Identification of Human Immunodeficiency Virus-infected Individuals by Delayed Type Hypersensitivity Skin Testing*†

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ABSTRACT

Objective: To determine if more useful information could be derived from delayed type hypersensitivity skin testing of human immunodeficiency virus (HIV)-infected patients by measuring the amount of induration in response to skin testing in contrast to assessing for the presence of anergy.

Design: Prospective, double-blind trial.

Setting: Two HIV clinics.

Patients: A sample of 60 ambulatory, HIV-infected patients and 18 normal controls.

Interventions: Delayed type hypersensitivity skin testing with a panel of recall antigens.

Main Outcome Measures: Anergy, total induration in response to the panel of antigens, and CD4 lymphocyte count.

Results: Anergy was present in only 28 of 60 (46.7 percent) HIV-infected subjects. A low skin test score (under 20 mm of total induration) was present in 43 of 60 (71.7...
percent) HIV-infected subjects (p < 0.01 vs anergy). One control patient was anergic, while two had a low skin test score (p = NS). Among HIV-infected subjects, there was poor correlation between CD4 lymphocyte count and skin test score (R² = 0.12).

Conclusions: Skin testing by this method identifies more HIV-infected patients than does assessing the presence or absence of anergy.

Introduction

The HIV-infected patient is at risk for several neoplasms as well as opportunistic infections such as mucocutaneous candidiasis and Pneumocystis carinii pneumonia (PCP), and the risk of these conditions can be predicted by the number of circulating CD4 lymphocytes. Measurement of the CD4 cell count has, therefore, become an indispensable tool for determining the appropriate timing of therapies such as PCP prophylaxis.

Delayed type hypersensitivity (DTH) skin testing, using a battery of recall antigens has been used in a number of clinical situations to assess cell mediated immune function. Skin testing has become accepted as one of the standard methods for assessing nutritional status and for predicting mortality in surgical patients. Not surprisingly, many groups have studied the utility of skin testing in assessing the degree of immunosuppression in patients with or at risk for HIV infection. The use of skin testing has potential advantages over the measurement of CD4 cell counts. Skin testing can be performed without the use of the relatively sophisticated equipment required for CD4 cell identification. Additionally, skin testing is less expensive. The potential importance of these factors becomes apparent when one considers the magnitude of the AIDS epidemic in developing countries where access to technology and funds for combatting AIDS are limited.

Prior investigators have demonstrated that up to 83 percent of HIV-infected patients who have progressed to AIDS are anergic to a battery of recall antigens. The presence of anergy correlates only roughly with the number of circulating CD4 cells, and up to 39 percent of patients with advanced HIV-infection have skin test reactivity, while a similar percentage of patients with early HIV-infection are anergic. Therefore, skin testing for anergy generally has not been useful as a method of determining the degree of immunosuppression in a patient infected with HIV. Skin testing for anergy also has not been found useful in identifying asymptomatic patients infected with HIV because most such patients will have preserved skin test reactivity. Additionally, because the presence of anergy is an all-or-nothing phenomenon, its use as the sole measured endpoint of DTH skin testing does not allow for the quantification of immunosuppression as does the measurement of CD4 cell counts.

It has been demonstrated that HIV-infected patients who are not anergic will nonetheless display smaller reactions in response to DTH skin testing. Similarly, in healthy Japanese individuals infected with HTLV-I who respond to purified protein derivative, the size of the response is smaller than those of normal patients.

The purpose of the present study was to determine if using the size of the reaction to DTH skin testing could provide a more sensitive indication of HIV-infection than does the endpoint of anergy. Additionally, it was sought to ascertain if the size of the reaction could yield more information regarding the degree of immunosuppression in a patient infected with HIV than does solely assessing the presence or absence of anergy.

Materials and Methods

This study was approved by the Human Subjects Committee at the University of California, San Diego Medical Center (UCSD). Informed consent was obtained from all subjects. Patients infected with HIV were recruited from the Owen Clinic at UCSD and
the Beach Area Clinic in San Diego. Normal volunteers were recruited from staff and students at UCSD. Subjects with a risk factor for HIV-infection were excluded from the control group. All HIV-infected patients had serologic evidence of infection.

