Journal of Immunological Methods 412 (2014) 35–41

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Evaluation of a rapid serological test for leprosy classification using human serum albumin as the antigen carrier

Rodrigo Scaliante Moura [a], Gerson Oliveira Penna [b], Tsuyoshi Fujiwara [c], Maria Araci de Andrade Pontes [d], Rossilene Cruz [e], Heitor de Sá Gonçalves [d], Maria Lúcia Fernandes Penna [f], Ludimila Paula Vaz Cardoso [a], Mariane Martins de Araújo Stefani [a], Samira Bührer-Sékula [a,*]

[a] Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil
[b] Núcleo de Medicina Tropical, Universidade de Brasília, Brasília, DF, Brazil
[c] Institute for Natural Science Nara University, Nara, Japan
[d] Centro de Dermatologia Dona Libânia, Fortaleza CE, Brazil
[e] Fundação de Dermatologia Tropical e Venereologia Alfredo da Matta, Manaus, AM, Brazil
[f] Departamento de Epidemiologia e Bioestatística, Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 13 March 2014
Received in revised form 21 June 2014
Accepted 23 June 2014
Available online 29 June 2014

Keywords:
Leprosy
BSA
Serology

ABSTRACT

The presence of anti-BSA antibodies may interfere in serological tests, as ELISA or immunochromatographic assays. BSA is frequently used as a blocking agent or as "inert" carrier of antigens, such as the NT-P-BSA, the semi-synthetic trisaccharide analogue of the PGL-I (phenolic glycolipid-I) antigen from the cell wall of the *Mycobacterium leprae*. PGL-I was prepared and linked to human serum albumin based in the hypothesis that replacing BSA by a human protein carrier would enhance the performance of leprosy serological tests. A total of 1162 serum samples were tested by ELISA and by the ML Flow rapid test using NT-P-BSA or NT-P-HSA antigens. When grouping leprosy patients as paucibacillary (PB) or multibacillary (MB) according to the Ridley & Jopling classification, ML Flow BSA and ML Flow HSA tests correctly allocated 70.9% and 68.6% of patients in the PB group, and 87% and 81% of patients in the MB group, respectively. Concordant results were found in 82.0% (953/1162) (kappa value = 0.637; sd = 0.023) of samples between ML Flow tests and 85.7% (996/1162) (kappa value = 0.703; sd = 0.021) between ELISA tests. ML Flow results were statistically similar and the same was true for ELISA tests using HSA or BSA. However, we noticed a tendency to decreased capacity to detect MB patients and an increased positivity among PB patients, HHC, TB patients and healthy controls by the HSA carrier in both ML Flow and ELISA. The PGL-I serology performed by the ML Flow test with BSA or HSA as antigen carriers can be a useful, friendly auxiliary tool to identify patients with higher bacterial load.

Abbreviations: BSA, bovine serum albumin; HAS, human serum albumin; ELISA, enzyme-linked immunosorbent assay; MB, multibacillary leprosy patient; PB, paucibacillary leprosy patient; PGL-I, phenolic glycolipid I; TT, tuberculoid leprosy patients; BT, borderline tuberculoid leprosy patients; BB, borderline borderline leprosy patients; BL, borderline lepromatous leprosy patients; LL, lepromatous leprosy patients; EA HC, endemic area healthy controls; EA TB, endemic area tuberculosis patients; HHC, household contacts of leprosy patients; NEA HC, non-endemic area healthy controls; NEA TB, non-endemic area tuberculosis patients; PPV, positivity predictive value; NPV, negative predictive value.

* Corresponding author at: Instituto de Patologia Tropical e Saúde Pública, Departamento de Imunologia, sala 335, Rua 235 s/n Setor Leste Universitário, Goiânia, GO, CEP 74605-050, Brazil. Tel.: +55 62 3209 6111.
E-mail address: samira.buhrer@gmail.com (S. Bührer-Sékula).

http://dx.doi.org/10.1016/j.jim.2014.06.014
0022-1759/

1. Introduction

The first report of antibody production against the bovine serum albumin (BSA) was made by Rothberg and Farr (1965). Studies have reported the presence of anti-BSA antibodies in the sera of healthy individuals, patients with liver disease, autoimmune diseases and diabetes mellitus patients (Karjalainen et al., 1992; Lenkei and Ghetie, 1977; Mihăescu et al., 1981; Saukkonen et al., 1994; Sjöwall et al., 2011). The antigenic properties of BSA have also been reported by numerous studies involving milk allergy (Goldman et al., 1963a, 1963b; Restani et al., 1995), allergy to animal hair (Prahl et al., 1978; Szépfalusi et al., 1993) or other antibody responses after BSA exposure (Mackensen et al., 2000; Macy et al., 1989; Morales et al., 1994). The decline in antibody titer with age has been related to tolerance induction and to a decrease in gut permeability (Scott et al., 1985; Vaarala et al., 1995), but could also be related to a better enzymatic digestion of dietary proteins.

