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Article/Artigo

Delayed-type hypersensitivity skin test responses to PPD and other antigens among BCG-vaccinated HIV-1-infected and healthy children and adolescents

Resposta de testes de hipersensibilidade tardia utilizando PPD e outros antígenos em crianças e adolescentes saudáveis e infectados pelo HIV-1 e vacinados com BCG

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ABSTRACT

Introduction: Among HIV-1-infected patients, CD4+ T cell counts are well-established markers of cell-mediated immunity. Delayed-type hypersensitivity (DTH) skin tests can be used to evaluate *in vivo* cell-mediated immunity to common antigens. **Methods**: DTH responses to tuberculin purified protein derivative (PPD), sporotrichin, trichophytin, candidin and streptokinase/streptodornase antigens were assessed. Thirty-six HIV-1-infected children/adolescents and 56 age- and sex-matched HIV-1/HIV-2-seronegative participants were tested. All participants had a BCG scar. Fisher's exact test was used to evaluate significant differences between groups ($p<0.05$). **Results**: The main characteristics of the HIV-1 patients were as follows: median age 8.1 years; 20/36 were males; 35 were vertical transmission cases; 34 were AIDS cases under antiretroviral therapy; median viral load = 3.04 log10 copies/ml; median CD4+ T cell count = 701 cells/μl. A total of 25% (9/36) and 87.5% (49/56) of HIV-1-infected and healthy participants, respectively, displayed DTH reactivity to at least one antigen ($p<0.001$). Among HIV-1-infected participants, reactivity to candidin predominated (8/36, 22.2%), while PPD positivity prevailed among healthy participants (40/56, 71.4%). PPD reactivity in the HIV-1-positive group was 8.3% ($p<0.01$). The median PPD induration was 2.5mm (range: 2-5mm) in the HIV-1 group and 6.0 mm among healthy participants (range: 3-15mm). There was no correlation between PPD positivity and age**.** No correlation between CD4+ T cell counts and DTH reactivity was observed among HIV-1-infected patients. **Conclusions**: DTH skin test responses, including PPD reactivity, were significantly lower among HIV-1-infected participants compared to healthy controls, which likely reflects advanced disease and T cell depletion.

Keywords: Delayed-type hypersensitivity. Children. HIV infection. Tuberculin test. Purified protein derivative.

RESUMO

Introdução: A contagem de células CD4+ representa marcador da resposta imune celular em pacientes infectados pelo HIV-1. Testes cutâneos de hipersensibilidade tardia (DTH) podem ser empregados para avaliar *in vivo* respostas celulares a antígenos comuns. **Métodos**: DTH para derivado proteico purificado de tuberculina (PPD), esporotriquina, tricofitina, candidina e estreptoquinase/estreptodornase foram realizados. Foram testados crianças/adolescentes infectados pelo HIV-1 (n=36) e indivíduos saudáveis (n=56), soronegativos para HIV-1/HIV-2 pareados por sexo-idade, todos com cicatriz vacinal por BCG. Teste exato de Fisher foi aplicado ($p<0,05$). **Resultados:** Entre as **c**rianças/adolescentes infectados pelo HIV-1, mediana de idade=8,1 anos; 20/36 eram do sexo masculino; 35 casos de transmissão vertical; 34 casos de AIDS sob terapia antirretroviral; mediana de carga viral = $3.04_{lc}10$ cópias/ml; mediana de contagem de células CD4+ = 701 células/μl. Entre os infectados e saudáveis a reatividade DTH a pelo menos um dos antígenos foi, respectivamente, 25% (9/36) e 87,5% (49/56) ($p<0,001$). Reatividade à candidina predominou nos infectados (8/36, 22%) e ao PPD nos indivíduos saudáveis (40/56, 71,4%). A reatividade ao PPD entre infectados foi de 8,3% ($p<0,01$). A mediana da indução ao PPD foi 2,5mm (variação: 2-5mm) entre infectados e 6,0mm (variação: 3-15mm) entre os saudáveis. Não observamos correlação entre positividade ao PPD e idade. No grupo de infectados, não observamos correlação entre contagens de células CD4+ e reatividade ao DTH. **Conclusões:** Respostas DTH significativamente diminuídas, incluindo a reatividade ao PPD foram observadas em crianças/adolescentes infectados pelo HIV-1 comparadas com controles saudáveis, provavelmente refletindo doença avançada e supressão da imunidade mediada por células T.

