PPD Skin Reactivity and Anergy in HIV-Infected Patients in Hawaii

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This study was a prospective screening study for PPD and anergy skin test reactivity in 304 HIV-positive individuals. A PPD positivity rate of 4.1% and an anergy rate of 50.5% were observed. The Hawaii HIV population has a relatively low prevalence of latent TB compared with the high prevalence of TB in the Hawaii population at large.

Introduction

Tuberculosis is a major cause of morbidity and mortality in HIV-infected individuals and constitutes an AIDS-defining opportunistic infection under CDC's Revised Classification System for HIV Infection.[1] The risk of progression from latent to active disease for a person co-infected with TB/HIV is estimated to be 7% to 10% annually as compared to a 10% lifetime risk in HIV-seronegative individuals.[2]

Among the opportunistic infections affecting HIV-infected individuals, tuberculosis is unique in that it is usually preventable and curable if detected early. Diagnosis of latent infection, however, may be difficult, as a high degree of anergy has been reported in the HIV-infected population, reducing the sensitivity of the tuberculin skin test.[3-5]

The CDC in April 1991 made several recommendations for the diagnosis of latent TB infection in HIV-infected individuals.[6] Specifically, recommendations called for companion testing with two delayed-type hypersensitivity (DTH) test antigens in association with the standard Mantoux method PPD tuberculin (5 TU) testing. A PPD cut off of ≥5 mm in this population was recommended as evidence of tuberculosis infection.

According to the CDC, in 1994 Hawaii had the highest TB case rate in the nation with 20.9 cases/100,000 individuals.[7] Little is known, however, about the rate of PPD reactivity in the HIV-infected population in Hawaii.

The aims of this study were to determine the rate of PPD reactivity in the HIV-infected community in Hawaii, to correlate the utility of PPD in this HIV population with a suspected high degree of anergy, and to correlate PPD and anergy result with immune status of these individuals as measured by peripheral blood absolute CD4 and CD8 lymphocyte subset determinations.

Materials and Methods

This study was a three year, prospective, single-center screening study for PPD and anergy skin test reactivity. HIV-infected persons age ≥12 years were recruited through AIDS service organizations and primary health-care providers for voluntary PPD and anergy skin testing. Participants were recruited from all major islands in the state. Demographic data including age, place of birth, ethnicity, HIV risk factors, history of previous PPD, and history of BCG vaccination were obtained for each participant. Three hundred individuals were targeted. Those with a history of sensitivity or intolerance to screening agents, history of previous positive PPD or history of tuberculosis were excluded.

The antigens and concentrations used to determine DTH were as follows: Tuberculin purified protein derivative (PPD) (Connaught Laboratories, Ltd, Willowdale, Ontario, Canada), 5 tuberculin units (TU) per 0.1 mL; mumps skin test antigen (MSTA) (Connaught Laboratories, Ltd, Willowdale, Ontario, Canada), 4 colony-forming units (CFU) per 0.1 mL; and tetanus toxoid fluid (Wyeth Laboratories, Inc, Marietta, Pennsylvania). The tetanus toxoid antigen was prepared by adding 0.2 ml of fluid tetanus toxoid to 1.8 ml of sterile albumin saline with phenol (Hollister Stier/Miles, Spokane, Washington), to provide a 1:10 dilution containing 0.1 LFU per 0.1 mL. Sterile albumin saline contains 0.9% sodium chloride, 0.03% albumin (human), and 0.4% phenol in water for injection. The skin tests were applied to the volar aspect of the right forearm using standard Mantoux technique. Skin tests were read once between 48 to 72 hours post-skin-test placement by trained personnel. The largest transverse diameter of palpable induration at each site was measured and recorded. A positive response was defined as ≥5 mm of induration in response to PPD and any induration in response to mumps or tetanus. Anergy was defined as negative response to all three antigens. Those individuals with less than 5 mm of induration to PPD underwent retesting with all three antigens.

Blood was drawn for T-lymphocyte subset analyses by flow cytometry within three months of skin testing. Patients with positive PPD and patients with anergy underwent medical evaluation by study personnel and chest x-ray examination for evidence of active tuberculosis. Those with positive PPD were referred for additional evaluation and treatment. They were offered enrollment into AIDS Clinical Trials Group 177 TB Prophylaxis study or referred to their primary physician or the Hawaii Department of Health.

