Immunology Letters 207 (2019) 6–16

Contents lists available at ScienceDirect

Immunology Letters

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

Notch signaling induces lymphoproliferation, T helper cell activation and Th1/Th2 differentiation in leprosy

Bhavyata Dua[a], Rajni Upadhyay[a], Mohan Natrajan[a,1], Mamta Arora[a], Kithiganahalli Narayanaswamy Balaji[b], Beenu Joshi[a,*]

[a] National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Agra, 282004, India
[b] Department of Microbiology and Cell Biology, Indian Institute of Science, Bangaluru, 560012 India

ARTICLE INFO

Keywords:
Notch1
Delta1
Jagged1
Jagged2
PGL-1
WCS
Leprosy

ABSTRACT

The present study evaluates role of Notch1 signaling in the regulation of T cell immunity in leprosy. Peripheral blood mononuclear cells from leprosy patients and healthy controls were activated with *Mycobacterium leprae* antigens along with activation of Notch1 signaling pathway and then lymphoproliferation was analyzed by lymphocytes transformation test and the expression of Notch1 and its ligands DLL1, Jagged1 and Jagged 2, T cell activation marker and Th1-Th2 cytokines on Th cells in PBMCs of study subjects were analyzed by flow cytometry. Further, these parameters were also analyzed after inhibition of Notch1 signaling pathway. Higher percentage of Notch1expressing Th cells were noted in TT/BT cases and higher percentage of DLL1 expressing Th cells in TT/BT and BL/LL cases. *M. leprae* antigens were found to induce the expression of Jagged1 on Th cells. Interestingly activation of Notch1 signaling pathway induced lymphoproliferation in BL/LL cases in response of PGL-1. Activation of Notch1 signaling was also found to induce the expression of T cell activation markers CD25, CD69 and Th1 cytokine IFN-γ in response to *M. leprae* antigens. Immunomodulation through Notch1 signaling seen in our study could be helpful in augmenting Th1 response in leprosy.

1. Introduction

Leprosy is a spectral disease which manifests itself in different clinical forms causing damage to the peripheral nerves resulting in sensory and motor nerve function impairment with characteristic deformity and disability. Tuberculoid form of the disease is the result of high cell mediated immunity with Th1 type of immune response whereas lepromatous form of leprosy is characterized by low cell mediated immunity with humoral Th2 type of immune response [1].

Notch is a single transmembrane receptor protein that regulates a broad range of cell fate decisions. In mammals, expression of four Notch genes (*Notch1-4*) and at least five of their ligands (*Jagged1 and 2; Delta1,3 and 4)* are reported [2]. The interaction between the Notch and its ligands Delta or Serrate/Jagged, constitutes an evolutionary pathway important for cell fate decisions [2]. Notch engagement with its ligands leads to the proteolytic cleavage of the notch domain by presenilin which moves to the nucleus where it interacts with the transcriptional repressor RBP-Jκ and converts its transcriptional activator [3,4]. Notch signaling has been described by different groups to promote such diverse outcomes as tolerance [5], regulatory T cell differentiation [6–8], enhancement of T cell expansion [9,10], inhibition of the cytotoxic T cell activity [8], regulation of T cell differentiation [10,11] and cytokine production [7,8]. Thus the role of Notch in T cell is complex and context dependent.

Notch1 signaling has been shown to play a role in induction of suppressor of cytokine signaling (SOCS)3 in macrophages [12], in modulation of gene expression pattern known to affect the function of mature macrophages [13], in regulation of T cell activation and proliferation [14] and in enhancing the expression of CD25 on T cells [9]. As in leprosy outcome of disease depends on the type of T cell response developed by patient, in the present study role of Notch1 signaling in lymphoproliferation or in regulation of Th1/Th2 response in leprosy

Abbreviations: BL, borderline lepromatous leprosy; BT, borderline tuberculoid leprosy; LL, lepromatous leprosy; *M. leprae*, *Mycobacterium leprae*; PBMCs, peripheral blood mononuclear cells; PGL, 1- Phenolic glycolipid-1; TT, tuberculoid leprosy; WCS, whole cell sonicate

* Corresponding author at: Department of Immunology, National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Tajganj, Agra, 282004, India.

E-mail addresses: bhavidua08@gmail.com (B. Dua), rajniupadhyay@gmaill.com (R. Upadhyay), mohannatrajan13@gmail.com (M. Natrajan), mamtaarora_aroram@rediffmail.com (M. Arora), balaji@mcbl.iisc.ernet.in (B. Kithiganahalli Narayanaswamy), beenuj2002@yahoo.co.in (B. Joshi).

[1] Present address: Department of Clinical Research, National Institute for Research in Tuberculosis, Chennai 6000031, India.

https://doi.org/10.1016/j.imlet.2019.01.003
Received 13 June 2018; Received in revised form 28 December 2018; Accepted 5 January 2019
Available online 07 January 2019

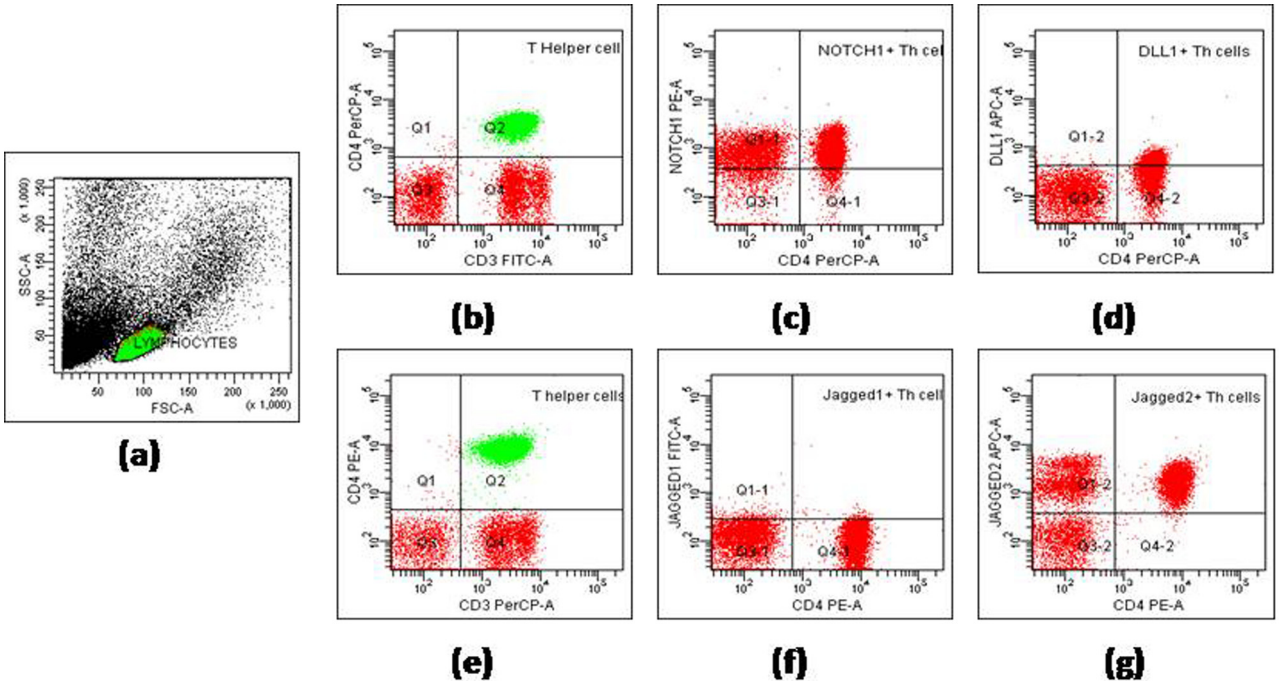


Fig. 1. Representing the gating criteria of Notch1, DLL1, Jagged1 and Jagged2 expressing T helper cells in different study groups. Gating of PBMCs **(a)**, gating of CD3 + CD4 + Th cells from PBMCs **(b)**, gating of Notch1 + cells from Th cells **(c)**, selection of DLL1 + cells from Th cells **(d)**, again gating of CD3 + CD4 + Th cells with different combination of dyes from PBMCs **(e)**, selection of Jagged1 cells from Th cells **(f)**, selection of Jagged2 cells from Th cells **(g)**.

was evaluated. For this purpose we activated and inhibited the Notch1 signaling pathway in peripheral blood mononuclear cells from leprosy patients and healthy controls along with stimulation by two different antigens of *Mycobacterium leprae* and then analyzed lymphoproliferation, expression of T cell activation marker CD25 and CD69 and Th1-Th2 cytokines IFN-γ and IL-4 by T helper cells. We also investigated the expression of Notch1 and its ligands DLL1, Jagged1 and Jagged 2 on Th cells in PBMCs of leprosy patients and healthy controls with or without stimulation with *M. leprae* antigens.

