ELSEVIER

DC-SIGN Interacts with *Mycobacterium leprae* but Sequence Variation in This Lectin Is Not Associated with Leprosy in the Pakistani Population

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ABSTRACT: The C-type lectin DC-SIGN is involved in early interactions between human innate immune cells and a variety of pathogens. Here we sought to evaluate whether DC-SIGN interacts with the leprosy bacillus, *Mycobacterium leprae,* and whether DC-SIGN genetic variation influences the susceptibility and/or pathogenesis of the disease. A case–control study conducted in a cohort of 272 individuals revealed no association between DC-SIGN variation and leprosy. However, our results clearly show that DC-SIGN recognizes *M. leprae,* indicating that mycobacteria recognition by this lectin is not as narrowly restricted to the *Mycobacterium tuberculosis* complex as previously thought. Altogether, our results provide further elucidation of *M. leprae* interactions with the host innate immune cells and emphasize the importance of DC-SIGN in the early interactions between the human host and the infectious agents. *Human Immunology 67, 102–107 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: Leprosy; DC-SIGN; binding; genetic susceptibility; polymorphism

ABBREVIATIONS

TT	tuberculoid leprosy
LL	lepromatous leprosy
BT	borderline tuberculoid
BB	borderline borderline
BL	borderline lepromatous
DCs	dendritic cells
Mϕs	macrophages
SNPs	single nucleotide polymorphisms
HIV	human immunodeficiency virus

INTRODUCTION

Leprosy is a chronic granulomatous disease caused by *Mycobacterium leprae* affecting essentially the superficial peripheral nerves, the skin, and the mucosal membranes of the upper respiratory tract. Depending on the degree to which cell-mediated immunity is expressed and on the extent of spread and multiplication of the bacilli, infection can result in a broad spectrum of clinical manifestations and outcomes. At one pole of the disease, patients with tuberculoid leprosy (TT) develop a strong cell-mediated immune response that contains the infection in few localized lesions with low bacillary counts and that often progresses to self-healing. At the opposite pole, lepromatous leprosy (LL) patients develop a weak cellular response and suffer multiple lesions with high bacillary loads. Intermediary types of leprosy, namely borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL), with various clinical manifestations and bacillary counts, are classified in between TT and LL types. Although factors influencing the type of leprosy developed upon infection remain poorly under-

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stood, genetic host factors have long been suspected to play a major role in the clinical outcome of the infection [1]. Indeed, polymorphisms in genes encoding important mediators of the immune response, such as Toll-like receptor 2, tumor necrosis factor-α, interleukin-10, NRAMP1, vitamin D receptor, and other genes, such as the Parkinson-related genes PARK2 and PACRG, have been reported to be involved in susceptibility to leprosy and/or to preferential development of either type of the disease (see [2] for a review).

In the context of host factors influencing infectious disease susceptibility or outcome, the innate immunity system may play a critical role. Polarization of the T lymphocyte response is tightly linked to early recognition of the pathogen by innate immunity cells, such as dendritic cells (DCs) and macrophages (Mϕs), and to subsequent signaling events resulting in cytokine secretion and antigen presentation. Thus, genetic variation in host genes whose products are involved in the early steps of pathogen recognition may have a broad range of influence in the pathogenesis of leprosy. In this context, C-type lectins play a crucial role in detecting pathogens by their characteristic carbohydrate structures and internalizing them for further antigen processing and presentation, inducing therefore adaptive immunity [3]. We and others have recently shown that the prototypic C-type lectin dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin DC-SIGN (also known as CD209) is a major receptor for *Mycobacterium tuberculosis* in human DCs [4, 5] and in alveolar Mϕs in patients with tuberculosis [6]. DC-SIGN not only mediates internalization of the bacillus by DCs but may also transduce intracellular signals leading to secretion of IL-10 and to partial DC deactivation upon recognition of the microbe [4]. In this view, DC-SIGN may be a key element in shaping an appropriate T-cell response against *M. tuberculosis* and possibly other mycobacteria, such as *M. leprae.* Our most recent results show that nucleotide variation in the *DC-SIGN* promoter region is associated to susceptibility to tuberculosis [7]. Here we sought to evaluate whether DC-SIGN interacts with the leprosy bacillus, *M. leprae,* and whether *DC-SIGN* genetic variation has an influence on the susceptibility and/or pathogenesis of the disease.

MATERIAL AND METHODS

Binding Experiments

The bacilli *M. tuberculosis* H37Rv and *Mycobacterium smegmatis* mc^2155 harboring the pLuxGFP plasmid (kind gift from G. R. Stewart, London, UK) were cultivated in 7H9 medium containing ADC supplement (Difco) and 50 μg/ml hygromycin. Suspensions of fresh, viable, nude mouse-derived Thai-53 strain *M. leprae* were obtained from the National Hansen's Disease Programs Laboratory at Louisiana State University in Baton Rouge (LA, USA). This isolate of leprosy bacilli is maintained in programmed serial passage in the foot pads of athymic nu/nu mice infected with 5×10^7 freshly harvested *M. leprae.* Briefly, bacilli were harvested from the foot pads 3–4 months after infection (at mid-log growth), washed by centrifugation in Middlebrook 7H12 medium (18,000*g* for 5 minutes) and enumerated by direct count according to Shepard's method. The relative viability of *M. leprae* in a suspension was evaluated using the LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes). For the present study pure preparations of bacilli, free of mouse footpad tissue, were obtained by treating the footpad suspension with 0.1 M NaOH for 5 minutes followed by neutralization with 0.1 M HCl and three washes with phosphate-buffered saline. The cell membranes of these pure bacilli were stained with green PKH67 dye (Sigma) according to the manufacturer's recommendations, recounted by the Shepard technique, resuspended in RPMI-1640 at 1×10^9 *M. leprae* per milliliter, and stored at 4°C. HeLa and DC-SIGN-expressing HeLa cells (HeLa::DC-SIGN) were cultivated in RPMI-1640 (Invitrogen) supplemented with 10% fetal calf serum (Dutcher). For binding experiments, cells were cultivated in six-well plates (BD-Falcon) until 75% confluency and infected with the bacilli at a multiplicity of infection of 1 bacterium/cell for 4 hours at 4°C. After three washes in RPMI, cells were gently collected, fixed in 4% paraformaldehyde, and analyzed by flow cytometry for green fluorescence using a Facscalibur apparatus (Becton). Four independent experiments were conducted to assess the ability of *M. leprae* to bind to DC-SIGN. In two of these experiments, *M. tuberculosis* and *M. smegmatis* were included as controls.

Subjects

The study cohort of the present study consisted of 272 adult Pakistani individuals, including 194 patients with leprosy and 78 ethnically matched healthy individuals. All individuals were volunteers from whom informed consent was obtained. Disease evaluation was based on clinical, bacteriological, and histological data and determined according to the presence and number of bacteria observed in skin smears taken from the right and left ears, right eyebrow, and right and left middle fingers. The bacteria were detected using AFB staining. The clinical forms of leprosy were classified in accordance with the Ridley and Jopling classification [8]. The leprosy individuals included 76 patients with LL, 33 with BL, 15 with TT, and 70 with BT. Given the absence of significant differences between LL versus BL and between TT versus BT, individuals were grouped into lepromatous patients (BL + LL; $n = 109$) and tuberculoid