Palavras-chaves: Testes de hipersensibilidade tardia. Crianças. Infecção pelo HIV. Teste de tuberculina. Derivado proteico de tuberculina.

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INTRODUCTION

Impaired cell-mediated immune responses are the hallmark of progression of human immunodeficiency virus type 1 (HIV-1) infection to acquired immune deficiency syndrome (AIDS)[1]. Peripheral CD4+ T cell counts are considered the gold standard measure for assessing the immune status of HIV-1-infected patients[2]. Delayed-type hypersensitivity (DTH) skin tests represent another tool to evaluate the specificity and functional status of memory cell-mediated immunity among HIV-1-infected patients *in vivo*[3].

The tuberculin skin test, which uses a purified protein derivative (PPD) of the tuberculosis bacilli, is one of the oldest and most widely used DTH skin tests[4]. PPD has been employed to screen for tuberculosis among high-risk populations, such as HIV-infected patients[5]. When interpreting PPD reactivity, neonatal vaccination for tuberculosis with Bacille Calmette-Guérin (BCG) must be considered. BCG vaccination is routinely performed in endemic countries, such as Brazil, and can enhance the tuberculin skin test response[6]. Nevertheless, tuberculin skin test positivity has mainly been associated with exposure to environmental mycobacteria[7].

The goal of this study was to evaluate DTH skin test responses to several antigens among BCG-vaccinated HIV-1-infected and healthy pediatric/adolescent populations, including PPD, sporotrichin, trichophytin, candidin and streptokinase/streptodornase.

METHODS

Thirty-six children and adolescents infected with HIV-1 (3-13 years), both symptomatic and asymptomatic, were randomly recruited among pediatric patients at the main regional reference center for HIV diagnosis and patient care: Dr. Anuar Auad Hospital (HAA/HDT/SUS), City of *Goiânia* - State of *Goiás*, Central Brazil. Clinical and laboratory data regarding CD4+ T cell counts (FACsCount,

Becton Dickinson, San Jose, CA) and viral loads (polymerase chain reaction (PCR) - Amplicor HIV-1 monitor test, version 1.5, Roche Diagnostic Systems, Branchburg N.J.; Nucleic Acid Sequence-Based Amplification (NASBA) - Organon, Holland - and Branched-DNA (b-DNA) - Chiron Diagnostics Emeryville, CA, USA) closest to the moment of the skin testing (1-3 months before or after the test) were retrieved from the medical files at the reference center (HAA/HDT/SUS). To avoid bias, a single member of the study team (Costa NMX) retrieved clinical and laboratory information using standardized forms. Highly active antiretroviral therapy (HAART) was defined as the association of one protease inhibitor and two non-nucleoside reverse-transcriptase inhibitors or two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor. CDC criteria were used for classification into asymptomatic or AIDS cases and for defining the immunological status of HIV-1-infected participants. In brief, immunosuppression was defined according to age range as $CD4^+$ T cells counts < 1,000/μl for patients up to 5 years old and $CD4^+$ T cells counts < 500/μl for patients older than 6 years.

The control group comprised fifty-six age- and sex-matched HIV-1/HIV-2 seronegative volunteers (anti-HIV-1/2 ELISA, Weiner Lab, Rosario, Argentina) who were recruited among scholars in the same setting. All participants (i.e., HIV-1-infected and healthy patients) had BCG scars and were recruited among a population with very high BCG vaccine coverage (close to 100%).

For both HIV-1-infected and healthy participants, DTH skin tests were performed in the left forearm by the inoculation of 0.1ml of each antigenic extract, with a distance of 3cm between each. The following antigenic extracts were used: PPD (2 UT/0.1ml), sporotrichin (100μg/ml), trichophytin (100μg/ml), candidin (100μg/ml) and streptokinase/streptodornase (100g/ml) (*Laboratorio de Extratos Alergenicos* - Rio de Janeiro, Brazil). As the main study population consisted of patients with advanced HIV-1 disease/AIDS and impaired cellular immunity, we adopted the lower cut off of 4mm for DTH reactivity. An individual was considered a reactor when an induration diameter ≥ 4mm to at least one of the five antigens tested was observed 48h after the antigen inoculation. Anergy was defined as the absence of reactivity for all tested antigens. All DTH tests and readings were performed by one specialist in clinical immunology (Moriya NXC).