BSA is a major allergen present in bovine milk and meat, which means that human exposure to BSA antigens certainly begins early in life. BSA is also present in significant amounts in preparations of vaccines and medications. Some studies have demonstrated the antibody response after an identified exposure to BSA in humans. In one study, 6 out of 7 patients presented anti-BSA antibodies after administration of a vaccine of dendritic cells prepared in tissue culture medium containing BSA (Mackensen et al., 2000). Another study reported anaphylaxis and anti-BSA IgE formation in a patient who received an autologous bone marrow infusion of stem cells prepared in BSA (Macy et al., 1989).

The presence of anti-BSA antibodies may interfere in serological tests, such as enzyme-linked immunosorbent assays (ELISA), which frequently use BSA as a blocking agent or as "inert" carrier of antigens (Sjöwall et al., 2011). The use of a blocking agent, such as BSA or other milk proteins, prevents unspecific antibody binding to microspaces in the microplate surface not occupied by the specific antigen. BSA is also used as a carrier of non-proteic antigens in immunoassay compositions, such as the NT-P-BSA, which is a semi-synthetic trisaccharide, analogous to the PGL-I from the cell wall of the *Mycobacterium leprae*, Hansen's disease causative agent. A rapid serological test, the ML Flow test, was developed for leprosy using the NT-P-BSA. The agreement between the ML Flow test and the ELISA using the same antigen was 91% (Bührer-Sékula et al., 2003). As a control, in the ELISA assay, each sample is tested in an additional well with BSA only, aiming to discount bindings to the carrier portion of the antigen.

In confirmed leprosy patients, high antibody levels correlate with high bacterial index and, on the other hand, the absence of specific antibodies implies a negative bacterial index (Klatser et al., 1996). Thus, after diagnosis of a leprosy patient, the antibody response to PGL-I can be used for the classification of patients as multibacillary (MB) or paucibacillary (PB) forms of the disease (Buhrer-Sekula et al., 2000). In addition, the test may be used as a marker of infection for research purposes.

Proper treatment is the key for the success of leprosy control programs and a uniform regimen for all patients would make classification for treatment purposes unnecessary, simplifying leprosy control and benefitting patients (Penna et al., 2012a, 2012b). The "*Clinical Trial for Uniform Multidrug Therapy regimen for leprosy patients in Brazil (U-MDT/CT-BR)*" is still in course and primary results showed that there is no statistical difference when comparing the frequency of reactions between patients under U-MDT regimen and the ones under present WHO regimen (Penna et al., 2012a, 2012b). Nevertheless, it is known that patients with high BI have a higher risk of developing reactions (Baohong, 2001); besides, the high BI other unknown factors play a role in the susceptibility to leprosy reactions since only some patients develop reactions. Therefore, tools to detect among leprosy patients the ones with higher risk of reactions remain an important research challenge.

Recent data, from a pilot study, showed a high positivity in the ML Flow test among healthcare workers in Rio Grande do Sul, south region of Brazil, a state known to present a low endemicity for leprosy (Calado et al., 2013). One hypothesis to explain this high positivity could be related to unspecific BSA bindings. Theoretically, the use of bovine serum albumin as the carrier of a semi-synthetic antigen could lead to false-positive results (Beretta et al., 2001; Restani et al., 2004) and if this is true, replacing BSA by a human protein carrier, as human serum albumin (HSA), can improve the specificity of the test.

This study investigated the effect of using HSA as the protein carrier upon the specificity of anti-PGL-I serology and therefore its performance to identify individuals with high bacterial load.

2. Methods

2.1. Study groups

Leprosy patients: 830 newly diagnosed leprosy patients were included, 49 tuberculoid (TT), 295 borderline tuberculoid (BT), 158 borderline borderline (BB), 189 borderline lepromatous (BL) and 139 lepromatous (LL) classified according to a modified R&J classification system taking into account clinical features, histopathological results of skin biopsies and the slit skin smear bacterial load; Mitsuda tests and BI of the skin biopsy were not performed (Ridley and Jopling, 1966). These leprosy patients were recruited from March 2007 until February 2012 at two national leprosy referral centers from two Brazilian states: "Centro Dermatológico Dona Libânia" (Fortaleza city, Ceará State northeast Brazil) and "Fundação Alfredo da Matta" (Manaus city, Amazonas State north Brazil) (Penna et al., 2012a, 2012b). For the analysis, MB and PB leprosy groups were defined based on the R&J classification in which BB, BL and LL leprosy patients were merged as MB and BT and TT leprosy patients were grouped as PB leprosy. Therefore, whenever serology results were positive for MB patients or negative for PB patients, serological tests were considered able to correctly classify patients.

Healthy control group from a leprosy endemic area (EA HC) included 68 volunteers (blood donors, police officers and healthcare workers) recruited at Eduardo de Menezes Hospital, which is the referral center for sanitary dermatology in Minas Gerais state, Brazil. Thirty healthy controls from a non-endemic area (NEA HC) were recruited at the internal medicine sector of the Hospital del Salvador, University of