DTH Responsiveness of HIV-Infected Thai Adults

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Abstract

A study was carried out in Thailand to determine the frequency of reactivity to delayed-type hypersensitivity (DTH) skin tests used for the staging of HIV patients in the United States. A four-antigen panel which included tetanus toxoid (1:10), *Candida* (1:10), mumps and *Trichophyton* antigens was assessed in 221 adult subjects from across the full immunological spectrum of HIV disease. Complete anergy was found in 38 per cent of 73 subjects with CD4 counts of 0-200 cells/ml and in 6 per cent of 78 subjects with 201-400 cells/ml. Partial anergy (response to 1 of 4 antigens) was found in 26 per cent of the 0-200 cell/ml group and decreased progressively with increasing CD4 cell count. Results suggested that a 3-member recall antigen panel would provide nearly all the clinically useful information gained by the more standard 4-member panel. In conclusion, DTH skin testing was confirmed to provide a method of assessing the integrity of cellular immune function of HIV-infected Thai adults which correlated with disease progression.

Key word : HIV-1, Skin Testing, Cutaneous Delayed-type Hypersensitivity

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Dysregulation of the cellular immune system is the central feature of the pathogenesis of HIV-related disease. Recognition of this led Walter Reed investigators in the United States to incorporate an *in vivo* assessment of cell-mediated immune (CMI) function into their staging system for HIV-infected individuals[1]. Cutaneous delayed-type hypersensitivity (DTH) reactions are assessed with a panel of intradermal skin tests utilizing recall antigens. Employing this system, DTH anergy has been shown to be an independent predictor of rate of disease progression[2,3].

In vitro tests of T-cell function have been shown to correlate with cutaneous DTH responses and to reflect similar associations with HIV disease progression[4]. However, such laboratory assays are both more artificial and costly than the use of DTH skin tests. Within the developing world, assessment of CMI in HIV/AIDS patients is often not possible due to resource limitations. In these settings, although skin testing is more feasible than most laboratory assays of immune function, it has been under utilized. Baseline data on DTH responses in HIV-infected populations of developing countries are lacking.

Based upon experience with the skin test panel utilized by the U.S. military as part of the Walter Reed HIV Staging System[2], a panel of antigens and concentrations were evaluated in Thai adults with HIV infection. These included tetanus toxoid, mumps, *Candida* and *Trichophyton* antigens, as well as PPD for help in diagnosis of tuberculosis. For the antigens to be useful, individuals have to have been immunized or exposed to these antigens in the past. Recall antigen reactivity is not well described for non-U.S. populations. In assessing a population in Southeast Asia, this study begins to address the necessity of documenting responses among populations where the frequency of tetanus and mumps vaccination, and of exposure to microbial antigens may differ from those in Western industrialized countries. This study population differed from that previously studied in its HLA allele frequency[4] and in that women were well represented. We sought to determine the frequency of complete and partial anergy across the immunological spectrum of HIV-infected individuals and to assess whether differences exist based upon gender.

METHODS

Subjects

Study subjects were Thai adults with HIV-1 infection documented by both antibody and nucleic acid analyses who enrolled in an HIV natural history study in Bangkok, Thailand. Volunteers gave written informed consent to participate in the study protocol which had been approved by institutional review boards of the Royal Thai Army Medical Department, the Thai Ministry of Public Health and the U.S. Army Medical Research and Development Command. Evaluations of volunteers included medical history, physical examination, CD4 T lymphocyte quantitation and DTH skin testing based upon the Walter Reed HIV Staging System[2].

Skin testing

Intradermal skin tests were applied to the forearms, at least 3-4 cm apart, of each patient using the standard Mantoux method. The antigens and concentration were as follows: *Candida albicans* (Hollister-Steir), 1:100 and 1:10 dilutions; tetanus toxoid (Connaught), 1:100 and 1:10 dilutions; mumps (Connaught), 40 colony-forming units per milliliter; *Trichophyton mentagrophytes* (Hollister-Steir), 1:30 dilution; purified protein derivative of tuberculin (Connaught), 5 TU. Test results were read at 48 hours, as mm of induration along perpendicular axes. (Erythema was ignored for the purpose of this measurement.) An average diameter of 5 mm or greater was defined as positive.