The HIV-infected subjects were placed into one of the following three categories based on a chart review and medical history: (1) Asymptomatic HIV-infected (chronic lymphadenopathy permitted); (2) Symptomatic HIV-infection; included patients who had at least one of the following: fatigue, night sweats, or diarrhea for greater than one month, oral candidiasis, hairy leukoplakia, herpes zoster, or unexplained weight loss greater than 10 lbs.; without an AIDS defining diagnosis; and (3) AIDS patients had a history of an AIDS defining opportunistic infection or malignancy.16 Absolute CD4 count was not considered an AIDS defining diagnosis for the purposes of this study. Subjects taking immunosuppressive drugs were excluded from the study.

Six antigens were used for this study: (1) Tuberculin Purified Protein Derivative (Mantoux) 5 TU per 0.1 ml, Lot 2308-12*; (2) Dermatophytin, allergenic extract prepared from Trichophyton spp., Lot H98H6098, diluted 1:30 v/v with buffered saline containing 0.4 percent phenol;† (3) Tetanus Toxoid USP, Lot 9B11051 diluted 1:100 v/v with buffered saline containing 0.4 percent phenol;‡ (4) Dermatophytin “O”, allergenic extract prepared from Candida albicans, Lot D09E61150; diluted 1:100 v/v with buffered saline containing 0.4 percent phenol; (5) Mumps Skin Test Antigen USP, Lots 9H01046 and 8D01162;§ (6) Candin, Candida albicans antigen preparation, Lot Ca10318902.§

As noted, two different preparations of Candida were used. Candin, a new skin test antigen for cellular hypersensitivity, was used because it has standardized potency from lot-to-lot. Because its use for DTH-skin testing had not yet been approved by the Food and Drug Administration, a second Candida preparation, Dermatophytin “O”, was included for comparative purposes.

All skin tests were placed by one of two skin test technicians, each of whom had over seven years of experience in the placing of skin tests. The six antigens were coded so that neither the skin test technician nor the subject knew the identity of the antigen. The skin test technicians were also blinded as to the patient group of the HIV-infected subjects.

Each subject was injected with all six antigens at a single sitting. Injections were made intradermally with 0.1 ml of the antigen, using a 27 gauge beveled needle. Three injections were made on the volar surface of each arm, at least 10 cm apart. Skin tests were read at 24 and 48 hours, using the palpation technique. The number of millimeters of induration in both the transverse and longitudinal axis was measured and the mean was calculated to obtain the final result. Whenever possible, the skin tests were read by both technicians so that an assessment of interobserver variability could be made. When the test was read by both technicians, the mean of the two results was used. Three skin tests were read only by one of the authors. At the time of the 48 hour reading, venous blood was obtained for a CD4 cell count.

Induration of 5 mm or greater in response to a specific antigen was defined as a positive response. However, because our past experience and that of other researchers17 has shown that the mumps antigen produces a larger reaction than other skin test antigens, 10 mm of induration was required for a positive response to mumps. Thus, a patient with less than 10 mm of induration in response to the mumps antigen and also less than 5 mm of induration to the other antigens was defined as anergic. A normal CD4 cell count at our laboratory is 500 to 1200 per mm3.

Analysis of variance was used to evaluate the difference between the ages, CD4 counts, and skin test scores for each patient group. The Wilcoxon rank order test was used to determine the difference between the 24 and 48
hour readings. The paired t-test was used to evaluate the difference between the reactions to Candin and Dermatophytin “O”. The unpaired t-test was used to compare the skin test scores of patients taking anti-retroviral therapy to those not. The Fisher’s exact test was used to determine the relationship of HIV status to skin test score, the relationship of skin test score and anergy to HIV infection, and the relationship of skin test score and anergy to CD4 cell count. Numbers are expressed as mean ± standard error of the mean. Significance was accepted at p < 0.05.

**Results**

Eighty-two subjects were entered into the study. Two were excluded because they did not complete the protocol, and two others entered into the control group were excluded because they were found to have a risk factor for HIV-infection. Thus, there were 78 subjects included in the analysis. In Table I are shown the number of subjects entered into each group, their mean ages, and their mean CD4 cell counts. There was excellent agreement between the readings of the two skin test technicians. Duplicate values were available for 61 percent of the readings. There was agreement within 2 mm for 95 percent of those readings.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age±</th>
<th>Male</th>
<th>CD4 Count±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>35.6±2.8</td>
<td>16</td>
<td>785.9±48.5</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>21</td>
<td>35.1±1.5</td>
<td>20</td>
<td>468.5±56.2</td>
</tr>
<tr>
<td>Symptomatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>20</td>
<td>38.1±2.0</td>
<td>20</td>
<td>241.4±34.8</td>
</tr>
<tr>
<td>AIDS</td>
<td>19</td>
<td>41.9±2.9</td>
<td>18</td>
<td>141.7±34.1</td>
</tr>
</tbody>
</table>

*p = NS for all groups.

