

Monitoring cellular immune function in HIV infection by the delayed hypersensitivity skin test: Alternative to the CD4⁺ T-cell count?

The delayed-type hypersensitivity (DTH) skin test is a reliable screening test for cell-mediated immunity; it can be used in outpatient settings, and it is relatively inexpensive.^{1,2} Studies have shown that a positive DTH test response correlates with cytokine production and lymphocyte proliferation to the same recall antigens used in the DTH test.³ Interpretation of DTH results depends on correct intradermal placement of the proper dose of recall antigens, accurate reading of the DTH skin site, and age considerations of the patient.^{4,5} Recall antigens commonly used are derived from *Candida albicans*, tetanus toxoid and tuberculin, mumps virus, and purified protein derivative.⁶ A positive reaction is defined as a certain size of induration at the injection site, which is maximal at 48 hours but still present at 72 hours.⁷ The cells responsible for the induration include the CD4⁺ T cell and macrophage, and inflammatory mediators include TNF- α , IFN- γ , IL-1, IL-6, and PGE₂.⁸

The use of the DTH to recall antigens has long served clinical immunologists in determining the degree of patient immunocompetence and infectious diseases specialists in determining the incidence of exposure to *Mycobacterium tuberculosis*.⁶ There is clearly a reciprocal relationship between these 2 goals in the case of patients debilitated by a serious infection, such as HIV infection.⁹ In immunocompetent subjects, for example, the DTH test will demonstrate positive results to recall antigens, such as mumps, tetanus toxoid, and *Candida* antigens, and to tubercular antigens if suitable exposure has occurred. Thus the DTH test can accurately describe both immunocompetency and exposure to *M tuberculosis* (with competent immunity). In immunoincompetent subjects, however, there are frequently reduced or absent DTH responses to both recall and tubercular antigens. Thus because of weakened immunity caused by primary or secondary immunodeficiency, an individual exposed to or actively infected by *M tuberculosis* may test negative to tubercular antigens.⁵ The first clear example of negation of the predictive power of tubercular antigen in the DTH was that recorded by Von Pirquet in 1907 and studied systematically by Helms and Helms,¹⁰ who noticed decreased reactivity in patients with previously positive reactions infected secondarily with measles.

In the current era, HIV infection has provided the opportunity to again examine the usefulness of the DTH

Abbreviations used

DTH: Delayed-type hypersensitivity
HAART: Highly active antiretroviral therapy

test as a measure of immunocompetence and exposure to *M tuberculosis*. Here too the usefulness of the DTH test to provide satisfactory answers is compromised by a serious infection. A quick reliable in vivo test of specific immune responses would obviously be an enormous benefit to clinicians dealing with a disease ravaging the immune system, and for a while the DTH test was thought to afford that benefit.¹¹ However, because of problems with reading the DTH test, the Centers for Disease Control declared that anergy testing is no longer recommended as a routine component of tuberculosis screening in HIV-infected persons in the United States.¹² In all testing, however, the method of testing is extremely important in interpretation of results. The usual indication of a positive DTH reaction is 10 mm of induration recorded at 48 to 72 hours. In HIV-infected subjects, the size of induration was reduced to 5 mm for recall antigens because of HIV-induced weakening of the cellular immune system.¹³

In the article by Klein et al¹⁴ located in this issue, an exemplary study of DTH reactions in a large number of women (721 HIV-seropositive and 358 HIV-seronegative) was performed in which compelling data argue for a reassessment of the use of the DTH test for the determination of immunocompetence and prediction of exposure to *M tuberculosis*. According to their findings, it may be possible to use a cutpoint of 2 mm of DTH induration to accomplish both goals. This new rule would maximize the difference between HIV-seropositive and HIV-seronegative individuals. In addition, the authors demonstrate an excellent correlation between degree of cutaneous anergy and CD4⁺ T-cell strata. Finally, the authors demonstrate a decreased rate of tubercular antigen DTH reactions in anergic HIV-seropositive subjects, emphasizing the importance of performing control antigen DTH to adequately interpret the results. The authors of this study should be commended for the excellent design and execution of a study that proves the utility of using a practical technology to produce important clinical information. An added benefit of this study is the participation of women, which has been missing in most similar trials. It is likely that the results of this trial in women may be applied to men. Indeed, other smaller DTH studies in women¹⁵ and men¹⁶ support the continued use of recall antigen testing with DTH in HIV-infected patients. Perhaps we should