2. Materials and methods

2.1. Study subjects

Ten clinically diagnosed untreated tuberculoid leprosy (TT/BT) patients, ten untreated lepromatous leprosy (BL/LL) patients attending OPD of National JALMA Institute for Leprosy and Other Mycobacterial Diseases were included in the study. All the patients were diagnosed on the basis of clinical signs and symptoms and bacteriological index (BI) grading. Ten students and staff working in the laboratories of NJIL & OMD were included in the study as healthy controls. All the healthy controls were examined clinically and were not suffering from any infectious disease. Eight milliliter peripheral blood was collected in heparinized vials from all study subjects after taking informed written consent and the study was approved by institutional human ethics committee.

2.2. Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from the venous blood by Ficoll-hypaque density gradient centrifugation method [15]. PBMCs were then suspended in RPMI-1640 (Sigma, USA) supplemented with 100 unit penicillin/ml, 100 µg streptomycin/ml (Sigma, USA), L-glutamine (Sigma, USA) and 5% heat inactivated fetal bovine serum (Sigma, USA).

2.3. Lymphocyte transformation test (LTT)

PBMCs were cultured in 96 well flat bottom plate (NUNC, Denmark) with 2×10^5 cells per well in 200 µl culture medium (RPMI-1640) with or without stimulation with optimum doses of different antigens/mitogen: phytoheamagglutinin (PHA) 5 µg/ml, whole cell sonicate of *M. leprae* (WCS) 10 µg/ml and phenolic glycolipid-1 (PGL-1) of *M. leprae* 10 µg/ml along with anti Notch1 antibody (Thermo Scientific, USA) 10 µg/ml. For inhibition of Notch signaling PBMCs were treated with Gamma Secretase Inhibitor (Calbiochem, USA) 5 nm for 30 min before addition of stimulants. Plates were then incubated at 37 °C in 5% CO_2 with humidified air for five days. Cells were then pulsed with 1µci ^{3}H-thymidine (Bhabha Atomic Research Centre, Mumbai India) and incubated for 16–18 hours. Cells were harvested with a cell harvester (Skatron, Instruments) on a glass fiber filter and ^{3}H-thymidine incorporation was determined in β-scintillation counter (LKB Rackbeta, Finland). Stimulation index (SI) for each antigen was measured by dividing mean count per minute (CPM) of stimulated cells by the mean CPM of unstimulated cells. PHA was included as stimulator of lymphoproliferation (data not shown).

2.4. Stimulation and staining of cells for flowcytometry

2×10^6 cells/ml were stimulated with plate bound anti-CD3 (BD Biosciences, USA) 2 µg/ml, anti-CD28 (BD Biosciences, USA) 2 µg/ml and with or without antigens WCL, PGL-1, plate bound anti-Notch1 antibody and GSI. Plates were incubated for 24 h at 37 °C in 5% CO_2 incubator with humidified air. Six hours before the termination of incubation cells were treated with monensin (4 nm). After incubation cells were stained for surface markers with anti-CD3 FITC (BD Biosciences, USA), anti-CD4 PECy5 (BD Biosciences, USA), anti-CD4 PerCP (BD Biosciences, USA), anti-CD4 PE (BD Biosciences, USA), anti-Notch1 PE (R&D, USA), anti-DLL1 APC (R&D, USA), anti-Jagged1 FITC (R&D, USA), anti-Jagged2 APC (R&D, USA), anti-CD25PECy7 (BD Biosciences, USA) and anti-CD69APC (BD Biosciences, USA) for 30 min in dark at 4 °C. Cells were then washed and fixed with 4% formaldehyde in phosphate buffer saline (PBS, pH-7.4). Fixed cells were then washed

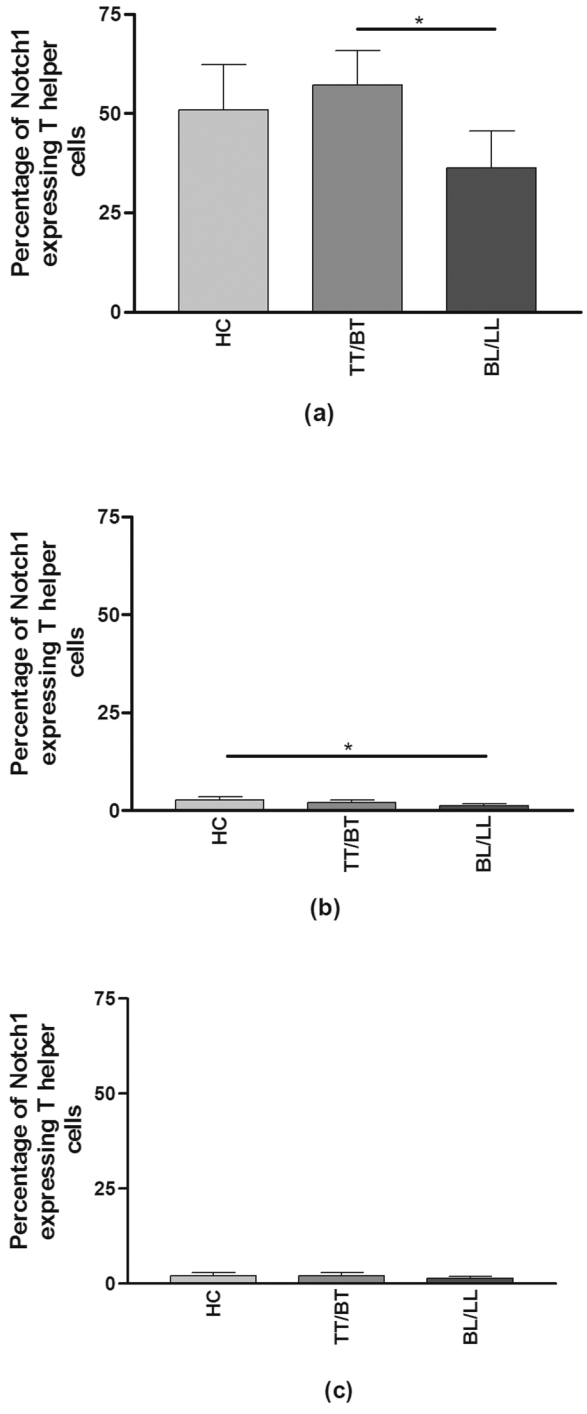


Fig. 2. Percentage of Notch1 expressing T helper cells in different study groups with or without stimulation. PBMCs of different study subjects were incubated with medium only **(a)**, stimulated with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)** and incubated for 24 h. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti-Notch1 antibodies and percentage of Notch1 expressing Th cells were determined by flowcytometry *p < 0.05 (n = 10).

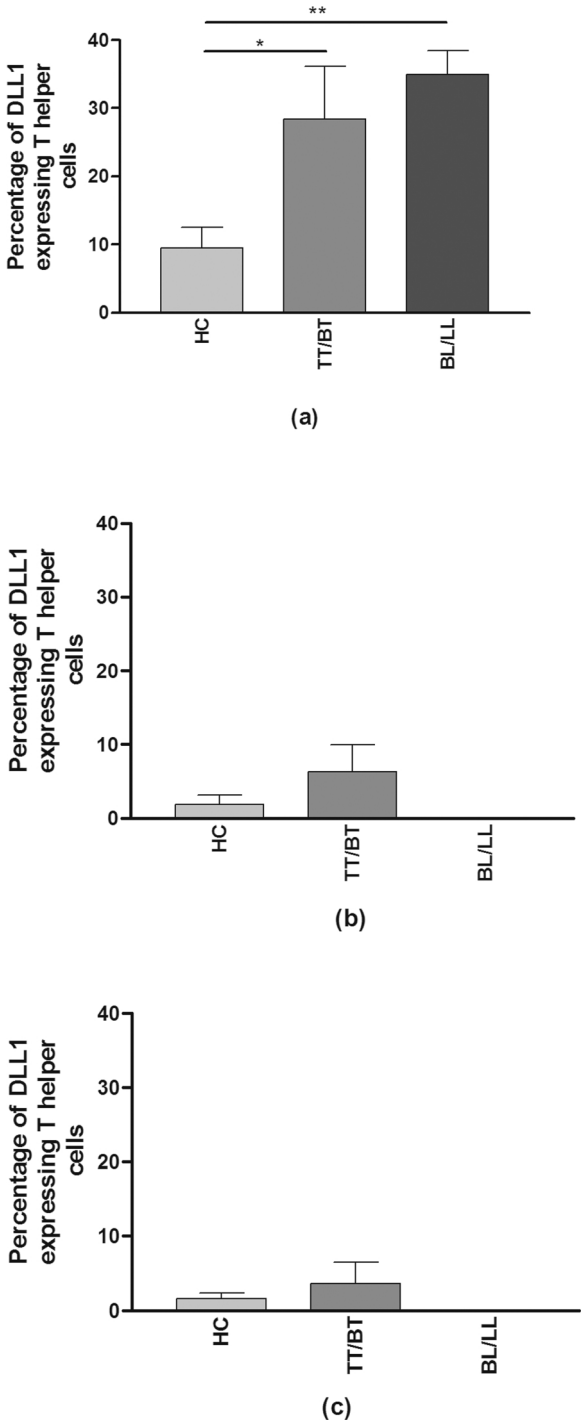
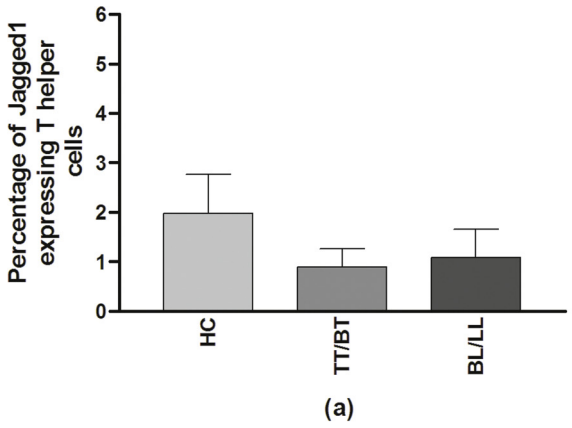