*p < 0.05 between all groups except between symptomatic HIV and AIDS.

There were no significant differences in the number of subjects responding to the two different preparations of *C. albicans* or in the size of the response. Therefore, only Candin was used for the data analysis because, unlike Dermatophytin “O”, it is standardized according to potency in DTH reactions and, therefore, allows valid comparisons with future studies.

Initial analysis of the data revealed that for some subjects, the 24 hour reading showed the greatest amount of induration, while for others the 48 hour reading had more induration. As these differences were statistically significant, the larger of the two readings was selected for analysis.

In Table II are demonstrated the number of control subjects who responded to the recall antigens. In agreement with prior investigators, we found that *Trichophyton* and purified protein derivative elicited few reactions in normal subjects (33.3 percent and 16.7 percent, respectively) and, therefore, were not useful as recall antigens. This contrasted with the larger number responding to candida, tetanus, and mumps (67.7 percent, 77.8 percent, and 88.9 percent, respectively). Therefore, the results from only the latter three antigens were used for deriving a skin test score for the final data analysis.

For each subject, a skin test score was derived. This score was the sum of the amount of induration in response to each of the three antigens used for the data analysis. A low score was defined as being under 20 mm of total induration, the lowest possible score that could be obtained with a positive response to each of the three antigens (5 mm each for candida and tetanus and 10 mm for mumps). This contrasts with our definition of anergy, which required that none of the responses were larger than the previously mentioned limits.

Anergy was observed in only 28 of 60 (46.7 percent) HIV-infected patients while 43 of 60 (71.7 percent) HIV-infected patients had a low skin test score of under 20 mm total induration (p < 0.01). Anergy was found in one control subject while a low skin test score was found in two of 18 (11.1 percent) normal subjects (p = NS) (Table III). Even when patients with a
CD4 cell count over 500 per mm$^3$ were excluded, a low skin test score was significantly more frequent than was anergy, (37 vs 20 of 49, p < 0.001). The skin test score was low in six of 11 HIV-infected patients with normal CD4 cell counts, while only two of these 11 patients were anergic.

In figure 1 it is demonstrated that the skin test scores for the three groups of HIV-infected patients were similar. The mean skin test score for asymptomatic HIV-infected patients was 15.2 ± 2.1, for symptomatic patients was 17.4 ± 2.0, and for AIDS patients was 14.7 ± 1.6. None of these results were significantly different from each other, but all were significantly different from the control population (mean score, 32.0 ± 2.4) (p < 0.0001).

Within both the asymptomatic HIV-infected group and the symptomatic group, patients taking anti-retroviral therapy and those not taking it had similar mean skin test scores. However, within the AIDS group, the mean skin test score was 19.6 ± 6.9 for patients taking anti-retroviral therapy, while it was 10.3 ± 3.8 for those who were not (p < 0.002).

Although there is a statistically significant relationship between CD4 count and skin test score among the HIV-infected subjects (p < 0.01), the correlation is poor (R$^2$ = 0.12). In figure 2 the derived regression results are demonstrated. If the nine patients with a CD4 count under 50 per mm$^3$ are excluded from the regression analysis (results not shown), no statistically significant relationship between CD4 cell count and skin test score is seen (p > 0.1).

