Delayed Hypersensitivity Skin Testing in the Thai Adult Population

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Objective: *The present study was carried out in healthy Thai subjects to determine the types and concentrations of standard antigens used in delayed-type hypersensitivity (DTH) skin testing as an evaluation of cell-mediated immunity.*
Material and Method: *One hundred subjects were tested with three antigens including tuberculin (purified protein derivative), tetanus toxoid, 1:10 and 1:100 dilutions, and* Candida albicans*, 1:10 and 1:100 dilutions.*
Results: *We found that 92% of the subjects responded to tuberculin and/or tetanus toxoid at a 1:10 dilution, 77% responded to tetanus toxoid at a 1:10 dilution, and 35% responded to a 1:100 dilution. There was only one subject who responded to* Candida albicans *at a 1:10 dilution, and none to a 1:100 dilution. The size of tuberculin reactions varied from 5 mm to over 20 mm without any evidence of active tuberculosis.*
Conclusion: *Tuberculin and tetanus toxoid at a 1:10 dilution are probably sufficient to be used in DTH skin testing to evaluate T-cell function.*

Keywords*: Delayed-type hypersensitivity, Skin testing, T-cell function, Cell-mediated immunity, Tuberculin, Tetanus toxoid*

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Cell-mediated immunity (CMI) is the major part of adaptive immunity working together with humoral immunity. T lymphocytes, particularly the $CD4^+$ cell, have a major role in CMI by recognizing the class II major histocompatibility complex (MHC class II) on antigen-presenting cells. T cells undergo several mitotic divisions in a process called transformation, and some of them then become memory T cells. After antigen re-exposure, previously sensitized memory T cells are presented with antigens in association with MHC class II by macrophages. Cytokines released by these T cells act on other cells including other lymphocytes, macrophages, and polymorphonuclear cells, resulting in inflammation. This phenomenon is called delayed-type hypersensitivity (DTH), which has typical histological figures of dermal vasodilatation and mixed cellular infiltration[1]. Defects in T cells may cause infections by certain organisms including viruses, fungi and protozoa.

Initial evaluation of T cells comprises the assessment of their numbers and functions. The amount of T cells can be measured by flow cytometry. The functions of T cells can be examined in vitro by lymphoproliferative response to mitogens. A more practical in vivo measurement is the DTH response test[2]. DTH response not only reflects $CD4^+$ T cell function but also the type of previously exposed antigens. The DTH skin test depends on several factors, including types and standards of antigens and the skill of testers[3].

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Approved standard antigens for DTH testing include Candida allergen extract, tetanus toxoid and tuberculin (purified protein derivative, PPD). These extracts are antigens to which most people have been previously exposed. Thus, a positive DTH response ensures grossly intact T-cell function[2].

DTH response is currently a standard and cost-effective test for evaluating T-cell function. Previous studies showed variations in the degree of DTH responses in different diseases. Transient depression of CMI occurs in viral infections including measles, infectious mononucleosis and influenza[4,5]. Prolonged depression of CMI occurs in autoimmune diseases[6], malignancies[7,8], disseminated infections[9] and AIDS[10]. In HIV-infected patients, a DTH skin test is directly associated with the number of $CD4^+$ T cells[11]. Nevertheless, a DTH skin test can be used as an independent predictor of progression and survival in patients with HIV infection[12,13]. In another study of the Western population, over 90% of subjects yielded positive in a DTH skin test to at least one standard allergen[14]. In Thailand, little is known about this useful test. The majority of the Thai population are vaccinated with BCG, which is not routinely performed in the United States. This may result in different DTH responses to tuberculin. Our study aims to identify the appropriate standard antigens and their concentrations used in DTH skin testing as an evaluation of T-cell function in the Thai population.

Material and Method

Study population

This study recruited 100 individuals, working in Siriraj Hospital, Bangkok, who gave written informed consents. The study protocol was approved by the Institutional Review Board of Siriraj Hospital. All subjects were in good health and free of known diseases. Pregnant or lactating women were excluded. It was decided to place the volunteers into two age-group categories: 15-34 and 35-60 years of age. Complete blood counts and $CD4^+$ T-cells were measured by using a flow cytometer (FACS Calibur, Becton Dickinson Biosciences, CA, USA) in the Division of Instruments for Research, Office for Research and Development, Faculty of Medicine, Siriraj Hospital, Mahidol University. The level of $CD4^+$ T-cells was used as a reference for the status of cell mediated immunity.

