Skin Test Reactivity and Cellular Immune Responses to *Mycobacterium avium* Sensitin in AIDS Patients at Risk for Disseminated *M. avium* Infection

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Skin tests and lymphocyte proliferation assays (LPA) were performed with *Mycobacterium avium* sensitin on patients with AIDS. Among 139 subjects, 13% had positive skin test results and 32% had positive LPA results. The LPA may be a more sensitive indicator of prior *M. avium* infection in this population.

Available data suggest that disseminated infection with organisms of the *Mycobacterium avium* complex (MAC) occurs when a mycobacterium-naive host encounters MAC organisms during the immunosuppression associated with advanced AIDS and that mycobacterium-experienced hosts with AIDS have a reduced risk of disseminated MAC infection. The protective role of prior mycobacterial infection is supported by data from the United States showing that AIDS patients with prior tuberculosis have a reduced risk of disseminated MAC infection and by data from Africa showing that MAC is present in the environment but that disseminated MAC infection is rare when background rates of latent tuberculosis are high (3, 4, 6). Further, human immunodeficiency virus (HIV)-infected persons in developed countries with lifelong exposures to soil or water (environmental sources of MAC) have a reduced risk of disseminated MAC compared to persons without such exposure (4). One prospective study showed a trend toward higher baseline levels of antibody to lipoarabinomannan (a common mycobacterial antigen) among AIDS patients who did not develop subsequent MAC infection than among those who did develop this infection, again suggesting that prior mycobacterial infection may protect against disseminated MAC infection during advanced AIDS (C. F. von Reyn, R. D. Arbeit, R. Waddell, et al., Abstr. 7th Conf. Retroviruses Opportunistic Infections, 2000). The evidence that disseminated MAC infection can result from a recently acquired infection comes from a study which used molecular epidemiology to confirm transmission of *M. avium* from persistently colonized hospital hot water to clusters of patients with AIDS (5).

Delayed-type hypersensitivity skin testing with *M. avium* sensitin (MAS) has been shown to be a sensitive and specific test for prior MAC infection in nonimmunosuppressed persons and provides a method to assess the hypothesis that preexisting MAC infection reduces the risk of new MAC infection among persons with advanced AIDS (7). In the present study, we performed MAS skin tests on a subset of HIV-positive subjects entering a prospective study of prophylaxis against disseminated MAC infection (AIDS Clinical Trials Group [ACTG] 362) (1). Informed consent was obtained from all subjects. Human experimentation guidelines of all participating institutions were followed in the conduct of clinical research. The objectives of the present substudy (ACTG 899) were to use MAS skin testing and lymphocyte proliferation assays (LPA) to estimate the proportion of HIV-infected persons with prior asymptomatic MAC infection and to prospectively evaluate the effect of such infection on the subsequent risk of new disseminated MAC infection.

Eligible subjects for the substudy were patients with no prior diagnosis of MAC infection who were receiving antiretroviral therapy and had a documented increase in CD4 cell count of 85% to more than 50 cells/mm³ on at least one occasion to more than 100 cells/mm³ on two sequential occasions at least 4 weeks apart. Subjects were randomized in a blinded fashion to receive either 1,200 mg of azithromycin or a placebo once weekly (362) (1). Informed consent was obtained from all subjects. Human experimentation guidelines of all participating institutions were followed in the conduct of clinical research. The objectives of the present substudy (ACTG 899) were to use MAS skin testing and lymphocyte proliferation assays (LPA) to estimate the proportion of HIV-infected persons with prior asymptomatic MAC infection and to prospectively evaluate the effect of such infection on the subsequent risk of new disseminated MAC infection.