Analysis of data to determine any significant differences or associations between variables was done using the chi-squared test of association with continuity correction and Fisher's exact test.

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This study is supported by the
Research Centers for Minority Institutions Grant No. RR 03061.

Results

A total of 304 individuals were enrolled in the study between January 1993 and March 1995 (Table 1). Complete results of skin test reactivity were available for 293 individuals. Results of T-lymphocyte subset data were available for 286 individuals. Not one case of active tuberculosis was identified.

A total of 133 individuals or 45.4% of all participants showed true negative PPD results (PPD negative, mumps and/or tetanus positive). A positive delayed-type hypersensitivity (DTH) response was observed most frequently with tetanus antigen (38.6%) followed closely by mumps antigen (36.2%). A positive reaction for both mumps and tetanus was seen in 25.9%. A total of 148 individuals showed no reaction to any of the three antigens, demonstrating an overall 50.5% rate of anergy (Table 2).

Some induration to PPD antigen was observed at 48 to 72 hours in 15 individuals (5.1%). Ten individuals had induration of ≥5 mm, meeting the CDC's criteria for a positive PPD in HIV-infected individuals.[6] The five individuals with less than 5 mm induration underwent repeat skin tests for all three antigens. One of these five individuals had an initial induration of 4 mm to tuberculin antigen and negative reaction to mumps and tetanus. Repeat tuberculin testing one month later revealed a 9 mm induration. Mumps and tetanus remained negative. He was counted as a PPD reactor. The remaining four individuals who were retested had a < 5 mm reaction or were nonreactive to PPD. Another individual initially showed no induration to tuberculin antigen at 48 hours but showed a delayed response to PPD 7 days later. He also was counted as a PPD reactor. Overall, a positive PPD was found for a total of 12 patients or 4.1% of the study cohort. None of these individuals reported a history of BCG vaccination.

Two of the 12 participants with positive PPD had negative reactions to mumps and tetanus. Seven had positive reactions to both mumps and tetanus. Two had a positive reaction to tetanus alone and one had a reaction to mumps alone.

A CD4 count less than 200/mm^3 was shown to be significantly associated with an anergic state as compared to a CD4 count greater than 200/mm^3 ($p < 0.05$). An inverse relationship of CD4 counts and anergy was seen (Fig 1).

Table 1.—Demographics

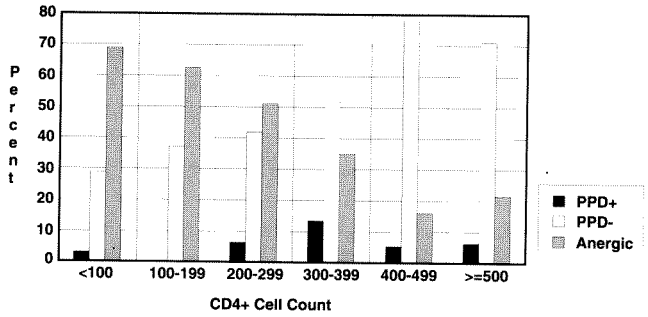
	No Patients (% total)
Patients entered	304
Age (Mean/Std dev)	40.5/8.0
Race	
African-American	6 (2.0)
Asian Pacific Islander	69 (22.8)
Caucasian	192 (63.4)
Latin/Hispanic/Portuguese	27(8.6)
Native American	10 (3.3)
Place of Birth	
Mainland	176 (57.9)
Hawaii	62 (20.4)
Foreign country	16(5.3)
Not given	50 16.4
Risk Behavior	
Bisexual	5 (1.6)
Blood transfusion	6 (2.0)
Heterosexual sex	19 (6.3)
Homosexual	222 (73.0)
Intravenous drug user	15 (4.9)
Sex with intravenous drug user or bisexual	3 (1.0)
CD444 count*	
≤50	72 (23.7)
51-199	65 (21.4)
200-499	100 (32.9)
≥500	49 (16.1)