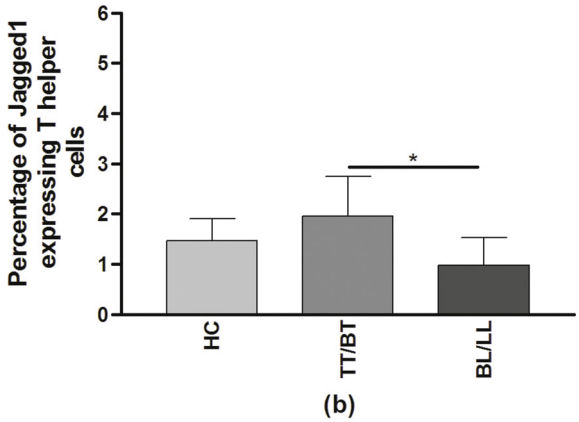
Fig. 3. Percentage of DLL1 expressing T helper cells in different study groups with or without stimulation. PBMCs of different study subjects were incubated with medium only **(a)**, stimulated with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)** and incubated for 24 h. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti-DLL1 antibodies and percentage of DLL1 expressing Th cells were determined by flowcytometry. *p < 0.05, **p < 0.01 (n = 10).

for intracellular staining, permeabilized and then stained for intracellular cytokine with anti-IFN-γPE (BD Biosciences, USA) and anti-IL-4PE (BD Biosciences, USA) for 30 min in dark at 4 °C. All the antibodies for flowcytometry were purchased from BD Biosciences, USA. Stained cells were acquired in BD FACS Aria and the percentage of cells was calculated using FACS Diva Software.

3. Calculation

Values after antigen stimulation were calculated by subtracting the unstimulated value from stimulated value. Data was statistically analyzed using Graph Pad Prizm software version 3.0 and differences among the groups were calculated by Mann Whitney test. P value less than 0.05 was considered as significant.





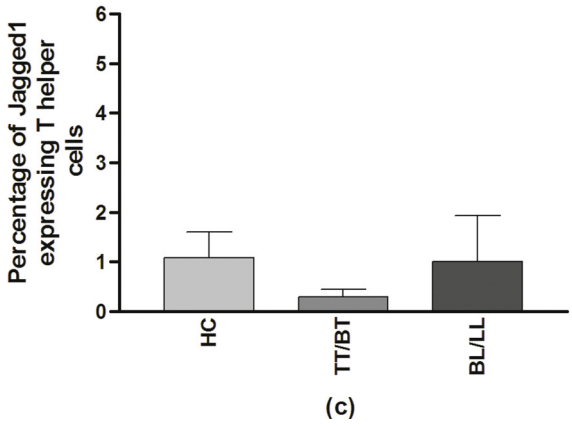
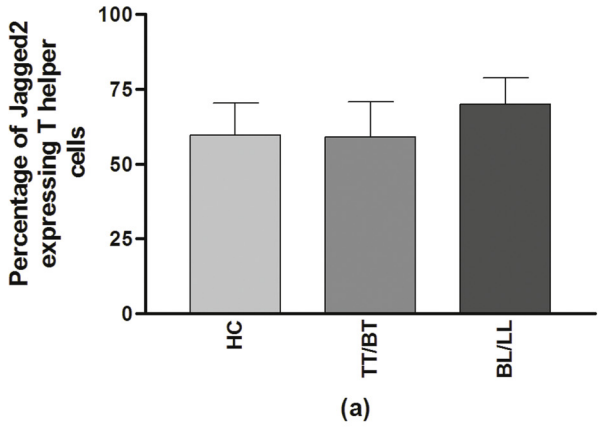
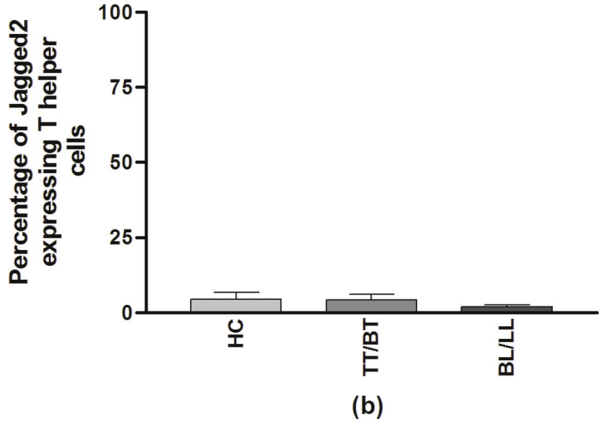


Fig. 4. Percentage of Jagged1 expressing T helper cells in different study groups with or without stimulation. PBMCs of different study subjects were incubated with medium only **(a)**, stimulated with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)** and incubated for 24 h. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti-Jagged1 antibodies and percentage of Jagged1 expressing Th cells were determined by flowcytometry. *$p < 0.05$(n = 10).





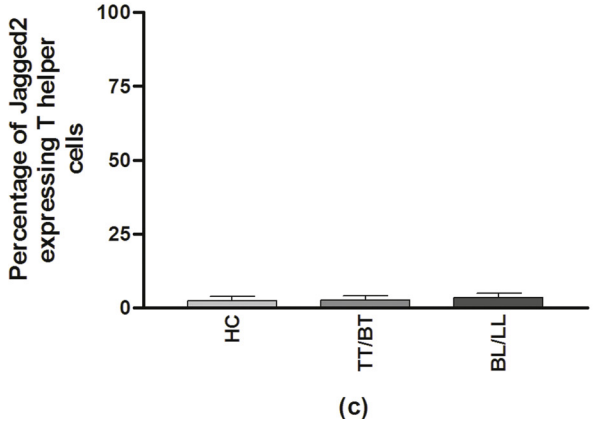


Fig. 5. Percentage of Jagged2 expressing T helper cells in different study groups with or without stimulation. PBMCs of different study subjects were incubated with medium only **(a)**, stimulated with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)** and incubated for 24 h. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti-Jagged2 antibodies and percentage of Jagged2 expressing Th cells were determined by flowcytometry (n = 10).