Frequency distributions and medians were calculated for the main variables of the enrolled HIV-1-infected patients and healthy controls. Fisher's exact test was used to compare groups, and p values < 0.05 were considered statistically significant. SPSS statistical package 14.0 was used for all data analyses (SPSS, Chicago, Illinois, USA). This study was approved by the ethical committee of the reference center (HAA/HDT/SUS), and for each participant informed consent was signed by a parent or a legal guardian.

RESULTS

The study group comprised 36 HIV-1-infected children and adolescents (median age: 8.1 years; range: 3-13 years); 20/36 were male. Perinatal transmission predominated (97.2%), except for one 13-year-old hemophiliac male patient infected by an HIV-1-contaminated blood transfusion. Most participants were AIDS cases (34/36), and 94.1% were treated with HAART. At the time of this study, all AIDS patients were symptom free. Two patients had a previous diagnosis of tuberculosis and had already concluded specific treatment by the time they were recruited for this study. Reviews of the medical files during the four years after this study indicated that two patients had discontinued follow-up at the reference center and that no new cases of tuberculosis were reported. Viral loads were detectable in 26/36 patients, ranging from 2.27 log10 copies/ml to 4.92 log10 copies/ml (median: 3.04 $\log_{10}$ copies/ml), and 20/36 patients had $CD4^+$ T cell counts within normal ranges. The HIV-seronegative control group consisted of 56 healthy volunteers (median age: 8.2 years; range: 3-13 years; 31 males).

DTH skin test reactivity to the five antigens was also examined. Of the HIV-1-infected participants, 25% (9/36) were considered reactors (induration ≥4mm), responding to at least one antigen, while 87.5% (49/56) of the HIV-1-negative controls were reactors ($p < 0.001$). Therefore, 75% (27/36) of the HIV-1-infected patients were anergic to the tested antigens. Among the HIV-1-infected participants, reactivity to candidin predominated (8/36, 22.2%). Conversely, PPD positivity prevailed (40/56, 71.4%) for healthy participants. PPD reactivity was 8.3% among HIV-1-infected patients and 71.4% among HIV-1-negative participants ($p < 0.01$) **(Table 1)**. The median induration sizes of the PPD reaction in the HIV-1-infected and healthy groups were 2.5mm (range: 2-5mm) and 6.0mm (range: 3-15mm), respectively. PPD reactivity was not age dependent for HIV-1-infected or healthy participants **(Figure 1)**.

Among HIV-1-infected children, there was no correlation between $CD4^+$ T cell counts and DTH reactivity. Normal $CD4^+$ T cell counts were observed in 66.6% of the DTH reactors (6/9) and 51.8% of the non-reactors (14/27) ($p = 0.442$). Seven out of nine reactors in the HIV-1-infected group had a detectable viral load (range: 3.29 log10 copies/ml - 4.36 log10 copies/ml).

TABLE 1 - Positive delayed-type hypersensitivity reactions to tested antigens among HIV-1-infected and healthy HIV-1-negative participants.

	DTH positivity HIV-1^+/AIDS (n=36)		HIV-1 negative (n=56)	
Antigens	n	%	n	%
Sporotrichin	-	0.0	2	3.5
Trichophytin	1	2.7	4	7.1
Candidin	8	22.2	9	16.0
Streptokinase/streptodornase	1	2.7	21	37.5
PPD	3	8.3	40	71.4

DTH: delayed-type hypersensitivity, **PPD:** purified protein derivative, **HIV:** human immunodeficiency virus, **AIDS:** acquired immune deficiency syndrome.

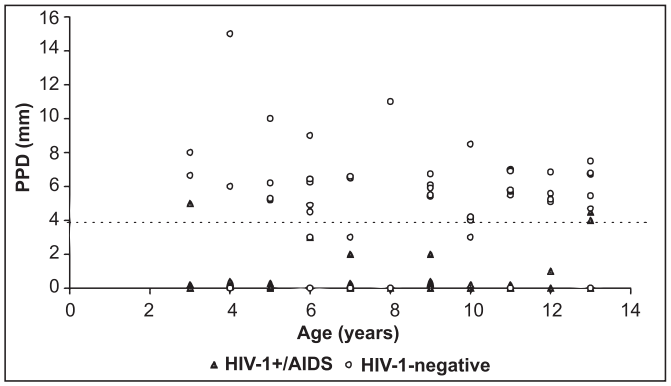

FIGURE 1 - DTH skin test results for PPD plotted in millimeters of induration (mm) for each age group (years). HIV-1+/AIDS individuals (▲, n=36). HIV-1-negative individuals (o, n= 56). PPD: purified protein derivative.
HIV: human immunodeficiency virus, **AIDS:** acquired immune deficiency syndrome. **DTH:** delayed-type hypersensitivity.

