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## Differential expression of CD1a and s-100 by dendritic cells in different forms of leprosy—possible role of dendritic cells in pathogenesis

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### Abstract

**Introduction:** Leprosy is one of chronic granulomatous disease that is caused by Mycobacterium leprae. This bacillus has a high affinity for skin and peripheral nerve cells. The clinical evolution of disease is determined by immune status of patients. Dendritic cells are one of the antigen-presenting cells (APCs) that play important role in initiating immune response to this pathogen. Langerhans cells and dermal dendrocytes are skin dendritic cells. These cells can be identified by using immunohistochemical markers such as CD1a and S-100.

**Aim:** To quantify the Langerhans cells and dermal dendrocytes by using immunohistochemical markers CD1a and S-100 in different forms of leprosy and to investigate the possible role of these dendritic cells in the pathogenesis of the disease.

**Methods:** In the present study, 30 skin samples from patients with tuberculoid (19 biopsies) and lepromatous (11 biopsies) leprosy were analyzed by using antibodies against CD1a and S-100.

**Results:** *CD1a immunostaining:* The mean number of positive Langerhans cells was  $3.4 \pm 0.7$  cells/hpf in tuberculoid leprosy (TT) and was  $0.4 \pm 0.5$  cells/hpf in lepromatous leprosy (LL). Dermal dendrocytes were also on higher side in TT with mean value of  $7.3 \pm 0.15$  and mean value of  $0.3 \pm 0.5$  cells in LL. (statistically significant,  $p=0.001$ ). *S-100 immunostaining:* The mean number of positive Langerhans cells was  $2.8 \pm 0.7$  cells/hpf in TT cases and was  $0.6 \pm 0.3$  in LL. The mean value of positive dermal dendrocytes was  $12.6 \pm 6.5$  cells in TT and  $1.5 \pm 2.3$  cells in LL. (statistically significant,  $p=0.001$ ). No significant difference was seen in Langerhans cell and dermal dendrocytes number between TT and BT and between LL and BL histological subtypes on CD1a and S-100 immunostaining.

**Conclusion:** Quantitative analysis showed a clear predominance of Langerhans cells and dermal dendrocytes in tuberculoid cases as compared to lepromatous cases. This indicates a role for dendritic cells in the cutaneous immune response and clinical evolution of leprosy.

**Keywords:** Leprosy, immunohistochemistry, langerhans cells, immunology

### Introduction

Leprosy is a chronic infectious disease that is caused by the obligate intracellular parasite, *Mycobacterium leprae*. This obligatory intracellular bacillus has a high affinity for skin and peripheral nerve cells. The disease can express itself in different clinicopathological forms depending on immune response of host<sup>[1]</sup>.

During infection by *M. leprae*, the bacillus is first processed by antigen-presenting cells i.e. macrophages and other antigen presenting cells (APCs) including dendritic cells<sup>[2]</sup>. A variety of cytokines are secreted. Depending on the type of cytokine secreted, T lymphocytes can either induce the development of milder disease<sup>[3]</sup> through a cell-mediated (Th1 type) response, or severe form of disease through humoral or Th 2 response<sup>[4, 5]</sup>. Th-1 cells produce IL-2 and IFN- $\gamma$  which activate macrophages<sup>[4, 6, 7]</sup>. The activated macrophages ingest and completely destroy the bacilli in tuberculoid (TT) and borderline tuberculoid forms (BT) (mild forms)<sup>[8, 9]</sup>. Humoral immune responses (TH2 type) predominate in midborderline (MB), borderline lepromatous (BL) and lepromatous leprosy (LL) (severe forms) with the production of cytokines that suppress the phagocytic response (IL-4 and IL-10)<sup>[10]</sup> and stimulates B lymphocytes to differentiate into plasma cells which produce immunoglobulins<sup>[11]</sup>.

Dendritic cells are antigen-presenting cells that possess numerous hair-like branched projections. In skin, dendritic cells are represented by epidermal Langerhans cells and dermal

dendrocytes. A number of surface markers are expressed by these cells; few of them are common such as CD1a and S-100. Langerhans cells present lipid antigen of *Mycobacterium leprae* to T cells with the help of CD1a and langerin [1]. dermal dendrocytes are found predominately in the papillary dermis and in the perivascular adventitial layer. So far very few studies have investigated the concomitant expression of these markers in skin lesions of patients with leprosy. The objective of the present study was to quantify the Langerhans cells and dermal dendrocytes by immunohistochemistry in different forms of the disease and to investigate the possible role of these dendritic cells in the pathogenesis of leprosy.

**Materials and methods**

**Study population**

Thirty patients with a clinical and histopathological diagnosis of leprosy were selected, irrespective of age and sex. 19(63%) cases had tuberculoid leprosy and 11(37%) had lepromatous leprosy.

**Histopathology and Immunohistochemistry**

The skin biopsies were immediately fixed in 10% formalin, processed routinely and was embedded in paraffin blocks. Sections of 2-3µm thickness were cut and were subjected to haematoxylin and eosin (H&E) staining, modified Ziehl-Neelsen and anti-CD1a and anti-S-100 immunostaining. The final diagnosis was made on the basis of histopathological criteria, bacilloscopy and clinical characteristics of the patient as proposed by Ridley and Jopling.