Laboratory testing.

CD4 cell counts were determined in peripheral blood specimens by 2-color flow cytometry using standard methodology[6]. Specimens were drawn consistently between 8 and 11 a.m. in order to avoid effects of diurnal variation in CD4+ T-cell circulation[7]. All specimens were analyzed within 4 hours of collection. The HIV-1 subtype was determined by differential polymerase chain reactions (PCR) of proviral DNA from peripheral blood mononuclear cells as previously described[8].

Statistical analysis

When data from more than one time point were available for a subject, only that from the first time point was used. Skin test results with four non-PPD antigens were combined to provide an assessment of DTH responsiveness: complete anergy, partial anergy or normal responsiveness, if

0, 1 or ≥ 2 of 4 tests were positive, respectively. Original data was entered and maintained in an Oracle database system and transferred for analysis to SPSS version 8.0. Comparisons of skin test response frequency within CD4 cell strata were made using the Chi-square test. The differences between CD4 values, grouped by specific skin test and gender, utilized the T-test for analysis.

RESULTS

From 1993 through 1996, 221 adult Thai subjects had DTH skin test responses evaluated as part of staging in an HIV natural history protocol. HIV-1 infections of these subjects were 90 per cent subtype E, 10 per cent subtype B, as determined by PCR of the *env* gene. Subjects, who were seen in association with out-patient visits to an infectious disease clinic, were representative of the immunological spectrum: CD4 cell counts of ≤ 200 (n = 73), 201-400 (n = 78), 401-600 (n = 46) and > 600 cells/ml (n = 24). Approximately two thirds of the subjects were male (M:F, 143:78).

The frequency of positivity to each antigen/concentration of subjects within CD4 cell-defined strata is summarized in Table 1. Skin test positivity increased progressively in association with increasing CD4 cell levels for both tetanus toxoid and *Candida* antigens, and for both, positivity was greater with the stronger concentration of antigen (dilution, 1:10 *versus* 1:100). For example, among subjects with CD4 cell counts of 200/ml or less, positivity increased from 11 to 27 per cent for tetanus toxoid and 32 to 49 per cent for *Candida* antigens, for the 1:100 and 1:10 dilutions, respectively. At the higher concentration, 90 per cent of subjects with CD4 counts above 600 cells/ml responded to tetanus toxoid and to *Candida* antigens. Only one third of subjects in this CD4 stratum responded to *Trichophyton* and two thirds to mumps antigen. Based on this assessment, analyses of anergy and stage of disease utilize the more concentrated of the two preparations of tetanus toxoid and *Candida* antigen.

The spectrum of DTH responsiveness was assessed by combining the results of skin tests with the four control antigens (utilizing the results with 1:10 dilutions of tetanus toxoid and *Candida*). The frequencies of complete anergy, partial anergy and normal responsiveness are summarized in Table 2 for each CD4 cell strata with subgrouping by gender. The proportion of subjects with complete anergy fell from 38 per cent in those with 200 CD4 cells/ml or less, to 4-6 per cent in those with 201-600 cells/ml, to 0 per cent in those with more than 600 cells/ml. A similar progression can be seen combining the complete and partial anergy groups as a percentage of the whole strata: complete/partial anergy fell from 64 per cent to 22-27 per cent to 8 per cent as CD4 counts rose from ≤ 200 to >600 cells/ml (Table 2).

The frequency of DTH skin test responsiveness was directly related to CD4 cell counts in these HIV-infected subjects (Fig. 1). The CD4 count (mean ± SD) rose as the number of positive skin tests increased: 0 of 4 reactive, 106 ± 145, 1 of 4, 253 ± 189, 2 of 4, 336 ± 214, 3 of 4, 404 ± 164,

Table 1. Frequency (%) of DTH skin test positivity of HIV-infected Thai adults (n = 221) to a panel of antigens, stratified by CD4 cell count.