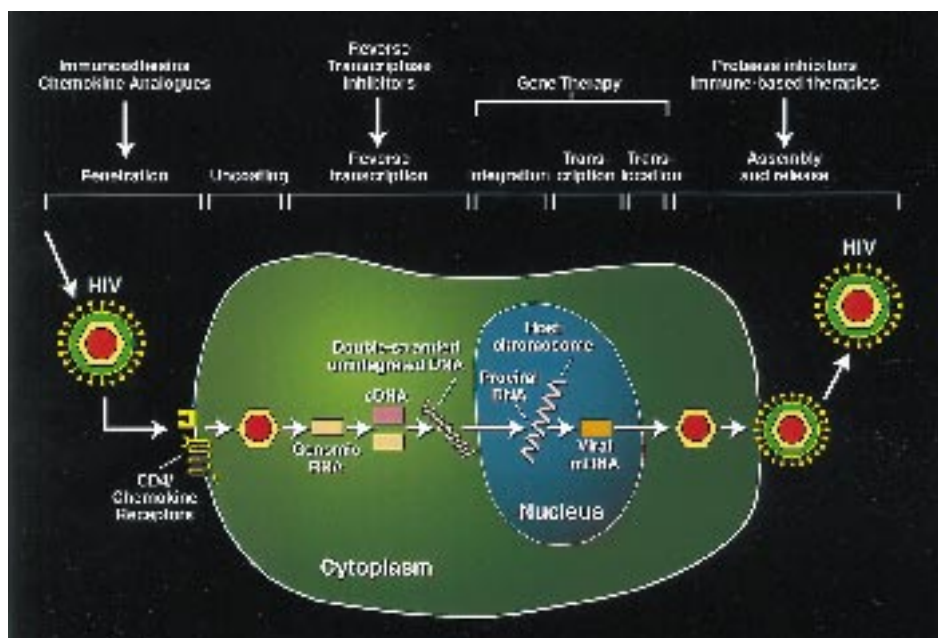


FIG 1. Diagram of the HIV replicative cycle with sites of action of some antiretroviral agents. Modified with permission from Kline MW, Shearer WT. HIV infection and AIDS in children. In: Rich RR, Fleisher TA, Schwartz BD, Shearer WT, Strober W, editors. Clinical immunology: principles and practice. St. Louis: Mosby; 1996.

reconsider routine DTH testing to recall and tubercular antigens in HIV-infected subjects by using the 2-mm cut off.

With the availability of 3-color flow cytometric analysis of CD4⁺ T cells and HIV RNA PCR measurements, one may question the wisdom of resurrecting the DTH as a means of monitoring cellular immunity in HIV-infected patients being treated with highly active antiretroviral therapy (HAART). Cutaneous anergy testing is acknowledged as an independent predictor of HIV disease progression, but it does not replace more sophisticated predictors, such as the CD4⁺ T-cell count and the HIV viral load. However, the fact is that most HIV infection takes place in locations where sophisticated technology is unavailable, including inner-city clinics in the United States and many parts of Africa and Asia. Application of the DTH test in such areas might well serve the need for a simple, but clinically relevant, technology to assess general immune function in patients and to monitor immune reactivity in patients placed on HAART treatment programs where possible. These new HAART regimens consist of nucleoside reverse-transcriptase inhibitors, nonnucleoside reverse-transcriptase inhibitors, and protease inhibitors.^{17,18} The reverse-transcriptase inhibitors prevent the HIV reverse-transcriptase enzyme from copying the HIV RNA genetic code into DNA code (Fig 1). Protease inhibitors prevent the glycoprotein packaging of the HIV virion. It is believed that unless immunocompetence is restored by HAART treatment, there is no hope of eradication of HIV from patients.¹⁹⁻²¹ Of course, it is possible to measure restoration of components of immunity with high-grade technology, such as CD4⁺CD45⁺RA⁺ naive T cells or V-β T-cell repertoire.²²

For clinicians coping with HIV-infected patients who may not have ready access to such technology to measure immune competence or the technology to monitor HIV burden,²³ the DTH assessment of immunity may be essential. The report by Klein et al¹⁴ brings home to us the importance of including practical assessments of immune responses in HIV clinical trials.