DISCUSSION

In our study, DTH skin test responses, including PPD reactivity, were significantly higher among BCG-vaccinated healthy children/adolescents compared to BCG-vaccinated age- and sex-matched HIV-1-infected participants. DTH skin test reactivity is influenced by several factors, such as age, nutritional status and the time interval between antigen exposure and the skin test[3,8]. Lower DTH reactivity rates or anergy have been described among HIV-1-infected adults and children[9-11].

Our study group comprised mainly AIDS cases with advanced disease under therapy, and 70% had $CD4^+$ T cell counts within a normal range close to the time of skin testing. Nevertheless, impaired DTH reactivity was observed. It is possible that a quantitative recovery of total $CD4^+$ T cell numbers by HAART, without a complete replacement of the memory cells for recall antigens, may explain the lack of correlation between $CD4^+$ T cell numbers and DTH reactivity. Additionally, this analysis is limited because the DTH tests were not performed simultaneously with the $CD4^+$ T cell counts. A recent study showed an important association between PPD DTH skin test reactivity and the levels of PPD-responsive IFNγ-producing $CD4^+$ T cells, highlighting the importance of functional $CD4^+$ T cell populations for DTH reactivity[12]. These results suggest that decreased functional $CD4^+$ T cell activity can also lead to reduced DTH reactivity among children/adolescents infected with HIV-1.

The PPD response was significantly different among BCG-vaccinated HIV-1-infected and healthy participants. Data regarding the influence of BCG vaccination on tuberculin skin tests are conflicting. A meta-analysis showed that neonatal BCG vaccination can increase the likelihood of a positive tuberculin skin test, indicating that the interpretation of a tuberculin test should take into consideration each patient's clinical context[6]. However, in our study, BCG vaccination was not a determinant factor for PPD reactivity because all participants (HIV-1-infected and healthy groups) had been vaccinated in the neonatal period and had a characteristic BCG scar. Some studies have shown that PPD reactivity in BCG-vaccinated individuals wanes with time[13], suggesting that environmental exposure to mycobacteria may be crucial to maintain PPD reactivity[7,14]. In our study, both natural environmental exposure to mycobacteria and BCG vaccination may have played a synergistic role to sustain PPD reactivity among immunocompetent healthy children and adolescents, regardless of their age[15,16].

Candidin was the most prevalent antigen recognized by the HIV-1-infected group. *Candida* sp. are naturally present in the human microbial flora, and therefore, a positive DTH test to candidin is expected in around 50-60% of the general population[3,17]. Blazevic et al.[18] have demonstrated that children infected with HIV-1 and treated with HAART have important recovery of cell-mediated immunity to candidin but not to tetanus toxoid. These results indicate a possible selective type of recovery in the cell-mediated response, which can be greater to those antigens to which the individual is primarily exposed.

Among BCG-vaccinated HIV-1-positive participants with similar exposure to mycobacteria, lower rates of PPD reactivity and many cases of anergy were observed. Incomplete restoration of mycobacteria-specific T cell-mediated immunity in severely immunocompromised HIV-1-infected patients, even with quantitatively normal levels of $CD4^+$ T cells, could be a possible explanation[19]. At the time this study was conducted, two patients had previously been diagnosed with tuberculosis. A review of the patient medical files during the 4 years following this study did not reveal any other tuberculosis cases, suggesting some degree of preserved cell-mediated immune response to mycobacteria in these patients. We acknowledge that the small sample size and the lack of extended clinical follow-up may limit the interpretation of our results. Additionally, the relatively small number of antigens employed for the DTH tests may have underestimated the *in vivo* cellular immune response to antigens in both groups.

This study showed marked differences in DTH reactivity between HIV-1-infected and healthy children. DTH tests can evaluate the *in vivo* specificity and functional status of memory T cell-mediated immunity to recall antigens. In this context, DTH tests could represent an additional tool for the follow-up evaluation and clinical prognosis of HIV-1-infected patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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