Skin testing procedure

Intradermal skin tests were applied to the forearm of each individual using the standard Mantoux method. The antigens and concentrations used were as follows: tuberculin purified protein derivative (PPD) 10 IU/0.1 ml (Thai Red Cross, Thailand), tetanus toxoid 10 lf/0.5ml (Biogenetech, Italy) at 1:10 and 1:100 dilutions, and Candida albicans allergen extract 20,000 PNU/ml, 1:10 w/v, (ALK Abello, USA) at 1:10 and 1:100 dilutions. Each antigen was injected into the left volar surface of the forearm, 5 cm apart, in a volume of 0.1 ml. Skin indurations were measured at 48 hours by an investigator. A diameter of 5 mm or greater measured by the same investigator was defined as positive.

Statistical analysis

A chi square for 2 x 2 table was used to compare the frequency of positive reactions for each of the tested antigens in the different age groups and genders. A Student's t test was used to compare the mean diameters of positive reactions between different antigens, genders and age groups. A ninety-five percent confidence interval was calculated as an estimated range of values for the overall incidence of positive reactions among different antigens and the incidence of tuberculin positive reactions.

Results

Prevalence of positive DTH reactions related to age and gender

The characteristics of 100 participants recruited into this study are shown in Table 1. The preva-

Table 1. Characteristics of the participants (n = 100)

Characteristics	
Gender: male/female	37/63
Age (years), mean $\pm$ SD	35.9 $\pm$ 12.2
White blood cell count ($\times 10^3$ cells/ml): mean $\pm$ SD	7.5 $\pm$ 1.7
$CD4^+$ T lymphocyte (cells/ml), median (min.-max.)	878 (334-1840)

SD = standard deviation

lence (percentage) of positive DTH reactions to individual antigens is shown in Tables 2 and 3. The prevalence varied from 0% for Candida albicans at a 1:100 dilution to 81% for tuberculin extract. Only one 50 year-old male subject reacted to Candida albicans at a 1:10 dilution. Seventy-seven subjects responded to tetanus toxoid at a 1:10 dilution and 35 subjects responded to tetanus toxoid at a 1:100 dilution. The prevalence of positive DTH reactions (5 mm or greater in size) to any antigens in the panel was 92% which was from a positive reaction to tuberculin and/or tetanus toxoid at a 1:10 dilution (Table 4). If a diameter of 3 mm was used as a cut-off point for a positive response, the prevalence of DTH reactions to tuberculin and/or tetanus toxoid at a 1:10 dilution was 99% (data not shown). Our older subjects had more DTH reactions to tuberculin and tetanus toxoid at a 1:100 dilution (Table 2). Males reacted more than females to tetanus toxoid at a 1:10 dilution (Table 3).

Difference in the mean diameter of DTH reactions related to age and gender

Overall, the mean diameters of DTH reactions to different antigens were not statistically different. This finding together with the findings in Table 2 suggested that even though more subjects from the older age group showed responses to tuberculin and tetanus toxoid at a 1:100 dilution, the sizes of reactions were similar between these two age groups.

Prevalence of positive DTH reactions to tuberculin with different diameter criteria

In 100 healthy subjects with no known history of tuberculosis, 81% of subjects were considered as having positive tuberculin tests. Using the induration of 10, 15, and 20 mm or more in diameter as criteria for positive response, 45%, 17%, and 3% of subjects respectively were considered as having positive tests (Table 5). One of the three subjects who had an indura-

Table 2. Positive DTH responses to antigens in different age groups

Antigens & Sizes*	Age (yr)		p-value
	15-34 (n = 53)	35-60 (n = 47)	
Tuberculin	35 (66%)	46 (98%)	0.0001
Diameter: mean ± SD	10.2 ± 4	10.7 ± 3.89	0.61
Tetanus (1:100)	12 (23%)	23 (49%)	0.01
Diameter: mean ± SD	6.2 ± 2.02	5.7 ± 1.08	0.34
Tetanus (1:10)	38 (72%)	39 (83%)	0.3
Diameter: mean ± SD	7.7 ± 2.98	8.2 ± 3.54	0.51
Candida (1:100)	0 (0%)	0 (0%)	-
Candida (1:10)	0 (0%)	1 (2%)	0.9