**Discussion**

Because of the severe defect in cell-mediated immunity associated with HIV-infection, many such patients show diminished levels of response to DTH skin testing. Sears$^5$ et al found 20 percent of asymptomatic HIV-infected homosexuals to be anergic. Lazzarin$^6$ et al found anergy in 26.7 percent of HIV-infected drug abusers at various stages of disease. MacDonell$^8$ et al found that 42 percent of HIV-infected men without AIDS were anergic. French$^{11}$ et al found anergy in 11 of

### TABLE II

<table>
<thead>
<tr>
<th>N</th>
<th>PPD</th>
<th>Trichophyton</th>
<th>Candin</th>
<th>Dermatophyton</th>
<th>Tetanus Toxoid</th>
<th>Mumps</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>3 (16.7)</td>
<td>6 (33.3)</td>
<td>12 (66.7)</td>
<td>13 (72.2)</td>
<td>14 (77.8)</td>
<td>16 (88.9)</td>
</tr>
</tbody>
</table>

### TABLE III

<table>
<thead>
<tr>
<th>Skin Test Results for Each Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Anergic</td>
</tr>
<tr>
<td>Score &lt; 20</td>
</tr>
</tbody>
</table>

$^a$p < 0.01 vs, number anergic.

$^b$p < 0.05 vs, number anergic.
24 patients at various stages of HIV infection. Fernandez-Cruz et al found 83 percent of 38 AIDS patients to be anergic. This wide disparity in results is probably accounted for by both differences in the populations being studied and differences in the skin test antigens and techniques. Because of the variable skin test results, and because many patients infected with HIV will have some degree of DTH evident, determining the presence or absence of anergy generally has not been useful for assessing immune status of HIV-infected patients. Nonetheless, some investigators have found that the presence of anergy predicts progression to AIDS among HIV-infected patients.13,14

Several groups have used the Multitest method of skin testing, which uses a multipuncture needle device to inject six antigens percutaneously. A score is derived based on the average size of induration of the positive reactions.20 These groups found that not only did the patients respond to fewer antigens than normal patients, but the positive reactions were smaller than expected.6,10,12 These results suggest that measuring the amount of
induration in response to skin testing could provide more useful information regarding the immune status of HIV-infected patients than only assessing the presence or absence of anergy. However, there have been reports of false negative results with the Multitest, as well as questions regarding the suitability of the antigens. These problems, as well as potential variability in the amount of antigen deposited in the skin have led the Centers for Disease Control and Prevention to discourage its use for the determination of anergy.

It has been confirmed by us, using the well-validated Mantoux technique, that the size of the reactions to a panel of three antigens is diminished in HIV-infected patients. It was also found that these measurements are more sensitive to the decrements in immune function associated with early phases of HIV infection than is the endpoint of anergy. Other groups have attempted to identify the defect in DTH associated with early HIV infection by defining a lack of response to some but not all of a panel of antigens as partial anergy. However, this method could result in a large number of uninfected subjects being falsely defined as partially anergic since many normal subjects will fail to respond to one or more of a panel of recall antigens.

Our method demonstrated alterations in DTH in 76.2 percent of asymptomatic HIV-infected patients, including five of nine who had normal CD4 cell counts. Despite the increased sensitivity of our method for detecting the defect in DTH associated with early HIV infection by defining a lack of response to some but not all of a panel of antigens as partial anergy. However, this method could result in a large number of uninfected subjects being falsely defined as partially anergic since many normal subjects will fail to respond to one or more of a panel of recall antigens.

Furthermore, this was accomplished with antigens which allow valid comparisons with future studies. Tetanus toxoid is the only one of our three antigens which is not standardized for DTH testing, however it has been reported by the Centers for Disease Control and Prevention that its use for anergy testing produces reliable results.

An unanticipated result of our study was that the decrement in the skin test score was just as profound for patients in the early stage of HIV-infection as it was for patients with AIDS. The CD4 cell counts of our asymptomatic group were generally only mildly diminished, suggesting that infection with HIV causes a decrement in DTH by a mechanism that may be unrelated to the CD4 cell count, at least in the early stages of infection.

The early, dramatic decline in DTH detected by our method suggests that DTH skin testing might serve as an inexpensive, simple adjunct in screening for HIV-infection. The use of this method, based on a low skin test score will clearly identify more HIV-infected people than will testing based on the standard definition of anergy. Because it is not as sensitive as serologic methods, DTH skin testing might be most useful in medically underserved areas of the world where HIV-infection is common yet there is limited technology or funds available for the use of serological screening methods.

Acknowledgment

Thanks are extended to the staff of the UCSD Skin Test and Tuberculosis Control Office; Ellen Logue, Teri Festa, and Brenda Farrell for their assistance with the skin testing.

References

7. Fernandez-Cruz E, Fernandez AM, Gutierrez C, et al. Progressive cellular immune impairment leading to development of AIDS: two year prospective study of