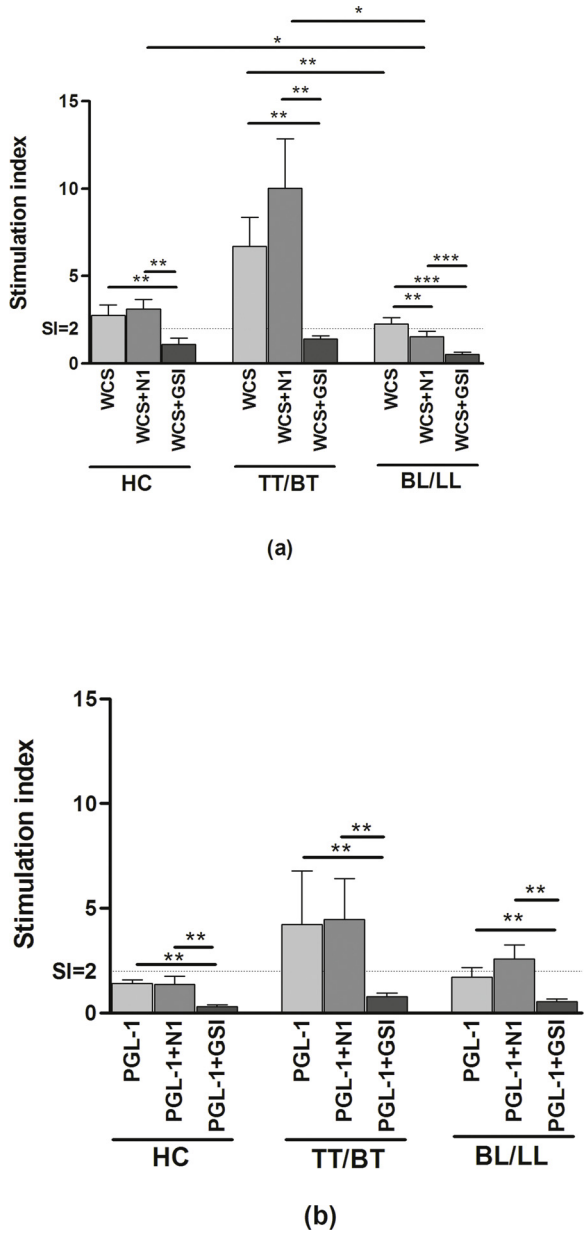


Fig. 6. Lymphoproliferative response after activation and blocking of Notch1 signaling pathway with antigenic stimulation in different study groups. Notch1 signaling pathway was activated with plate bound anti-Notch1(N1) antibodies and inhibited by the treatment of cells with GSI in PBMCs of different study subjects and cells were stimulated with whole cell sonicate **(a)**, phenolic glycolipid-1 **(b)** of *M. leprae.* Cultures were incubated at 37 °C for 5 days and then pulsed with ^{3}H thymidine for 16–18 hrs. After incubation cells were harvested on glass fiber filter paper and ^{3}H thymidine incorporation was measured by β-scintillation counter. Stimulation index (SI) for each antigen was measured by dividing mean count per minute (CPM) of stimulated cells by the mean CPM of unstimulated cells. $^{**}p < 0.01$ $p^{***} < 0.005$ (n = 10).

4. Results

4.1. Expression of Notch1 and its ligands DLL1, Jagged1 and Jagged2 on T helper cells

Notch1 and its ligands DLL1, Jagged1 and Jagged2 expressing Th cells were analyzed by Flowcytometry in different categories of study subjects (Fig. 1). Notch1 expressing cells were significantly higher in TT/BT patients than BL/LL patients and WCS significantly increased the Notch1 expressing cells in HC than BL/LL patients (Fig. 2).

DLL1 expressing Th cells were significantly higher in TT/BT and BL/LL patients than HC. None of the antigens significantly induced the DLL1 expression among different categories of subjects (Fig. 3). Jagged 1 and Jagged 2 expressing cells were not significantly different among different categories of study subjects (Figs. 4 and 5). WCS significantly increased Jagged1 expressing Th cells in TT/BT patients than BL/LL cases (Fig. 4). None of the antigen tested induced the percentage of Jagged2 expressing cells after stimulation (Fig. 5).

4.2. Notch signaling regulates lymphoproliferation

Proliferative response of lymphocytes of different categories of study subjects was measured after the stimulation of Notch1 signaling pathway in PBMCs with anti-Notch1 antibody and inhibition of Notch signaling pathway by treatment of PBMCs with GSI with or without stimulation with *M. leprae* antigens by lymphocyte transformation test. Results were represented as stimulation index (SI) and SI two or more than two was considered as proliferation. Inhibition of Notch signaling by GSI significantly inhibited lymphoproliferation mediated by WCS and PGL-1 with or without activation of Notch1 signaling by anti Notch1 antibody in all categories of study subjects. No effect was noted on the proliferation after activation through Notch receptor in presence of WCS in HCs and BT/TT however, higher S.I was noted in BL/LL after stimulation with PGL-1 (Fig. 6a and b). Lymphoproliferative response by the activation of Notch1 signaling with WCS was significantly higher in HCs and BT/TT in comparison to BL/LL (Fig. 6a). (discussion)

4.3. CD25 expression on T helper cells by Notch1 signaling

Activation of T cells leads to expression of CD25 which is α chain of IL-2 receptor and is necessary for the binding of IL-2. CD3/CD28 mediated expression of CD25 was analyzed on the T helper cells after activation of Notch1 signaling pathway by anti Notch1 antibody and inhibition of Notch1 signaling pathway by GSI in presence of *M. leprae* antigens by flowcytometry (Fig. 7). Inhibition of Notch signaling significantly reduced CD25 expression on T helper cells mediated by CD3/CD28 with or without stimulation with anti-Notch1 antibody in all categories of study subjects. Activation of Notch1 signaling through anti-Notch1 antibody significantly increased WCS mediated CD25 expression on T helper cells in HC and WCS and PGL-1 mediated CD25 expression on T helper cells in BL/LL patients (Fig. 8).

4.4. CD69 expression on T helper cells by Notch1 signaling

CD3/CD28 mediated expression of early T cell activation marker CD69 was also analyzed on T helper cells after activation and inhibition of Notch1 signaling pathway in presence of *M. leprae* antigens by flowcytometry (Fig. 9). Activation of Notch1 signaling significantly induced CD69 expression on T helper cells in HC and BL/LL cases. Inhibition of Notch signaling significantly reduced CD69 expression on T helper cells mediated by CD3/CD28 with or without stimulation with anti-Notch1 antibody in all categories of study subjects. Activation of Notch1 signaling by anti-Notch1 antibody significantly increased WCS mediated CD69 expression on T helper cells in HC and WCS and PGL-1 mediated CD69 expression on T helper cells in BL/LL patients (Fig. 10).

4.5. Notch1 signaling mediated regulation of IFN-γ expression in T helper cells

Th1 cytokine IFN- γ is important for anti mycobacterial immunity in mycobacterial diseases such as leprosy and TB. CD3/CD28 mediated expression of IFN-γ was analyzed in T helper cells after activation or inhibition of Notch1 signaling pathway in presence of *M. leprae* antigens by flowcytometry (Fig. 11). Inhibition of Notch1 signaling significantly decreased CD3/CD28 with or without anti Notch1 mediated expression of IFN-γ in all categories of study subjects with or without

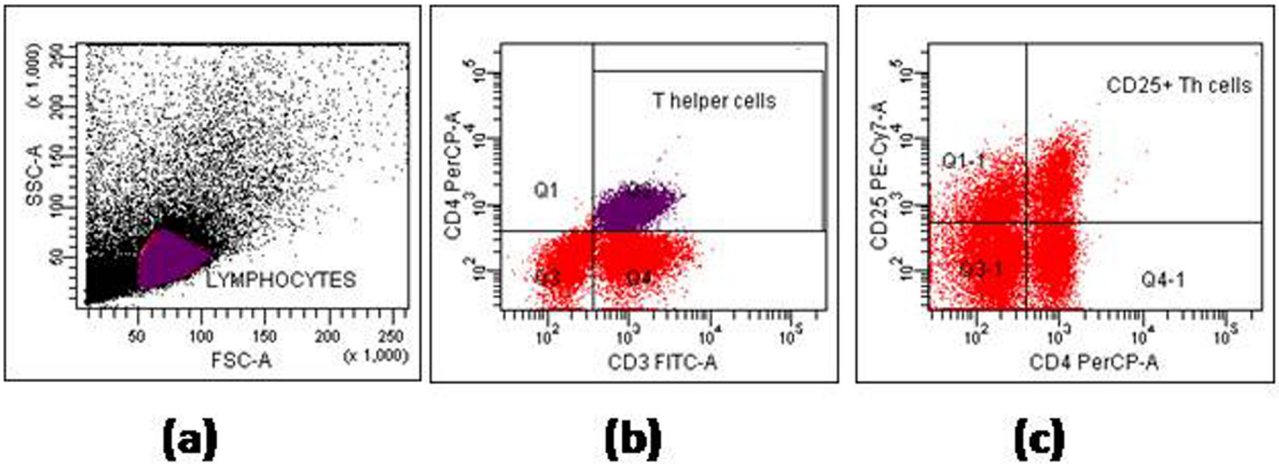


Fig. 7. Representing the gating criteria of CD25 expressing T helper cells in different study groups. Gating of PBMCs **(a)**, gating of CD3 + CD4 + Th cells from PBMCs **(b)** and gating of CD25 + Th cells **(c)**.

