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IL-10 production from dendritic cells is associated with DC SIGN in human leprosy

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ABSTRACT

The defective antigen presenting ability of antigen presenting cells (APCs) modulates host cytokines and co-stimulatory signals that may lead to severity of leprosy. In the present study, we sought to evaluate the phenotypic features of APCs along with whether DC SIGN (DC-specific intercellular adhesion molecule-grabbing nonintegrin) influences IL-10 production while moving from tuberculoid (BT/TT) to lepromatous (BL/LL) pole in leprosy pathogenesis. The study revealed an increased expression of DC SIGN on $CD11c^+$ cells from BL/LL patients and an impaired form of CD83 (~50 kDa). However, the cells after treatment with GM-CSF+IL-4+ManLAM showed an increased expression of similar form of CD83 on DCs. Upon treatment with ManLAM, DCs were found to show increased nuclear presence of NF-κB, thus leading to higher IL-10 production. High IL-10 production from ManLAM treated PBMCs further suggested the role of DC SIGN in subverting the DCs function towards BL/LL pole of leprosy. Anti-DC SIGN treatment resulting in restricted nuclear ingression of NF-κB as well as its acetylation along with enhanced T cell proliferation validated our findings. In conclusion, *Mycobacterium leprae* component triggers DC SIGN on DCs to induce production of IL-10 by modulating intracellular signalling pathway at the level of transcription factor NF-κB towards BL/LL pole of disease.

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and it progresses with defective cell mediated immune response (Scollard et al. 2006). The mechanism of how *M. leprae* modulates the cell mediated immune response is still a matter of investigation for researchers as well as clinicians. A proper interaction between dendritic cells (DCs) and T cells is an important factor in generating antigen specific immune response (Lipscomb and Masten 2002). Studies have also demonstrated the involvement of CD83, a member of the immunoglobulin super family generally expressed on mature/immature DCs, in modulation of antigen presenting capability of DCs for the development of effective T cell response (Aerts-Toegaert et al. 2007; Cao et al. 2005).

DC SIGN is a calcium-dependent carbohydrate-binding protein, generally expressed at the surface of DCs subpopulations. DC SIGN poses a large functional gamut acting as an adhesion receptor, required for endothelial cells to induce ICAM-2 dependent tethering and trans-endothelial migration of DCs. It is known to cross-talk with naive T cells through binding of ICAM-3 in the lymph nodes of various tissues. Immature DCs consisting DC SIGN affects the T cell proliferation (Gijzen et al. 2007). *Mycobacterium* (*M. tuberculosis* and *M. bovis*), except *M. smegmatis*, specifically target DC SIGN through ManLAM ligation with an aim to impair DCs maturation and to induce production of the anti-inflammatory cytokine IL-10, and TGF-β to some extent for their survival and multiplication in the host (Geijtenbeek et al. 2003). Moreover, a maturation defect of DCs in *Helicobacter pylori* infection and consequent induction of Th1 cytokine due to DC SIGN binding defect in *Neisseria meningitidis* infection further suggested the possible role of DC SIGN ligation in immune suppression (Gringhuis et al. 2009; Al-Bader et al. 2003).

Toll-like receptor 2 (TLR2) is considered as principal mediator of DCs activation in response to mycobacterium infections, and its signalling mechanism is far distinct from lipopolysaccharide (LPS)-induced signalling *via* TLR4 (Kang et al. 2004; Bochud et al. 2003). Previously, expression and activation of Toll-like receptors (TLRs) were found to be associated with the type of immune response mounted towards *M. leprae* (Krutzik et al. 2003). Furthermore, mutation in TLR2 (Arg677Trp) led to low production of IL-2, IL-12, IFN-γ, and TNF-α by *M. leprae*-stimulated PBMCs (Bochud et al. 2003).

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Our previous work showed that *M. leprae* infections lead to alteration of lipid raft scaffold in APCs *via* combinatorial effects of cholesterol depletion and positive feedback loop by inducing the secretion of immune suppressive cytokines in environment (Kumar et al. 2011a). Furthermore, we had shown the predominance of immature DCs with altered surface phenomenon during progression of disease (Kumar et al. 2011a). Upon pathogen invasion, the DCs migrate towards secondary lymphoid organs and activate naive T cells to initiate adaptive immune responses. On the other hand, immature DCs direct the immunological signalling towards generation of T regs, leading to T-cell anergy (Rutella et al. 2006). DCs, depending on ligation of specific cell surface receptor(s), selectively promote T helper 1 (T_H1)-, T helper 2 (T_H2) or regulatory T-cell responses (Kapsenberg 2003).

In the present study, we have investigated the dynamic expression of DC SIGN and CD83 on dendritic cells and their possible role in the development of hyporesponsiveness towards BL/LL pole of leprosy. Since both DC SIGN and TLR2 function through activation of NF-κB (Gringhuis et al. 2007) therefore, we have hypothesized that DC SIGN mediated ligation of *M. leprae* might be playing a role in production of IL-10 by reprogramming the NF-κB mediated events in chromatin remodelling. The principal objectives of the present study were to investigate DC SIGN mediated IL-10 production, and role of Histone acetyl transferases (HATs), p300 and CBP associated gene activation including interaction of p300 at IL-10 locus during *M. leprae* infections. Immature and mature DCs were used to unravel the relevance of DC SIGN in generation of T cell mediated immune responses. The results showed that blocking of DC SIGN was associated with low IL-10 production, and thereby increased T cell proliferation in DCs obtained from BL/LL patients. Furthermore, we have demonstrated that ManLAM ligation increases nuclear migration of NF-κB as well as NF-κB-p300 complexes while moving towards BL/LL pole in leprosy. So far, the study reports a novel finding endorsing the DC SIGN enriched DCs to be involved in the production of IL-10 during progression of leprosy which in turn helps in development of T cell hyporesponsiveness and persistence of pathogen in the host.

