral therapy for HIV infection: comparison of cohort studies with randomized trials. AIDS 13:2075–2082.

12. Raffi, F., J. Franck, H. Pelloux, F. Derouin, V. Reliquet, P. Ambroise-Thomas, J. P. Abouik, C. Leport, and H. Dumon. 1999. Specific antitoxoplasmic IgG antibody immunoblot profiles in patients with AIDS-associated Toxoplasma encephalitis. Diagn. Microbiol. Infect. Dis. 34:51–56.

13. Richards, F. O., J. A. Kovacs, and B. J. Luft. 1995. Preventing toxoplastic encephalitis in persons infected with human immunodeficiency virus. Clin. Infect. Dis. 21(Suppl. 1):S49–S56.

14. U.S. Public Health Service and Infectious Diseases Society of America. 1999. USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. Morb. Mortal. Wkly. Rep. 48:1–9.

15. Weiss, L. M., S. A. Udem, H. Tanowitz, and M. Wittner. 1988. Western blot analysis of the antibody response of patients with AIDS and Toxoplasma encephalitis: antigenic diversity among Toxoplasma strains. J. Infect. Dis. 157:7–13.

16. Zangerle, R., F. Allerberger, and P. Pohl. 1991. High risk of developing toxoplasmic encephalitis in AIDS patients seropositive to Toxoplasma gondii. Med. Microbiol. Immunol. 180:56–66.