	CD4 Cells/ml			
Antigen	≤ 200 (n = 73)	201-400 (n = 78)	401-600 (n = 46)	>600 (n = 24)
Tet 1:100	11	40	54	83
Tet 1:10	27	53	63	88
Candida 1:100	32	65	72	79
Candida 1:10	49	82	91	92
Mumps	23	49	52	63
Trichophyton	16	31	24	33
PPD	19	24	30	38

Notes:

Tet: tetanus toxoid

PPD: purified protein derivative (of tuberculin)

and 4 of 4, 406 ± 241 cells/ml. The Figure also groups those with 2 or more positive skin tests, since such a cut-point has been shown to have prognostic value in studies of disease progression (2,3). But it is noteworthy that within this study group, mean CD4 counts continued to increase with skin test positivity beyond this cut-point (3 of 4 compared with 2 of 4 antigens).

Table 2. Distribution of DTH skin test responsiveness (anergy, partial anergy, normal) of HIV-infected Thai adults (n = 221) grouped by CD4 cell count and sex.

CD4 cells/ml	Sex	N	Percentage		
			Complete Anergy	Partial Anergy	Normal Response
0-200	F	16	50	19	31
	M	57	35	28	37
	Both	73	38	26	36
201-400	F	35	6	29	66
	M	43	7	14	79
	Both	78	6	21	73
401-600	F	16	6	13	81
	M	30	3	20	77
	Both	46	4	17	78
> 600	F	11	0	0	100
	M	13	0	15	85
	Both	24	0	8	92

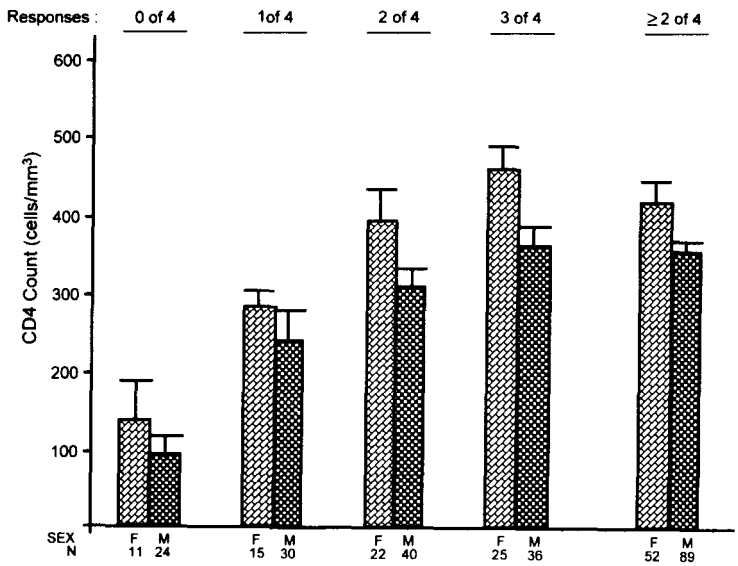

Fig. 1. Bar graph showing mean CD4 count of HIV-infected Thai adults grouped by frequency of response to a 4-antigen skin test panel and by sex. Complete anergy was defined as 0 of 4 responses; partial anergy as 1 of 4 responses; normal response was defined as 2 or more of 4 responses. (Those with 4 of 4 responses are not shown separately because of the small sample size.) Error bars represent SEM; the n for each group is noted below bars.

Table 3. Percentage of participants with complete anergy, partial anergy and normal response detected by DTH skin test panels: comparison of 3- and 4-antigen panels.

		CD4 cells/ml				
	# Antigens	≤ 200 (n = 73)	201-400 (n = 78)	401-600 (n = 46)	> 600 (n = 24)	All (n = 221)
Complete Anergy	4	38%	6%	4%	0%	16%
	3	40%	8%	4%	4%	17%
Partial Anergy	4	26%	21%	17%	8%	20%
	3	29%	31%	20%	8%	25%
Normal Response	4	36%	73%	78%	92%	64%
	3	32%	62%	76%	88%	58%

Definitions: anergy – 0 skin tests positive; partial anergy – 1 skin test positive; normal – 2 or more skin tests positive.