**Quantification of CD1a and S-100 positive dendritic cells**

The epidermal (Langerhans) and dermal cells showing cytoplasmic positivity for anti CD1a and nuclear and cytoplasmic positivity for anti-S-100 were taken positive. The number of epidermal and dermal cells expressing CD1a and S-100 was evaluated using a conventional optical microscope in all 30 cases. In the epidermis, the total number of positive cells was counted using a large field (40 X) objective and the average CD1a+ and S-100 + cells per high power field was calculated and the results were interpreted as Mean ± SD. S-100 positive cells on the basement membrane were excluded as melanocytes also show positivity for the same. In the dermis, the number of positive cells was evaluated by counting their total number in the whole biopsy section.

**Statistical analysis**

Anova and Post hoc test were applied for statistical analysis of the observed data.

**Results**

**Clinical characteristics**

Out of 30 patients, 23 (76%) were males and 7 (24%) were females. The mean age of presentation was 33.7 years. The most common presentation was hypopigmented patch (53.3%). Other common complaints were erythematous patch, nodules and macules. The lesions in all the 30 cases were either hypoaesthetic or anaesthetic.

**Histopathological findings**

Histopathological analysis of tuberculoid cases showed characteristic epithelioid cell granulomas with peripheral

cuffing by lymphocytes and langhans giant cells. Lepromatous forms showed sheets and granulomas of parasitized foamy histiocytes. Globii also noted at places (Figure 1).

**Immunohistochemical findings**

**a) Quantification of CD1a and S-100 positive Langerhans cells**

The use of CD1a and S-100 markers revealed the presence of Langerhans cells in the epidermis. These cells had an irregular appearance and long and thin cytoplasmic prolongations and were most often localized in the suprabasal layers between keratinocytes.

Quantitative analysis of CD1a and S-100 immunostaining showed larger mean number of positive Langerhans cells i.e.  $3.4 \pm 0.7$ cells/hpf and  $2.8 \pm 0.7$  cells/hpf respectively in patients with tuberculoid leprosy (TT). The mean value for CD1a and S-100 positive cells was  $0.4 \pm 0.5$ cells/hpf and  $0.6 \pm 0.3$ cells/hpf respectively in lepromatous leprosy (LL) form. The difference between TT and LL was statistically significant for both CD1a and S-100 ( $p = 0.001, 0.031$  respectively) (Table 1) (Figure 2 & 3). However the difference between tuberculoid (TT) and borderline tuberculoid (BT) and between lepromatous (LL) and borderline lepromatous (BL) cases was not statistically significant.

**b) Quantification of CD1a and S-100 positive Dermal dendrocytes**

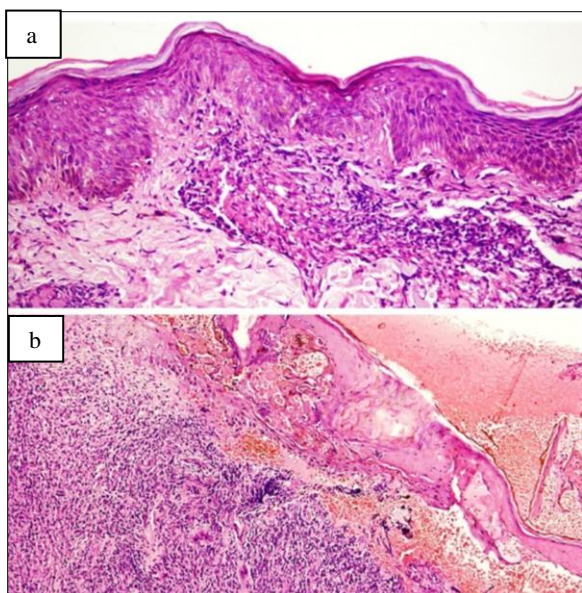
Dermal dendrocytes expressing CD1a and S-100 were also evaluated and quantified. These cells were aggregated in the papillary dermis which is often localized around superficial capillaries and around granulomas. Although the dermal CD1a and S-100+ dermal dendrocytes also varied in number, their expression followed the same pattern as that observed in the epidermis with mean value of  $7.3 \pm 03.2$  CD1a positive cells and  $12.6 \pm 6.5$  S-100 positive cells in tuberculoid leprosy (TT). The mean value was  $0.31 \pm 0.5$  cells for CD1a and  $1.5 \pm 2.3$ cells for S-100 in lepromatous leprosy (LL) form. The difference was statistically significant for both the markers ( $p= 0.000, 0.001$  respectively) (Table 2) & (Figure 2 & 3). However no significant difference was seen in the expression between tuberculoid (TT) and borderline tuberculoid (BT) and between lepromatous (LL) and borderline lepromatous (BL) histological subtypes.