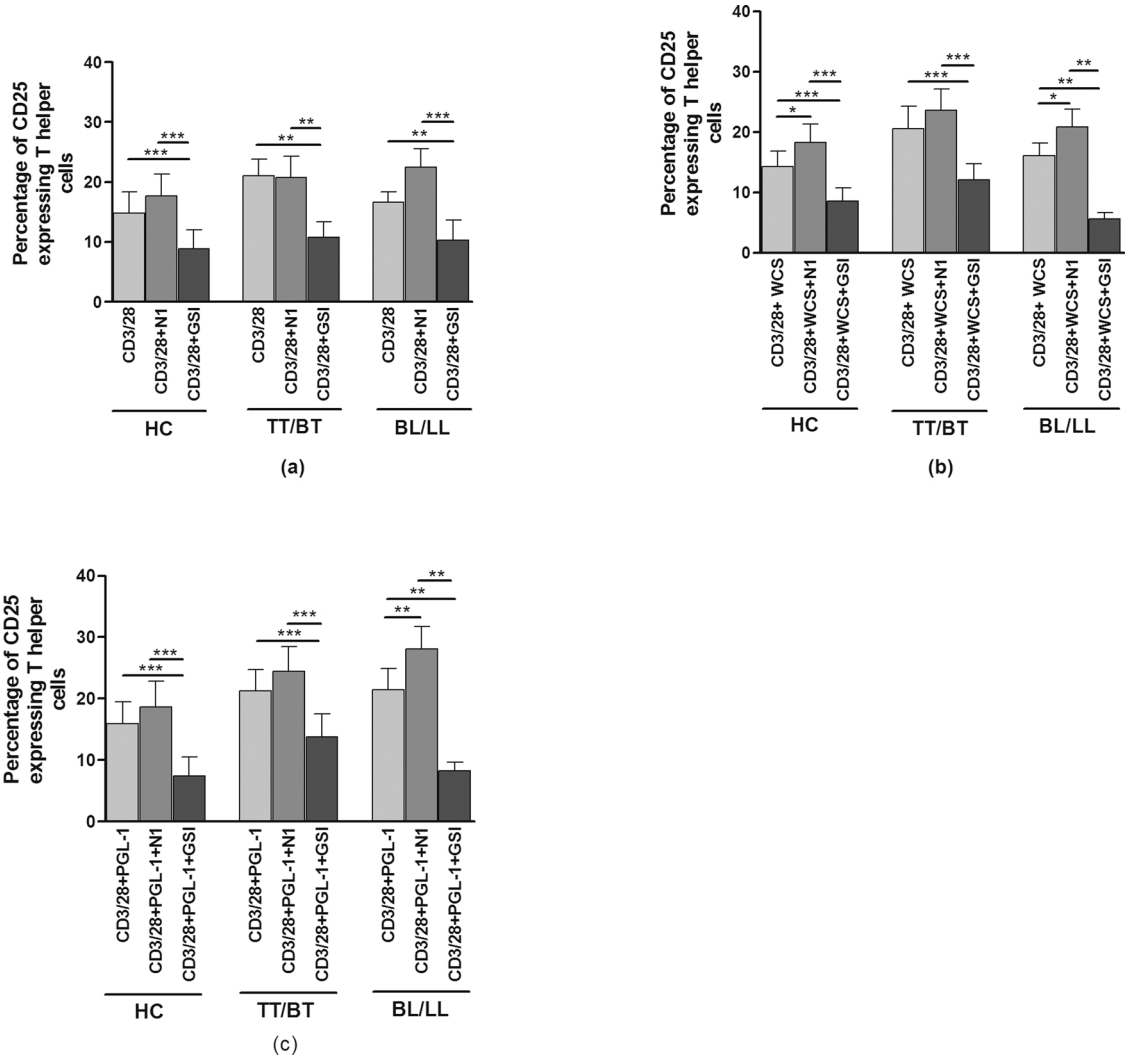


Fig. 8. Percentage of CD25 expressing T helper cells after activation and blocking of Notch1 signaling pathway in different study groups with or without stimulation with antigen. Notch1 signaling pathway was activated with plate bound anti-Notch1(N1) antibodies and inhibited by the treatment of cells with GSI in PBMCs of different study subjects and cells were incubated for 24 h with medium only **(a)**, with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)**. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti-CD25 antibodies and percentage of CD25 expressing Th cells were determined by flowcytometry. *$p < 0.05$**$p < 0.01$ p*** < 0.005 ($n = 10$).

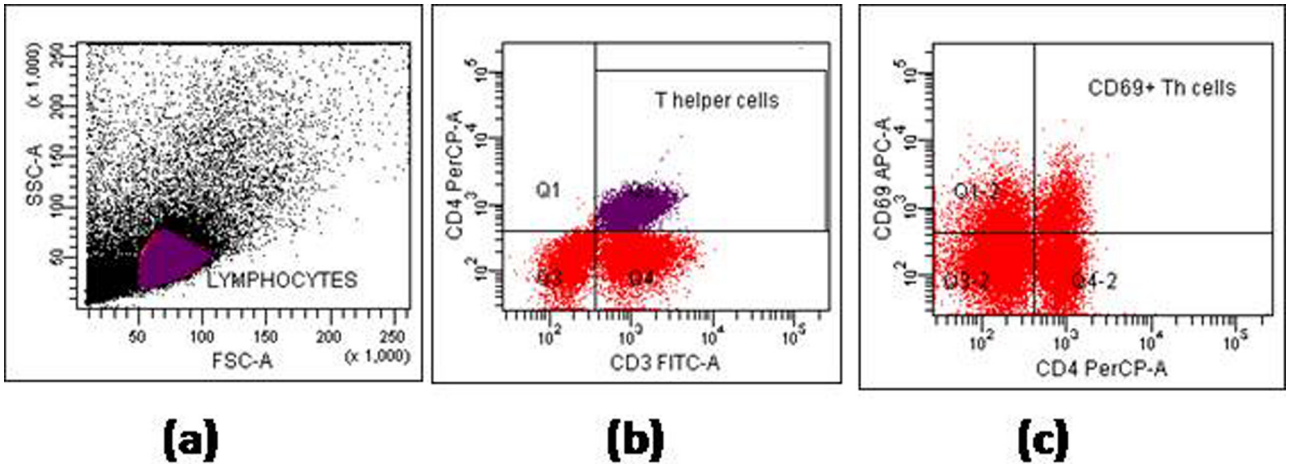


Fig. 9. Representing the gating criteria of CD69 expressing T helper cells in different study groups. Gating of PBMCs **(a)**, gating of CD3 + CD4 + Th cells from PBMCs **(b)** and gating of CD69 + Th cells **(c)**.

stimulation with WCS and PGL-1. Activation of Notch1 signaling significantly increased CD3/CD28 mediated expression of IFN-γ in response to PGL-1 in BL/LL cases (Fig. 12).

4.6. Notch1 signaling mediated regulation of IL-4 expression in T helper cells

CD3/CD28 mediated expression of Th2 cytokine IL-4 was analyzed in T helper cells after activation and inhibition of Notch1 signaling pathway in presence *M. leprae* antigens by flowcytometry (Fig. 13). Inhibition of Notch1 signaling pathway by GSI significantly decreased CD3/CD28 mediated expression of IL-4 in T helper cells in TT/BT patients and CD3/CD28 and Notch1 mediated expression in healthy controls. Further, inhibition of Notch signaling significantly decreased WCS mediated IL-4 expression in T helper cells in TT/BT patients and WCS and anti-Notch1 mediated IL-4 expression in T helper cells in HC and BL/LL patients. Activation of Notch1 signaling increased IL-4 expression in T helper cells in presence of PGL-1 in HC. Inhibition of Notch signaling decreased IL-4 expression in T helper cells mediated by PGL-1 and anti-Notch-1 antibody in TT/BT and BL/LL patients (Fig. 14).

5. Discussion

Notch1 signaling has been reported to play a role in regulation of T cell activation, proliferation and differentiation. Therefore, keeping these facts in consideration in the present study we tried to evaluate the role of Notch signaling in the regulation of T cell responses in leprosy. As TCR stimulation considerably increases expression of four Notch receptors within 24 h [7], expression of Notch1 and its ligands DLL-1, Jagged1 and Jagged2 on Th cells of leprosy patients and healthy controls after 24 h of activation with *M. leprae* antigens was analyzed. We found higher percentage of Notch1 expressing Th cells in TT/BT case than BL/LL cases which suggested that Notch1 may be playing a role in restriction of infection and regulation of immune response during leprosy. Percentage of DLL1 expressing Th cells was found significantly higher in TT/BT and BL/LL cases as compared to healthy controls which could be due to exposure of these patients to *M. leprae* antigens. Expression of Jagged1 on Th cells was induced after the stimulation with WCS in TT/BT cases which suggests that *M. leprae* antigens induces the expression of Jagged1 that could have a role in Th1 type of response seen in tuberculoid patients. Expression of Notch and its ligands was not evaluated on APCs and it will be interesting to study the same to correlate their role in favoring Th1/Th2 differentiation during leprosy.