The relationship between functional cellular immunity as measured by DTH skin tests and the level of circulating CD4+ lymphocytes appears to differ with gender. Table 2 summarizes the frequency of complete and partial anergy of subjects grouped by CD4 strata and sex. In those with the lowest CD4 counts (0 - 200 CD4 cells/ml), 50 per cent of 16 women compared with 35 per cent of 57 men were anergic ($p = 0.28$). The CD4 concentration in blood is higher for females at each level of skin test reactivity (Fig. 1). For 0, 1, 2, 3 or ≥ 2 of 4 skin tests positive, mean (SE) CD4 counts for females and males are: 137 (52) and 92 (27) [$p = 0.41$], 279 (28) and 241 (40) [$p = 0.53$], 394 (45) and 303 (33) [$p = 0.11$], 462 (30) and 363 (27) [$p = 0.02$], and 420 (26) and 347 (21) [$p = 0.04$], respectively.

To assess the possibility of further simplifying the skin test panel, anergy was determined by using three versus four antigens. Since reactivity was least frequent to *Trichophyton*, anergy was calculated based on the remaining three antigens compared with the 4-antigen panel described above (Table 3). Of the 221 study subjects, the proportion interpreted as anergic rose from 16 per cent to 17 per cent, and as partially anergic from 20 per cent to 25 per cent. Those reacting to two or more antigens ("normal response") fell from 64 per cent to 58 per cent.

DISCUSSION

The assessment of cell-mediated immune function by cutaneous delayed-type hypersensitivity reactions has been shown to be an independent predictor of survival in HIV-infected adults in the United States[2,3]. The assessment of cellular immune responses to recall antigens correlates with *in vitro* assays of T cell reactivity[4], but is more comprehensive since it incorporates antigen processing and presentation, and effector responses by cells residing in their natural micro-environment. Langerhans cells, the antigen presenting cells of the skin, participate in DTH skin tests; their frequency appears to be independent of the stage of HIV disease[9].

In this study of Thai subjects with a predominance of subtype E HIV infections, a panel of antigens for DTH skin testing was selected and assessed based upon the model within the Walter Reed HIV Staging System[2,3]. The antigens studied included two different strengths of both tetanus toxoid and *Candida*. The higher concentration of each antigen augmented the sensitivity of the skin tests (Table 1), a finding similar to that previously reported by Birx and colleagues[2]. The 88-92 per cent reactivity to these antigens by the group with more than 600 CD4 cells/ml is comparable to the 83-98 per cent reactivity in HIV seronegatives[2]. Reactivity to mumps antigen was present in nearly two thirds of subjects with 600 or more CD4 cells, a frequency which is lower than that reported in U.S. patients[3], possibly reflecting less complete utilization of mumps vaccine. One third of the subjects in this CD4 stratum were reactive to *Trichophyton*, similar to that in U.S. patients[3].

Based upon these findings, a panel for assessment of DTH anergy was defined as the four antigens, tetanus toxoid, *Candida*, mumps and *Trichophyton*, utilizing the first two in the stronger of the two concentrations evaluated (1:10 dilution). With this skin testing panel, the frequency of anergy and partial anergy among HIV-infected Thai subjects is described. Thirty-eight per cent of Thai subjects with 0-200 CD4 cells were found to be anergic. This is the same finding reported by Birx and colleagues[2] among 259 U.S. adult patients in the same cell strata. In contrast, Blatt and colleagues[3], using a variation of this antigen panel, reported that 59 per cent of 64 patients were anergic. Similar to results in the U.S. studies[2,3], the frequency of anergy among Thai subjects decreased with rising CD4 cell count. Among the 24 with 600 or more CD4 cells/ml, no anergic subjects were detected, though 8 per cent were partially anergic (responsive to only one of the four antigens).

Our analysis suggests that a 3-antigen, skin test panel would be very useful in the Thai population. Decreasing the number of antigens leads to an over-diagnosis of anergy. The removal of *Trichophyton* from the panel here changes 9 per cent of the 141 subjects with normal responses to an interpretation of complete or partial anergy. In certain settings, this may be an acceptable cost in exchange for the increased ease of testing. In contrast to laboratory assays of immune function, skin testing is much more practical and affordable[10] and thus potentially of greater utility within the developing regions of the world where HIV is most prevalent[11].