*Available for 286/304 pts

Table 2.—Skin Test Results

	PPD	Anergy Panel (one or both)	N (%)
Positive PPD	+	+	10(3.4)
	+	-	2(0.7)
True Neg PPD	-	+	133(45.4)
Anergic	-	-	148(50.5)
	Total		293 (100)

Fig 1.—Frequency of antigen response according to CD4 T-Lymphocyte



Of the 12 individuals with positive PPD skin tests, four had CD4 counts of 200/mm^3 to 299/mm^3, three had CD4 of 300 cells/mm^3 to 399 cells/mm^3, one had CD4 of 400 cells/mm^3 to 499 cells/mm^3, and three had CD4 of ≥500 cells/mm^3. Only one individual had a CD4 count of less than 200 cells/mm^3. This individual had a CD4 count of <6 cells/mm^3, and was the individual previously described with a initial 4 mm reaction to tuberculin increasing subsequently to 9 mm on boosting. A significant association was found between having a CD4 count greater than 200/mm^3 and a positive PPD ($p=0.004$).

An interesting phenomenon of delayed skin-test positivity was observed in five patients. The original case involved a 36-year-old HIV-positive Caucasian man from Maui who was seen in September 1994. No induration was noted at the site of his PPD at 48 hours post-skin-test placement. He did have a 13 mm reaction to tetanus and a 4 mm reaction to mumps. The patient phoned our research office with information that he had noted a swelling in the region of his PPD seven days post placement of the skin tests. The positive reaction was confirmed and read as a 7 mm induration by the local HIV care coordinator nurse who is certified in tuberculosis testing. Unfortunately, no repeat PPD was done and the patient later died of non-TB-related causes.

All patients subsequent to this case were asked to call if indurations developed after the initial 48 to 72 hour reading. No other delayed reaction to PPD was reported. However, calls were received from four participants describing delayed reactions to tetanus and/or mumps from five to ten days post placement of the skin

tests. Two of these individuals underwent repeat skin testing. One of these individuals was initially anergic but reported a reaction to tetanus antigen at day eight. He underwent repeat skin tests two months later and had a positive tetanus reaction of 3 mm. No reaction was observed to mumps or tuberculin. The second individual, also initially anergic, reported a positive reaction to mumps and tetanus at day ten. He was retested four months later and had a 3 mm reaction to tetanus, and negative reactions to mumps and PPD.

Discussion

Hawaii has the highest tuberculosis case rate in the nation with 20.9 cases/100,000 individuals.[7] According to Richard Vogt MD of the Hawaii Department of Health in September 1995, of the 247 cases of active TB reported to the Hawaii Department of Health in 1994, 224 (91%) were Asian/Pacific Islanders and only 20 (8%) were listed as Caucasians. One hundred ninety five patients (79%) were foreign-born individuals with 127 patients born in the Philippines, 17 in Southeast Asia, and 11 from Korea. Tuberculosis in Hawaii is a problem largely of the immigrant population especially of Asian/Pacific Islander ancestry and not of the larger local population.

This study found a PPD reactivity rate of 4.1% among Hawaii's HIV-infected population, a rate much lower than expected given the high overall case rate in the state. Our study cohort consisted of 63.4% Caucasians and 22.8% Asian/Pacific Islanders, an ethnic composition closely matching the ethnic distribution of AIDS cases reported to the Hawaii Department of Health (66% Caucasians and 25% Asian/Pacific Islanders),[9] but very different from the ethnic distribution of persons with active TB in the state. Thus the HIV population in Hawaii appears to have a relatively low prevalence of latent TB and is a population probably distinct from the immigrant Asian/Pacific Islander population responsible for the high prevalence of tuberculosis in Hawaii.

