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

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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 tuberculopurified 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 induction was 2.5mm (range: 2-5mm) in the HIV-1 group and 6.0mm 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. Tuberculopurified 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), esporotricina, tricotofina, candidina e estreptokinase/estreptodornase foram realizados. Foram testados crianças/adolescentes infectados pelo HIV-1 (n=36) e indivíduos saudáveis (n=56), soronegativos para HIV-1/HIV-2 pesados por sexo-idade, todos com cicatriz vacinal por BCG. Teste exato de Fisher foi aplicado (p<0,05). Resultados: Entre as crianças/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 antiretroviral; mediana de carga viral = 3,04·10 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). A reatividade à candidina predominou nos infectados (8/36, 22%) e ao PPD nos indivíduos saudáveis (40/56, 71,4%). A reatividade ao PPD entre os infectados foi de 8,3% (p<0,01). A mediana da indução ao PPD foi 2,5mm (variação: 2-5mm) entre os saudáveis. Não observamos correlação entre reatividade 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 pela células T.


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). Peripheral CD4+ T cell counts are considered the gold standard measure for assessing the immune status of HIV-1-infected patients. 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.

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. PPD has been employed to screen for tuberculosis among high-risk populations, such as HIV-infected patients. 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. Nevertheless, tuberculin skin test positivity has mainly been associated with exposure to environmental mycobacteria.

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 Auda Hospital (HAA/HDT/SUS), City of Goiânia, State of Goiás, Central Brazil. Clinical and laboratory data regarding CD4+ T cell counts (FACsCount,
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 log10 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 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: 3.29log10 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.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>HIV-1/AIDS (n=36)</th>
<th>HIV-1-negative (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTH positivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Sporotrichin</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Trichophytin</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>Candidin</td>
<td>8</td>
<td>22.2</td>
</tr>
<tr>
<td>Streptokinase/streptodornase</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>PPD</td>
<td>3</td>
<td>8.3</td>
</tr>
</tbody>
</table>

DTH: delayed-type hypersensitivity; PPD: purified protein derivative; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome.
**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 test15. Lower DTH reactivity rates or anergy have been described among HIV-1-infected adults and children8,11.

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 normal range close to the time of skin testing. Nevertheless, impaired DTH reactivity may have been described among HIV-1-infected adults and children8,11.

A recent study showed an important association between PPD DTH skin test reactivity and the levels of PPD-responsive IFN-γ-producing CD4+ T cells, highlighting the importance of functional CD4+ T cell populations for DTH reactivity12. 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 context8. 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 time13, suggesting that environmental exposure to mycobacteria may be crucial to maintain PPD reactivity13,14. 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 age13,16.

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 population17. 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 explanation19. 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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**REFERENCES**