* Unit of diameter: millimeter (mm)

Table 3. Positive DTH response to antigens according to gender (n = 100)

Antigens & Sizes*	Male (n = 37)	Female (n = 63)	p-value
Tuberculin	32 (86%)	49 (78%)	0.4
Diameter: mean ± SD	10.9 ± 4.1	10.1 ± 4.1	0.2
Tetanus (1:100)	16 (43%)	19 (30%)	0.3
Diameter: mean ± SD	5.8 ± 1.3	6.1 ± 1.7	0.34
Tetanus (1:10)	33 (89%)	44 (70%)	0.04
Diameter: mean ± SD	7.9 ± 3.8	8 ± 3.7	0.45
Candida (1:100)	0 (0%)	0 (0%)	-
Candida (1:10)	1 (3%)	0 (0%)	0.8

* Unit of diameter: millimeter (mm)

Table 4. Percentage of positive DTH responses

Antigens	Positive responses (n = 100)	95%CI[#]
Tuberculin + Tetanus (1:10)	92%	85%-95.9%
Tuberculin	81%	72.2%-87.5%
Tetanus (1:10)	77%	67.8%-84.2%
Tetanus (1:100)	35%	26.4%-44.7%

[#] CI = confidence interval

Table 5. Percentage of positive tuberculin DTH according to different diameter criteria

Diameter*	Positive Responses	95%CI[#]
≥ 5	81%	82.1%-94.6%
≥ 10	45%	35.6%-54.8%
≥ 15	17%	10.9%-25.5%
≥ 20	3%	1.0%-8.5%

* Unit of diameter: millimeter (mm)
[#] CI = confidence interval

tion of 20 mm had a remote history of exposure to tuberculosis by a relative several years before, but she reported no history of any major chronic illnesses. Chest radiography was obtained in all 17 subjects who had tuberculin DTH indurations of 15 mm or more, and all were reported to be negative i.e. neither infiltrates nor scars.

Discussion

In this study, 92% of 100 healthy Thai subjects responded to tuberculin and/or tetanus toxoid at a 1:10 dilution. This indicates that these two antigens are probably sufficient to be used in DTH skin testing to evaluate T-cell function. Seventy-seven percent of subjects responded to tetanus toxoid at a 1:10 dilution while 35% responded to a 1:100 dilution suggesting that a 1:10 dilution may be more appropriate than a 1:100 dilution. For Candida extract, there was only one subject who responded at a 1:10 dilution and none at a 1:100 dilution. The magnitude of tuberculin reaction did not correlate with active tuberculosis. Of note, $CD4^+$ cell counts were normal for all participants, except for a 29 year-old man whose $CD4^+$ cell count was 334 cell/mm^3 (normal 470-1404 cell/mm^3) without any evidence of immunodeficiency disease. However, his DTH response was comparable to others.

DTH skin testing is a standard and cost-effective *in vivo* method for initial screening of CMI[2]. Negative DTH skin testing, or anergy, had been found to be associated with all-cause mortality in the elderly[15]. In HIV-infected patients, anergy is a significant risk factor for developing tuberculosis[16]. DTH skin testing is also a helpful predictor of progression to clinical end-stage HIV infection[11]. Previous studies showed a positive correlation between DTH skin testing and other in-vitro tests including macrophage and leukocyte migration inhibition assays[17] and lymphocyte transformation assays in response to mitogens[14]. However, these in-vitro tests are rather expensive and technically difficult to perform.