Materials and methods

Patients, controls and their ethical approval

Newly diagnosed leprosy patients (age range 18–50 years; 40 males and 20 females) were recruited from Department of Dermatovenerology, AIIMS, New Delhi, India, after receiving approval from Institute's Ethics Committee and taking informed consent from individual patients. Leprosy patients were categorized based on Ridley–Joplin classification method (Ridley and Jopling 1966). Both clinical as well as histopathological details were considered while categorizing the patients in paucibacillary (BT/TT) and multibacillary (BL/LL) classes. Age matched healthy volunteers were recruited as controls (HC) after getting their written consent.

Reagents

Rat monoclonal anti-human CD209 (DC-SIGN) purified/FITC (clone eB-h209), anti-human IL-10 purified (JES3-9D7), mouse monoclonal anti-human CD11c PE (clone 3.9), anti-human/Mouse CD282 (TLR2) purified (clone T2.5), and rabbit polyclonal anti-NF kappa B p65 were procured from eBiosciences, San Diego, CA, USA. Mouse monoclonal anti-human CD83APC (clone HB15e) was obtained from BD Pharmingen, CA, USA and recombinant human GM-CSF, and IL-4 were purchased from R&D Systems, MN, USA. Mouse monoclonal anti-human p300 (clone 3G230) and anti-human monoclonal TBP (nuclear loading control) were obtained from Abcam, MA, USA.

Preparation of liposomes

Mannose-capped lipoarabinomannan (ManLAM) was generously gifted by Dr. Patrick Brennan (Colorado State University, Colorado) and Tuberculosis Research Materials and Vaccine Testing Organization, NIH/NIAID, USA. Multilamellar vesicles (MLVs) were prepared by freeze and thaw method (Sridevi et al. 2003). Briefly, cholesterol, phosphatidyl choline (PC) and phosphatidyl glycerol (PG) (Sigma–Aldrich, St. Louis, MO, USA) were dissolved together in 20:27:10 (w/v) mixture to obtain the lipid film. ManLAM (1 μg/μl) was added to the lipid film and the sample was kept at shaking for at least 2 h. The suspensions were then dipped in liquid nitrogen (−196 °C) for rapid cooling followed by thawing at 30 °C for 5 min each. After 5 min in water bath, the lipids were frozen again and this procedure was repeated one to ten times as indicated above to obtain freeze-thawed MLVs with a maximal trapping efficiency of 40–45%. Residual solvents were removed by centrifugation at 10,000 × *g* for 30 min.

Isolation and culture of PBMCs, monocytes, DCs, and T cells

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Sigma–Aldrich, St. Louis, MO, USA) density centrifugation of heparinized venous blood (20 ml), taken from leprosy patients and healthy controls. Cells (1×10^6 cells/well) were cultured in complete RPMI 1640 medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% foetal calf serum (Sigma–Aldrich, St. Louis, MO, USA), 100 U/ml penicillin, and 50 μg/ml streptomycin (Sigma–Aldrich, St. Louis, MO, USA) in 96-well tissue culture plates in duplicates wells at 37 °C (5% CO_2) for 72 h. For monocytes, PBMCs (1×10^7 cells/3 ml per well) were resuspended in complete RPMI 1640 media as described above, in a 6-well plates (Costar, Cambridge, MA, USA) and cultured at 37 °C for 2 h. Adherent monocytes were harvested after washing the cells two times. The PBMCs were used for preparation of DCs (Blood Dendritic Cell Isolation Kit II) and isolation of different subset of T cells ($CD4^+$ T Cell Isolation Kit) using MACS® Technology (Miltenyi Biotec GmbH, Germany) as per manufacturer's instructions. To prepare DCs, adherent cells isolated from PBMCs were cultured for 6 days in presence of GM-CSF (20 ng/ml) and IL-4 (20 ng/ml) with one-half of the medium being replaced by fresh culture medium every other day. Isolated DCs (1×10^5 cells) were cultured alone or co-cultured with T cells (5×10^5 cells) in 96-well V-bottom tissue culture plates (Greiner Bio-One GmbH, Germany) in triplicate wells at 37 °C for 72 h in aforementioned RPMI 1640 media. Following amounts of anti-DC SIGN (20 μg/ml), anti-TLR2 (20 μg/ml), anti-IL-10 (20 μg/ml) or isotype-matched control, ManLAM (10 μg/ml), and PHA (2 μg/ml) were used in respective experiments. ^{3}H-thymidine (0.5 μCi; specific activity 6.7 Ci/mmol, Bhabha Atomic Research Centre, Trombay, Mumbai) was added into each well 12–16 h before completion of 72 h culture duration. Cultures were harvested onto glass fibre disc and incorporation of radioactivity was measured by β-liquid scintillation counter. Results were expressed as cell count per minute (c.p.m.).

Flow cytometry

PBMCs from leprosy patients and healthy controls were stained with various anti-human antibodies with optimal titrated concentrations. For DCs analysis, anti-CD11c-PE, anti-CD209-FITC and CD83-APC and their respective isotype controls were used. Approximately, 2×10^4 cells were acquired to perform the above analysis. Stained cells were fixed with 2% paraformaldehyde and were