There is a special interest in the DTH results among women, since they make up an estimated 41 per cent of the number of people living with HIV/AIDS in the world[11], but less than 10 per cent of the two military populations previously studied[2,3]. Mean CD4 cell counts increased in both genders in direct association with the number of positive skin test responses (Fig. 1). At each level of cutaneous DTH responsiveness, women had higher levels of CD4 cells than men. This difference was statistically significant in those with normal DTH skin test responses ($\geq$ 2 of 4 antigens). A careful study of normal CD4 values in healthy Bangkok adults has shown that women have significantly higher values than men[6]. Just as the normal range of CD4 counts must be determined in a population, so a skin test panel for detecting anergy must be validated, if either are to be used for as the basis for therapeutic decisions.

Assessment of DTH skin test responsiveness in HIV-infected subjects has prognostic value, and may be useful in monitoring immune reconstitution associated with drug therapy. We conclude that DTH skin testing can provide a practical tool to assist in the staging of HIV patients in resource-poor countries. In addition, the determination of cutaneous DTH status will also assist in the diagnosis of tuberculosis, the ancient disease now again rampant due to the HIV pandemic.

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การตอบสนองแบบ DTH ในผู้ติดเชื้อเอ็ชไอวี ในประเทศไทย

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การศึกษาอัตราการตอบสนองของร่างกายทางผิวหนังต่อน้ำยากระตุ้น (antigen) ชนิดพึ่ง T cell lymphocyte (delayed type of hypersensitivity) ในคนไทยที่ติดเชื้อเอ็ชไอวี (HIV) ซึ่งเป็นวิธีหนึ่งที่ใช้แบ่งระยะการดำเนินโรคในประเทศสหรัฐอเมริกา น้ำยากระตุ้นมี 4 ชนิดที่ใช้ในการศึกษาครั้งนี้ คือ tetanus toxid (1:10), *candida* (1:10), mumps และ *Trichophyton* จากการศึกษาในผู้ป่วยผู้ใหญ่ (อายุมากกว่าหรือเท่ากับ 18 ปี) จำนวนทั้งสิ้น 221 คน พบว่าร้อยละ 38 ของผู้ป่วย 75 รายที่มี CD4 อยู่ระหว่าง 0–200 ตัวต่อลบ.มม. ไม่มีการตอบสนองต่อน้ำยากระตุ้นทั้ง 4 ชนิด เมื่อทดสอบทางผิวหนัง ตรงกันข้ามกับผู้ป่วยที่มี CD4 อยู่ระหว่าง 201–400 ตัวต่อลบ.มม. มีเพียงร้อยละ 6 เท่านั้นจากจำนวนผู้ป่วย 78 ราย มีคนไข้บางส่วนที่ตอบสนองเพียงหนึ่งในสี่ของสารกระตุ้น (partial anergy) คือร้อยละ 26 ในผู้ป่วยที่มี CD4 อยู่ระหว่าง 0–200 และจะเพิ่มการตอบสนองดีขึ้นเมื่อ CD4 สูงขึ้น นอกจากนี้ยังได้แสดงให้เห็นว่า การใช้น้ำยากระตุ้นเพื่อทดสอบปฏิกิริยาทางผิวหนังเพียง 3 ชนิดก็เพียงพอที่จะให้ข้อมูลเกี่ยวกับภูมิคุ้มกันชนิดพึ่งเซลล์ (cell mediated immunity) ได้ใกล้เคียงกับวิธีมาตรฐานที่ต้องใช้น้ำยา 4 ชนิดดังกล่าวเบื้องต้น โดยสรุปการทดสอบปฏิกิริยาทางผิวหนัง (delayed type hypersensitivity) สามารถประเมินภูมิคุ้มกันชนิดพึ่งเซลล์ในผู้ป่วยผู้ใหญ่ที่ติดเชื้อเอชไอวี และยังสัมพันธ์กับการดำเนินโรค

คำสำคัญ : เอ็ชไอวี–1, การทดสอบปฏิกิริยาทางผิวหนัง, การตอบสนองแบบ DTH

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