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REFERENCES

1. Buckley RH. Immunodeficiency diseases. *J Am Med Assoc* 1992;268: 2797-806.
2. Pacheco SE, Shearer WT. Laboratory aspects of immunology. *Pediatr Clin North Am* 1994;41:623-55.
3. Smith DL, Deshazo RD. Delayed hypersensitivity skin testing. In: Rose NR, Conway De Macario E, Friedman H, et al, editors. *Manual of clinical laboratory immunology*. 4th ed. Washington (DC): American Society for Microbiology; 1992. p. 202-6.
4. Paul ME, Shearer WT. Approach to the evaluation of the immunodeficient patient. In: Rich RR, Fleisher TA, Schwartz BD, Shearer WT, Strober W, editors. *Clinical immunology: principles and practice*. St. Louis: Mosby; 1996. p. 609-20.
5. American Academy of Pediatrics. Tuberculosis. In: Peter G, editor. 1997 Red Book: Report of the Committee on Infectious Diseases. 24th ed. Elk Grove Village (IL): American Academy of Pediatrics; 1997. p. 541-62.
6. Klimas N. Delayed hypersensitivity skin testing. In: Rose NR, de Macario EC, Folds JD, Lane HC, Nakamura RM, editors. *Manual of clinical laboratory immunology*. 5th ed. Washington (DC): American Society for Microbiology; 1997. p. 276-80.
7. Shearer WT, Buckley RH, Engler RJM, Finn AF Jr, Fleisher TA, Freeman TM, et al. Practice parameters for the diagnosis and management of

- immunodeficiency. *Ann Allergy Asthma Immunol* 1996;76:282-94.
8. Sell S. Immunopathology. In: Rich RR, Fleisher TA, Schwartz BD, Shearer WT, Strober W, editors. *Clinical immunology: principles and practice*. St. Louis: Mosby; 1996. p. 449-77.
9. Noroski LM, Shearer WT. Short analytical review: Screening for primary immunodeficiencies in the clinical immunology laboratory. *Clin Immunol Immunopathol* 1998;86:237-55.
10. Helms S, Helms P. Tuberculin sensitivity during measles. *Acta Tuberc Scand* 1956;35:166-71.
11. Centers for Disease Control. Purified protein derivative (PPD)-tuberculin anergy and HIV infection: guidelines for anergy testing and management of anergic persons at risk of tuberculosis. *MMWR Morb Mortal Wkly Rep* 1991;40(RR-5):27-33.
12. Centers for Disease Control and Prevention. Anergy skin testing and preventive therapy for HIV-infected persons: revised recommendations. *MMWR Morb Mortal Wkly Rep* 1997;46(RR-15):1-10.
13. Hopewell PC. Tuberculosis in persons with human immunodeficiency virus infection. In: Saude MA, Volbertine PA, editors. *The medical management of AIDS*. 5th ed. Philadelphia: WB Saunders Co; 1997. p. 311-325.
14. Klein RS, Flanigan T, Schuman P, Smith D, Vlahov D, for the HER Study Group. Criteria for assessing cutaneous anergy in women with or at risk for HIV infection. *J Allergy Clin Immunol* 1998;103:93-8.
15. Mofenson LM, Rodriguez EM, Hershow R, Fox HE, Landesman S, Tuomala R, et al. *Mycobacterium tuberculosis* infection in pregnant and non-pregnant women infected with HIV in the Women and Infants Transmission Study. *Arch Intern Med* 1995;155:1066-72.
16. Maas JJ, Roos MTL, Keet IPM, Mensen EAM, Krol A, Veenstra J, et al. In vivo delayed-type hypersensitivity skin test anergy in human immunodeficiency virus type 1 infection is associated with T cell nonresponsiveness in vitro. *J Infect Dis* 1998;178:10242.
17. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. *N Engl J Med* 1996;334:1011-7.
18. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. *JAMA* 1997;277:145-53.
19. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* 1997; 277:112-6.
20. Kelleher AD, Carr A, Zaunders J, Cooper DA. Alterations in the immune response of human immunodeficiency virus (HIV)-infected subjects treated with an HIV-specific protease inhibitor, ritonavir. *J Infect Dis* 1996;173:321-9.
21. Lefeuvre A, Chouraqui M, Hittinger G, Poggi C, Delbeke E. Lymph node expansion of CD4+ lymphocytes during antiretroviral therapy. *J Infect Dis* 1997;176:1378-82.
22. Lane HC. HIV pathogenesis. In: Volberding PA, editor. *Improving the management of HIV disease*. Vol 5. San Francisco: International AIDS Society - USA; 1997. p. 4-7.
23. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167-70.