There are several reports addressing the effect of Notch molecules on T cell proliferation, but so far reports are inconsistent. Some reports by different groups suggested that activation of Notch through its ligands induce tolerance or suppress T cell proliferation [6,11,14]. On the contrary two reports indicated that chemical γ-secretase inhibitor, which were inhibitors of Notch signaling decreased T cell proliferation [9,10]. It has also been reported that over expression of Notch1-IC enhances CD4 + T cell proliferation [9] Taking these facts in consideration for the first time role of Notch1 signaling in T cell proliferation in leprosy was evaluated. Inhibition of Notch1 signaling significantly decreased *M. leprae* antigen mediated lymphoproliferation in all categories of study subjects. In BL/LL cases activation of Notch1 signaling increased PGl-1 mediated lymphoproliferation and SI increased to more than 2. It could be inferred that Notch1 signaling pathway regulate lymphoproliferation in leprosy and lymphoproliferative response to PGL-1 in lepromatous leprosy.

Notch1 molecule has also been reported to play a role in Th1/Th2 differentiation. Some studies suggested role of notch in Th1/Th2 differentiation [11,16] In contrast to these studies, another study showed that CD4 + T cells lacking only Notch1 or Notch2 had no defect in Th2 differentiation, demonstrating that these Notch receptors are redundant in this context [17]. Notch signaling has also been linked to Th2 differentiation by other groups [18,19]. Potential role of Notch signaling in T cell activation has also been reported in few studies [11,14]. Overall discovery of contribution of Notch1 signaling in T cell activation and Th1/Th2 differentiation has opened a new pathway about the regulation of T cell responses in infectious diseases, therefore in the present study we tried to evaluate the role of Notch signaling in T cell activation and Th1/Th2 differentiation in leprosy. For this purpose CD3/CD28 and *M. leprae* antigen mediated expression of T cell activation markers CD25 and CD69 and expression of Th1/Th2 cytokines IFN-γ and IL-4 in Th cells was studied after activation and inhibition of Notch1 signaling pathway. GSI decreased CD25, CD69 and IFN-γ expression in *M. leprae* antigen activated Th cells (with or without anti Notch1 antibody) in leprosy patients and healthy controls. Inhibition of CD25 and CD69 expression of on T cells by blocking of Notch1 pathway has also been shown previously [10]. Further, activation Notch1 signaling was noted to enhanced T cell activation in response to WCS and PGL-1 in BL/LL cases by increasing expression of activation markers CD25 and CD69 on Th cells BL/LL cases in our study. Enhanced expression of CD25 after constitutive activation of Notch1 has also been reported [8]. IFN-γ expression in response to PGL-1 was also increased after activation of Notch1 signaling in BL/LL cases. Activation of Notch1 signaling did not increase IL-4 expression rather inhibition decreased *M. leprae* antigens mediated IL-4 expression in Th cells in leprosy patients.

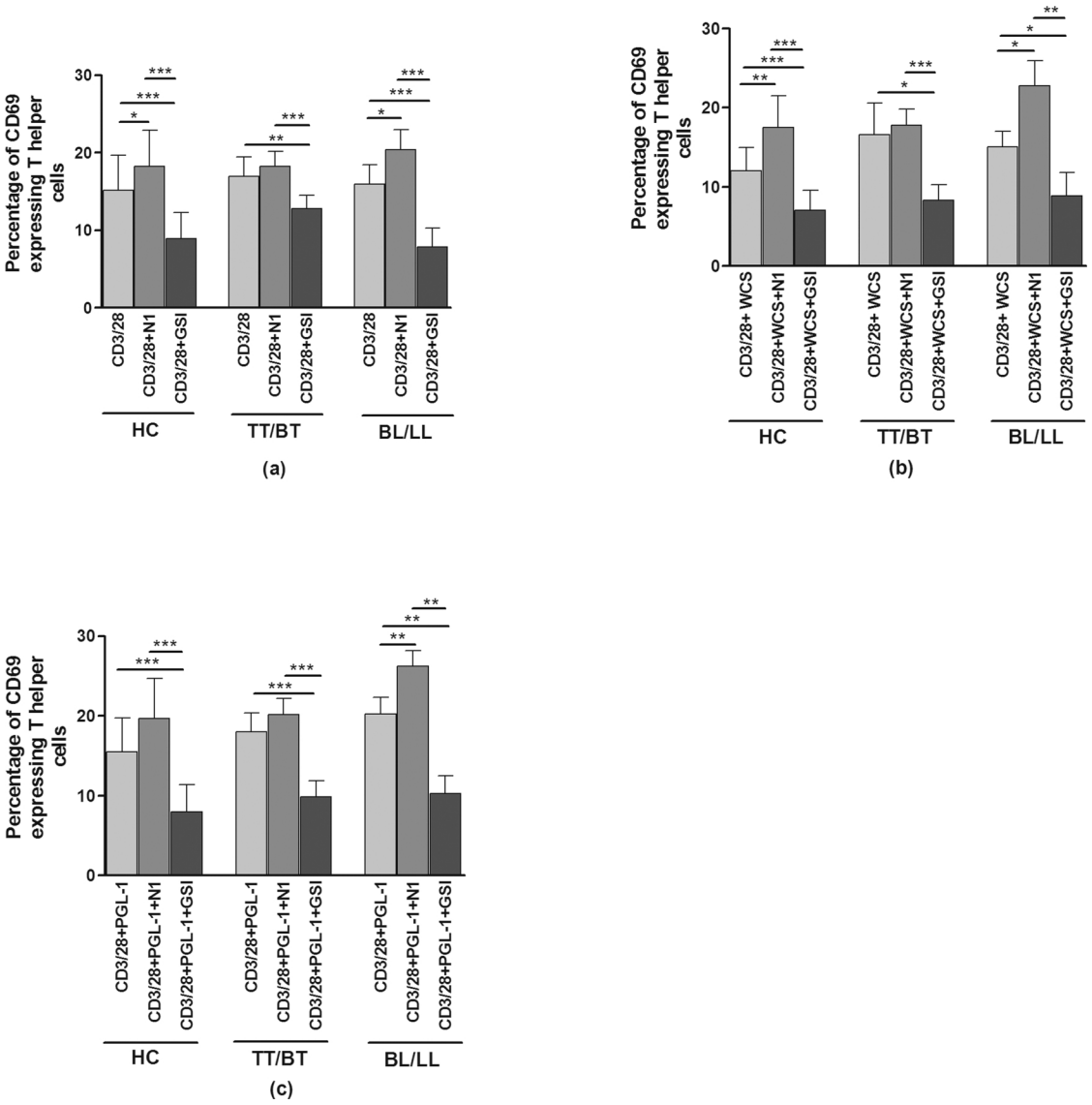


Fig. 10. Percentage of CD69 expressing T helper cells after activation and blocking of Notch1 signaling pathway in different study groups with or without stimulation with antigen. Notch1 signaling pathway was activated with plate bound anti-Notch1(N1) antibodies and inhibited by the treatment of cells with GSI in PBMCs of different study subjects and cells were incubated for 24 h with medium only **(a)**, with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)**. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti-CD69 antibodies and percentage of CD69 expressing Th cells were determined by flowcytometry. *p < 0.05**p < 0.01 p*** < 0.005 (n = 10).