Eligible subjects for the substudy were patients with no prior diagnosis of MAC infection who were receiving antiretroviral therapy and had a documented increase in CD4 cell count of less than 50 cells/mm³ on at least one occasion to more than 100 cells/mm³ on two sequential occasions at least 4 weeks apart. Subjects were randomized in a blinded fashion to receive either 1,200 mg of azithromycin or a placebo once weekly and were monitored every 8 weeks for the development of opportunistic infections. Skin tests were performed at weeks 0 and 24 using a 0.1-ml intradermal injection of each of two test antigens on a different forearm. Antigens included MAS (MAS 102, filling lot 68; State Serum Institute, Copenhagen, Denmark) and purified protein derivative (PPD) (Tubersol; Aventis Pasteur, Swiftwater, Pa.). Skin tests were read at 48 to 72 h as millimeters of induration in both the transverse and longitudinal diameters. All skin test readers received standardized training; readings were not blinded to skin test placement. For each subject, a set of four LPA replicates was reported for MAS (filling lot 35, State Serum Institute) at week 0 and week 24. The stimulation index was defined as the median of the...
No significant differences in MAS skin test reactivity were observed across geographic regions (data not shown). Table 2 shows the correlation between MAS skin test results and LPA responses.

In this study of patients with AIDS receiving potent antiretroviral therapy, 13% of patients had positive baseline MAS skin test results and 31% had in vitro lymphocyte responses to MAS indicative of prior MAC infection, but none developed disseminated MAC infection over a median follow-up period of 6 months. These data reflect the potent protection afforded by contemporary antiretroviral therapy. Because disseminated MAC infection did not develop in either the placebo group or the chemoprophylaxis group, we were unable to test the hypothesis that asymptomatic acquisition of MAC infection before the onset of advanced AIDS protects against subsequent disseminated MAC infection. Nor could the present study prove that disseminated MAC infection does not develop from reactivation, although the absence of such cases in a substantial number of skin test- or LPA-positive persons in the placebo group is consistent with that hypothesis.

The percentage of positive MAS skin test results among the HIV-positive patients in this study is lower than that observed in healthy subjects in the northern and southern United States (13 and 39%, respectively) (C. F. von Reyn, unpublished data). Since MAC infections are typically acquired in childhood (2), actual rates of MAC infection in the present patient cohort should mirror those in the general population. By analogy with PPD testing, the low percentage of positive MAS skin test results among the present subjects is likely due to false-negative results in patients with diminished CD4 cell function. Interestingly, the percentage of positive results with the MAS in vitro LPA among AIDS patients in this study is similar to the percentage of positive MAS skin test results among the general population (31 and 39%, respectively), suggesting that in vitro assays of cellular immune response (e.g., LPAs and gamma interferon assays) might provide a more sensitive method than that of skin tests for assessing prior or latent mycobacterial infection in persons with AIDS.

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| TABLE 1. Results of skin tests using MAS or PPD |
| Test and result | No. (%) of subjects at week: |
| MAS | |
| Negative | 117 (84) |
| Positive | 18 (13) |
| Not done | 4 (3) |
| 0 (n = 139) | 97 (81) |
| 24 (n = 120) | 17 (14) |
| Not done | 6 (5) |
| PPD | |
| Negative | 129 (93) |
| Positive | 1 (1) |
| Not done | 9 (6) |
| 0 (n = 139) | 109 (91) |
| 24 (n = 120) | 3 (3) |
| Not done | 8 (7) |

a A positive result was based on induration ≥5 mm.

| TABLE 2. Agreement between results of MAS skin test and of MAS LPAa |
| Stimulantb | LPA response | No. of subjects with MAS test result | % Agreement between tests | Kappa statistic | Confidence interval |
| MAS 0.5 | Negative | 40 | 3 | 43 | 74 | 0.29 | 0.039–0.537 |
| Positive | 13 | 6 | 19 |
| Total | 53 | 9 | 62 |
| MAS 1.0 | Negative | 38 | 3 | 41 | 71 | 0.25 | 0.011–0.483 |
| Positive | 15 | 6 | 21 |
| Total | 53 | 9 | 62 |

a Data are from 34 patients and combine results from testing at week 0 and week 24.
b In micrograms per milliliter.