Although DTH skin testing is easy and practical, accuracy depends upon the types and concentration of antigens as well as the technical skill of testers. Standard antigens consist of tuberculin, tetanus toxoid, Candida albicans allergen extract, mumps, and trichophyton[14,18]. These 5 antigens are generally used simultaneously in each patient since no single antigen has been shown to give a positive DTH response in all patients[3,18]. A previous study demonstrated that over 90% of patients responded to at least one antigen[14]. The present study in healthy Thai subjects found that tuberculin yielded a positive response in 81% of subjects while another study in the United States showed a positive response in only 30% of subjects[14]. The different results are probably explained by the high exposure to tuberculosis and use of BCG vaccination

in Thailand. Seventy-seven percent of our subjects responded to tetanus toxoid (1:10 dilution) which was comparable to the previous study[19]. Interestingly, our study shows only 1 subject who responded to a 1:10 dilution of Candida albicans compared to the response of 90% of adult subjects using 1:100 dilution and 64% of paediatric subjects using undiluted extract in other documented studies[14,20]. However, previous studies used Candida albicans extract from different pharmaceutical companies. In this regard, two additional DTH skin tests with the same vial of candida albicans at full strength were performed, both of the subjects had positive responses. It is possible that if we had used a full strength candida albicans, we would have had more positive DTH responses. The present study also demonstrated that the older group had more positive responses to all tested antigens (except Candida albicans) than the younger group with a statistical significance for tuberculin and tetanus toxoid (1:100 dilution). The result suggested that the older age group might have had more previous exposure than the other group. With regard to the gender influence on DTH response, the present study showed that males were more likely than females to respond to tuberculin and tetanus toxoid (1:10 and 1:100 dilution). Although the DTH responses were affected by gender[21], the exact reason remains to be determined.

Besides the advantage of evaluating CMI, tuberculin has been used to detect previous exposure and surveillance of tuberculosis. Our study showed a response to tuberculin of up to 81% of subjects with the diameter ranging from 5 mm to over 20 mm. We had chest radiography performed in 17 subjects whose tuberculin responses were 15 mm in diameter or greater. All had neither pulmonary infiltration nor scar suggesting that tuberculin skin testing may not be a helpful tool to screen or diagnose tuberculosis in endemic countries. Our relatively high prevalence of positive tuberculin skin testing in contrast to the Western population is probably caused by a high exposure to tuberculosis and BCG vaccination.

In conclusion, our study suggested that tuberculin and tetanus toxoid at a 1:10 dilution be used as standard antigens for DTH skin testing to evaluate T-cell function.

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การทดสอบการตอบสนองแบบ delayed hypersensitivity ในประชากรไทย

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การตอบสนองทางผิวหนังแบบ delayed-type hypersensitivity (DTH) เป็นการประเมินการทำหน้าที่ของภูมิคุ้มกันด้านเซลล์ การวิจัยในประชากรไทยปกติครั้งนี้เพื่อศึกษาว่าจำนวนและความเข้มข้นของ antigen ที่จะนำมาใช้นั้นควรเป็นอย่างไร อาสาสมัครจำนวน 100 คนได้รับการทดสอบด้วย antigen 3 ชนิดได้แก่ tuberculin, tetanus toxoid (ความเข้มข้น 1:10 และ 1:100) และ Candida albicans (ความเข้มข้น 1:10 และ 1:100) อาสาสมัครร้อยละ 92 ตอบสนองต่อ tuberculin และ /หรือ tetanus toxoid (ความเข้มข้น 1:10) ร้อยละ 77 ตอบสนองต่อ tetanus ความเข้มข้น 1:10 ขณะที่ร้อยละ 35 ตอบสนองต่อ tetanus ความเข้มข้น 1:100 มีอาสาสมัครเพียง 1 ราย ที่ตอบสนองต่อ Candida albicans ความเข้มข้น 1:10 และไม่พบผู้ที่ตอบสนองต่อ Candida albicans ความเข้มข้น 1:100 ขนาดของการตอบสนองทางผิวหนังต่อ tuberculin มีตั้งแต่ 5 มิลลิเมตร จนถึงมากกว่า 20 มิลลิเมตร โดยที่ภาพรังสีทรวงอกอยู่ในเกณฑ์ปกติ โดยสรุปการใช้น้ำยา tuberculin และ tetanus ความเข้มข้น 1:10 น่าจะเพียงพอสำหรับใช้ทดสอบการตอบสนองทางผิวหนังแบบ DTH เพื่อใช้ในการประเมินภูมิคุ้มกันด้านเซลล์ในประชากรไทย