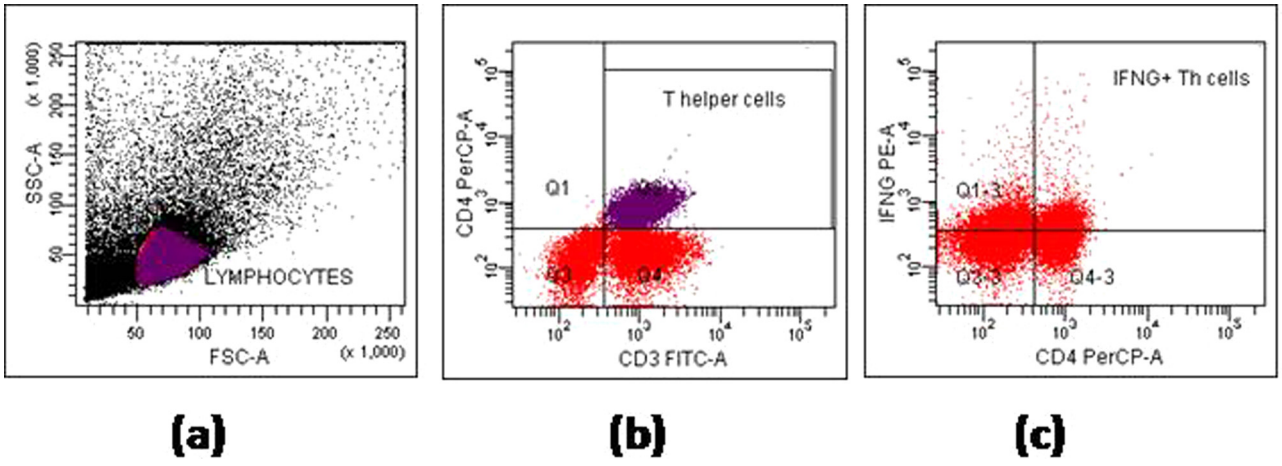


Fig. 11. Representing the gating criteria of IFN-γ expressing T helper cells in different study groups. Gating of PBMCs **(a)**, gating of CD3 + CD4 + Th cells from PBMCs **(b)** and gating of IFN-γ + Th cells **(c)**.

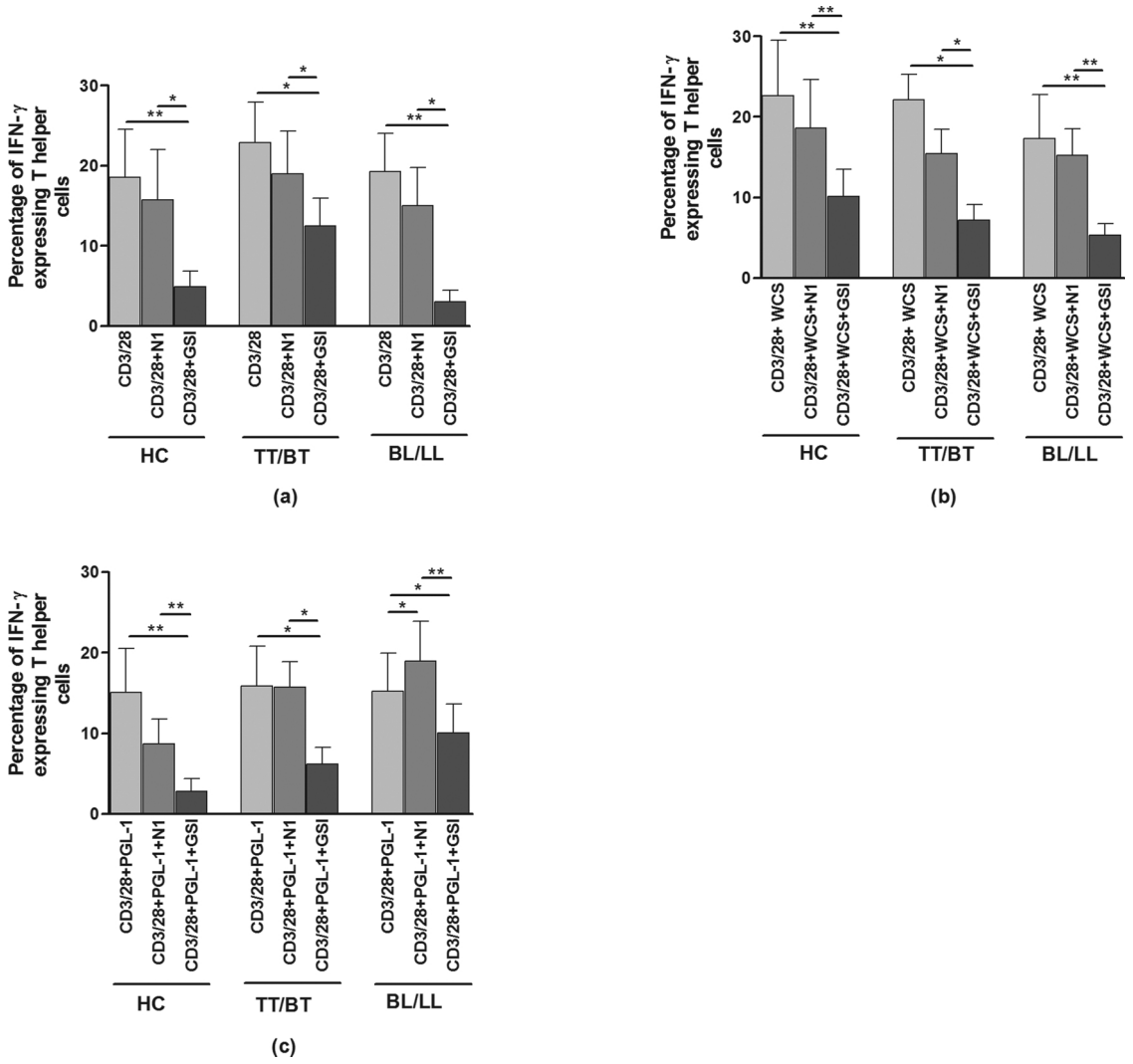


Fig. 12. Percentage of IFN-γ expressing T helper cells after activation and blocking of Notch1 signaling pathway in different study groups with or without stimulation with antigen. Notch1 signaling pathway was activated with plate bound anti-Notch1(N1) antibodies and inhibited by the treatment of cells with GSI in PBMCs of different study subjects and cells were incubated for 24 h with medium only **(a)**, with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)**. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti- IFN-γ antibodies and percentage of IFN-γ expressing Th cells were determined by flowcytometry. *$p < 0.05$**$p < 0.01$ ($n = 10$).

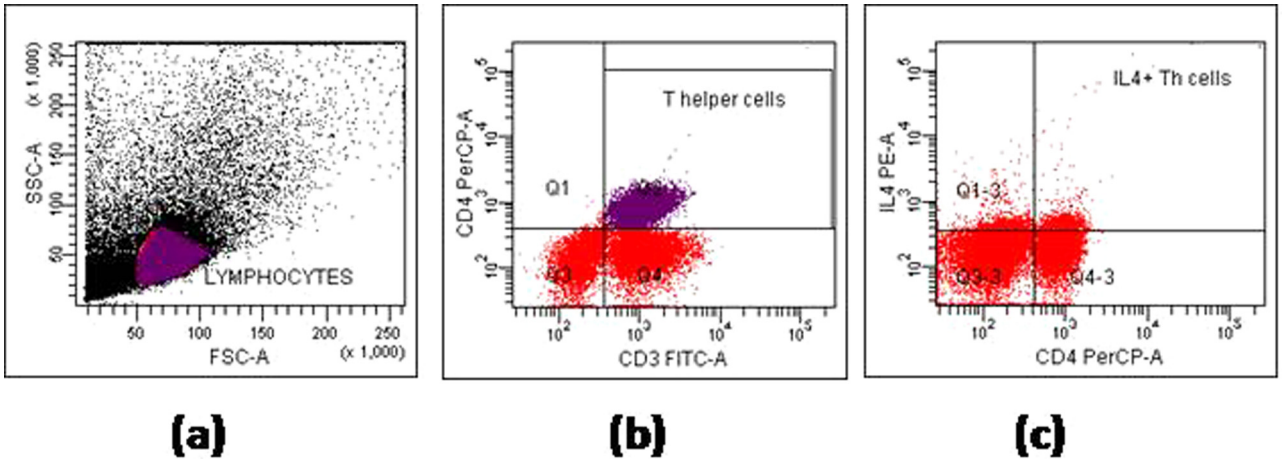


Fig. 13. Representing the gating criteria of IL-4 expressing T helper cells in different study groups. Gating of PBMCs **(a)**, gating of CD3 + CD4 + Th cells from PBMCs **(b)** and gating of IL-4 + Th cells **(c)**.