An overall 50.5% anergy rate was found in our study. A strong inverse association between the lack of response to DTH testing and degree of immunosuppression as represented by CD4 counts was demonstrated. The rate of anergy varied from 22% in individuals with CD4 counts $\geq 500/mm^3$ to 72% in those individuals with a CD4 count of $<50/mm^3$. This high rate of anergy and inverse correlation with CD4 counts are consistent with other published reports.[3-5,10] Huebner et al studied a total of 479 HIV-infected persons at an HIV clinic in Florida and a tuberculosis clinic in New Jersey and found that anergy was four times and 15 times as likely for persons with CD4 T-lymphocyte counts of $200/mm^3$ to $400/mm^3$ and $<200/mm^3$, respectively, as for persons with >499 CD4 T-lymphocytes/mm^3.[4] Similarly, Graham et al reported an inverse linear trend for PPD positivity and CD4 lymphocyte count in a cohort of 260 individuals with history of intravenous drug use in Baltimore.[10] Brix et al reported a 5% anergy rate in HIV-infected individuals with CD4 counts $>500/mm^3$ while among individuals with ≤ 200 cells/mm^3 the anergy rate increased to 38%.[3]

Individuals with a positive reaction to PPD were likely to have a positive reaction to anergy testing. Ten of the 12 participants with positive PPD had a positive reaction to DTH antigens. This result is in contrast to the finding of Markowitz et al who reported no association between the response to control antigens and tuberculin reactivity.[5] Furthermore the ability to mount a positive reaction to PPD appears heavily dependent on the CD4 count of these individuals. Eleven of 12 participants in our study (92%) with a positive PPD had CD4 counts ≥ 200. Only one individual with a positive PPD was detected in the cohort with CD4 counts <200 despite the fact that close to 50% of the entire cohort had CD4 counts less than 200.

DTH testing is recommended by the 1991 CDC guidelines as an adjunct to tuberculosis skin testing in the diagnosis of tuberculosis.[6] The value of anergy testing, however, has been questioned lately especially in view of the recent study demonstrating fluctuation of DTH reaction over time in many HIV-infected individuals. An anergy study in a cohort of 923 injection drug users at methadone clinics in Baltimore between 1991 and 1994 demonstrated an initial anergy rate in HIV-seropositive individuals of 36% and in HIV-seronegative individuals of 14%. Among those who initially were DTH-positive, anergy developed in 24% of seropositive and 15% of seronegatives at the next study visit. Among those who initially were anergic, changes to a positive test took place in 15% of seropositive and 11% of seronegatives.[11] Our study supports the view that DTH testing is imprecise. Neither low CD4 counts nor anergy to DTH testing is completely predictive of a negative PPD as demonstrated by our two patients with negative responses to DTH who nevertheless had a positive reaction to PPD. One of these individuals had a CD4 count of <6. Our results argue that PPD skin testing should be considered in all HIV-infected individuals regardless of their state of anergy or CD4 count.

Recent studies have shown some, although limited, utility in boosting for those patients with insignificant (<5 mm) PPD and anergy. Huebner et al[6] found seven individuals from a cohort of 130 patients with initially negative PPD tests who responded to boosting. Webster et al[12] found 18 patients in a cohort of 709 HIV-infected patients who demonstrated a booster effect. These boosted responses were seen in 8 (2.1%) anergic patients, 6 (4.5%) nonanergic patients and 4 (2.5%) with anergy status unknown. In our study, five participants with <5 mm reaction to tuberculin antigen underwent skin testing for the second time. One individual was found to have a 9 mm response to the second PPD. Although numbers were limited in this study, boosting by repeat PPD may be of value in those with minimal induration (<5 mm) to tuberculin antigen.

A possible delayed reaction to PPD and anergy testing was observed in this study, with positive reactions developing after the standard 48 to 72 hours after placement of the skin tests. In one instance, 7 mm induration developed at the site of the original PPD seven days after placement. Unfortunately, no biopsies were performed to demonstrate a delayed hypersensitivity reaction. Subsequently, the two other participants self-reported delayed positive reaction to DTH. On retesting, both showed positive reactions to DTH antigen. We believe this phenomena should be studied further and that there may be utility in instructing patients to return if a delayed response is noted in order to confirm a positive reaction.

In conclusion, our study demonstrates that the HIV population in Hawaii has a relatively low prevalence of latent TB compared with the high prevalence of TB in the immigrant Hawaii population. Despite relatively advanced immunosuppression, PPD skin testing identified patients for TB prophylaxis. Skin test anergy was inversely associated with CD4 counts although no level of immunosuppression could be identified that could reliably predict skin test anergy. A phenomenon of delayed reaction to skin testing was observed and merits further study.

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