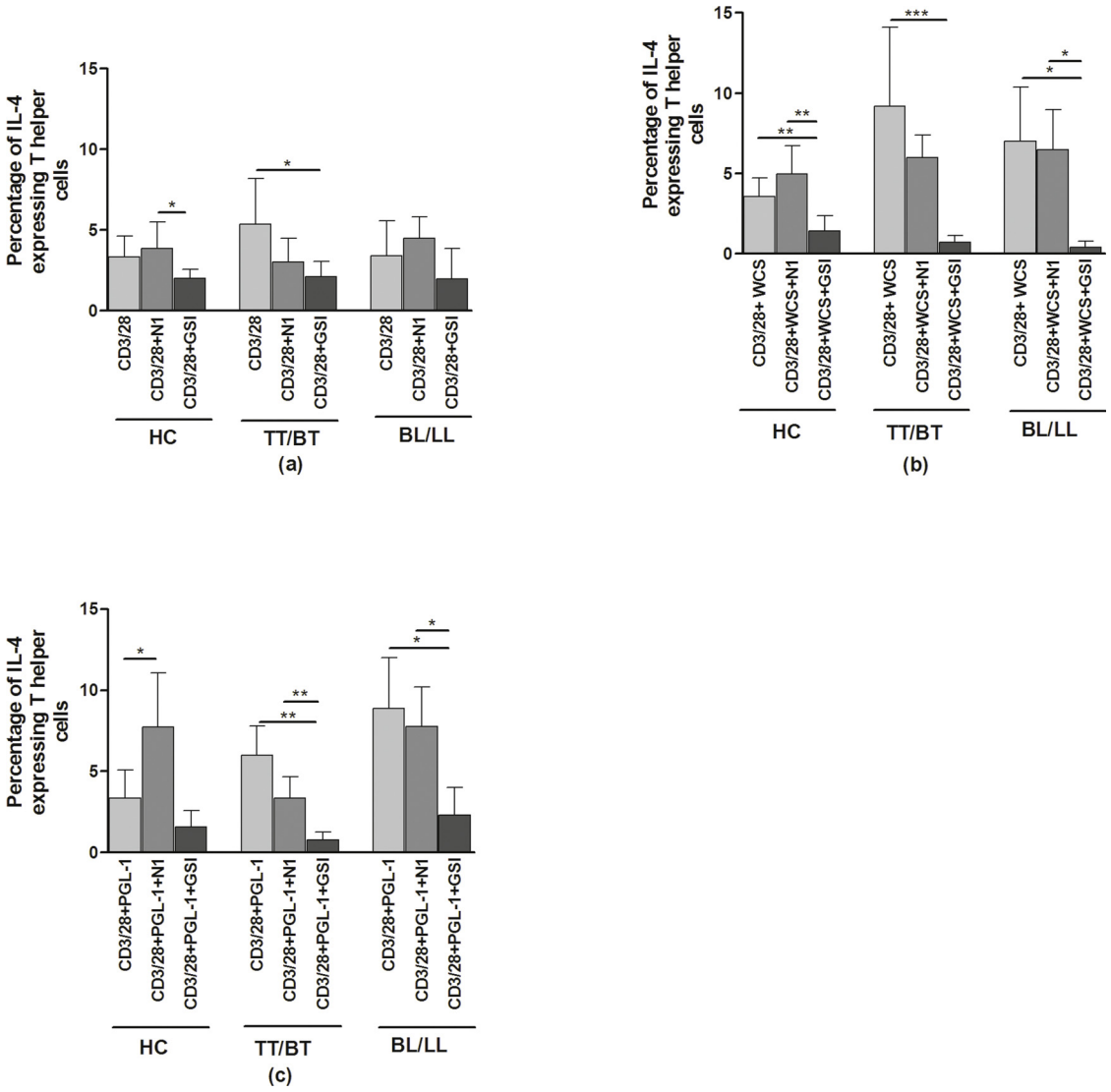


Fig. 14. Percentage of IL-4 expressing T helper cells after activation and blocking of Notch1 signaling pathway in different study groups with or without stimulation with antigen. Notch1 signaling pathway was activated with plate bound anti-Notch1(N1) antibodies and inhibited by the treatment of cells with GSI in PBMCs of different study subjects and cells were incubated for 24 h with medium only **(a)**, with whole cell sonicate of *M. leprae* **(b)**, with phenolic glycolipid-1 of *M. leprae* **(c)**. After incubation cells were washed and stained with fluorescent labeled anti-CD3, anti-CD4 and anti- IL-4 antibodies and percentage of IL-4 expressing Th cells were determined by flowcytometry. *p < 0.05**p < 0.01, p*** < 0.005 (n = 10).

6. Conclusions

Overall our findings suggest that activation of Notch1 signaling could help in restoring the cell mediated immune response in lepromatous patients as it promotes the expression of CD25, CD69 and IFN- γ –by Th cells of BL/LL cases and can evoke T cell activation and Th1 response. The role of Notch signaling in T cell activation or immunomodulation in lepromatous leprosy patients needs to be re-validated in future studies.

Funding

This work was supported by Indian Council of Medical Researches (ICMR) Delhi (Grant No.5/8/3(9)2009-ECD-1)

Conflict of interest

Authors have no conflict of interest.

Acknowledgements

We are grateful to Dr. P J Brennan, Colorado State University, USA for providing M. leprae antigens used in the present study. Authors acknowledge ICMR, Delhi for funding the present study. Help given by Mr. Hariram Dwivedi and Mr. Dalveer, Para Medical workers, Mr. Brajendra Singh, Medical Social Worker, Mr. MM Alam, Mr. Malikhan Singh Tomar and Mr. Ravi Rawat, Lab Technicians, Immunology Division, NJIL & OMD, Agra for sample collection is also acknowledged.

References

[1] R.L. Modlin, Th1-Th2 paradigm: insight from leprosy, J. Invest. Dermatol. 102 (1994) 828–832.
[2] T.S. Artanavis, M.D. Rand, R.J. Lake, Notch signaling: cell fate control and signal integration in the development, Science 284 (1999) 770–776.
[3] G. Struhl, I. Greenwald, Presenilin is required for activity and nuclear access of Notch in Drosophila, Nature 398 (1995) 522–525.
[4] S. Jarriant, C. Brau, F. Lageat, et al., Signaling downstream of activated mammalian Notch, Nature 377 (1995) 355–358.
[5] G.F. Hoyne, Roux I. Le, J.M. Corrin, et al., Serrate induced Notch signaling regulates the decision between immunity and tolerance made by peripheral CD4+ T cells,

Int. Immunol. 12 (2000) 177–185.
[6] G.F. Hoyne, M.J. Dallman, J.R. Lamb, Linked suppression in peripheral T cell tolerance to the house dust mite derived allergen Der p1, Int. Arch. Allergy Immunol. 188 (1999) 122–124.
[7] K.K. Wong, M.J. Carpenter, L.L. Young, et al., Notch ligation by Delta -1 inhibits peripheral immune response to transplantation antigen by a CD8+ cell-dependent mechanism, J. Clin. Invest. 112 (2009) 1741–1750.
[8] S. Vigouroux, E. Yvon, H.J. Wanger, et al., Induction of antigen specific regulatory T cells following over expression of Notch ligands by human B lymphocytes, J. Virol. 77 (2003) 10872–10880.
[9] S.H. Adler, E. Chiffloeau, L. Xul, et al., Notch signaling augments T cell responsiveness by enhancing CD25 expression, J. Immunol. 171 (2003) 2896–2903.
[10] T. Palaga, L. Miele, T.E. Gollde, et al., TCR mediated Notch signaling regulates proliferation and IFN-γ production in peripheral T cells, J. Immunol. 171 (2003) 3019–3024.
[11] Y. Maekawa, S. Tsusukomo, S. Chiba, et al., Delta1-Notch3 interaction bias the functional differentiation of activated CD4+ T cells, Immunity 19 (2003) 549–559.
[12] Y. Narayanna, K.N. Balaji, Notch1 upregulation and signaling involved in *Mycobacterium bovis* BCG induced SOCS-3 expression in macrophages, JBC 283 (2008) 12501–12511.
[13] E. Monsalvee, A.P. Miguel, R. Antonio, et al., Notch1 upregulation and signaling following macrophage activation modulates gene expression pattern known to affect antigen presentation capacity and cytotoxic activity, J. Immunol. 176 (2006) 5362–5373.
[14] T.N. Eagar, Q. Tang, M. Wolfe, et al., Notch1 signaling regulates peripheral T cell activation, Immunity 20 (2004) 407–415.
[15] A. Boyum, Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear ceels by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1g, Scand. J. Clin. Lab. Invest. 97 (1968) 77–89.
[16] D. Amsen, A. Antov, R.A. Flavell, The different faces of Notch in T-helper cell differentiation, Nat. Rev. Immunol. 9 (2009) 117–124.
[17] D. Amsen, A. Antov, D. Jankovic, et al., Direct regulation GATA3 expression determines the T helper differentiation potential role of Notch, Immunity 27 (2007) 89–99.
[18] T.C. Fang, Y.Y. Ohtani, C. De Biano, et al., Notch directly regulates GATA3 expression during T-helper2 differentiation, Immunity 27 (2007) 100–110.
[19] J.S. Yuan, P.C. Kousis, S. Suliman, et al., Function of Notch signaling in the immune system: consensus and controversies, Ann Rev Immunol. 28 (2010) 343–365.