**Table 1:** CD1a and S-100 positive Langerhans cells per high power field (Mean ± SD)

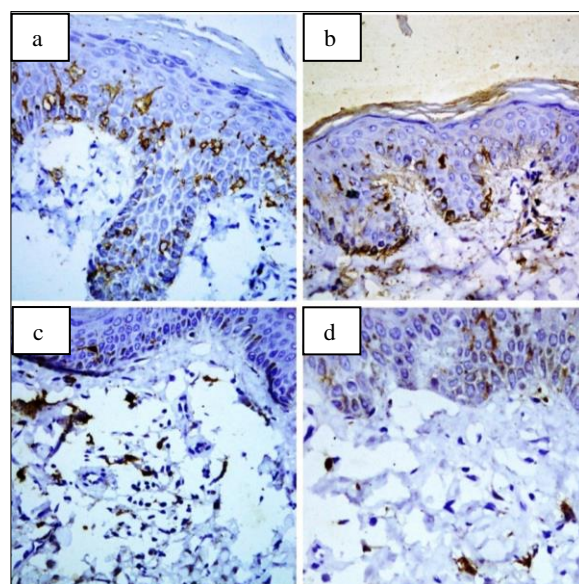
Histopathological subtype	CD1a	S-100
Tuberculoid leprosy	$3.4 \pm 0.7$	$2.8 \pm 0.7$
Borderline tuberculoid	$3.1 \pm 1.1$	$2.6 \pm 1.1$
Borderline lepromatous	$1.1 \pm 0.6$	$1.1 \pm 0.8$
Lepromatous leprosy	$0.4 \pm 0.5$	$0.6 \pm 0.3$

**Table 2:** CD1a and S-100 positive dermal cells (Mean ± SD)

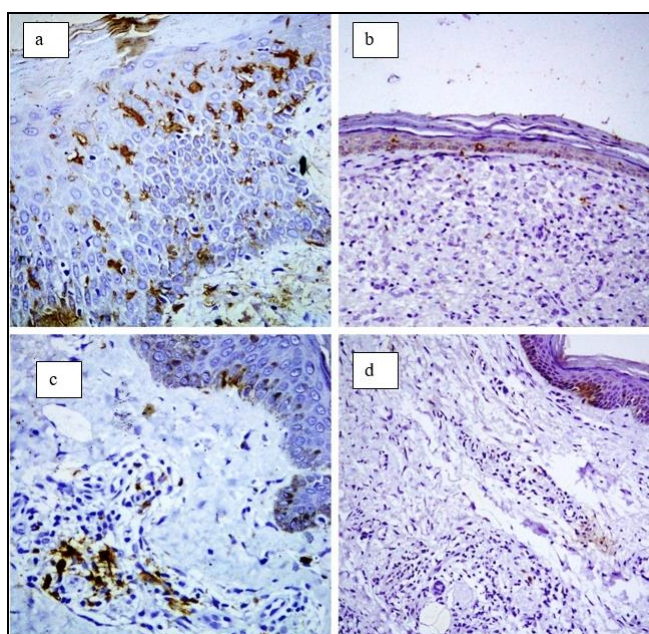
Histological subtype	CD1a	S-100
Tuberculoid leprosy	$7.3 \pm 3.2$	$12.6 \pm 6.5$
Borderline tuberculoid	$2.5 \pm 1.$	$4.6 \pm 3.5$
Borderline lepromatous	$0.4 \pm 0.5$	$0.3 \pm 0.5$
Lepromatous leprosy	$0.3 \pm 0.5$	$1.5 \pm 2.3$



**Fig 1:** a) Tuberculoid leprosy-Characteristic epithelioid cell granulomas with lymphocytes, in the dermis; granulomas encroaching the epidermis (H&E; X40). b) Lepromatous (histoid) leprosy- Sheets of spindle shaped histiocytes in the dermis (H&E; X40).



**Fig 2:** CD1a immunohistochemistry pattern. CD1a positive Langerhans cells in tuberculoid leprosy (a) (IHC; X40) and lepromatous leprosy (b) (IHC; X40). CD1a positive dermal dendrocytes in tuberculoid leprosy (c) (IHC; X40) and lepromatous leprosy (d) (IHC; X40).



**Fig 3:** S-100 immunohistochemistry pattern. S-100 positive Langerhans cells in tuberculoid leprosy (a) (IHC; X40) and lepromatous leprosy (b) (IHC; X40). S-100 positive dermal dendrocytes in tuberculoid leprosy (c) (IHC; X40) and lepromatous leprosy (d) (IHC; X20).

**Discussion**

Leprosy is a chronic infectious disease of great epidemiological importance. The immune response of individual determines the manifestation of disease. It has been documented that APCs of skin i.e. Langerhans cells depending on numbers, are able to initiate the effector mechanisms towards specific T helper responses [12, 13]. Dermal dendrocytes are normally present mainly in the papillary dermis around blood Vessels [14]. According to Pagliari and Sotto, this perivascular location is ideal for the interaction with T lymphocytes and initiating an immune response [15]. So, present study was conducted to quantify Langerhans cells and dermal dendrocytes by immunohistochemistry and to study their possible role in

pathogenesis of leprosy.

The study showed statistically significant higher value of CD1a+ Langerhans cells at tuberculoid pole as compared to lepromatous pole ( $3.4 \pm 0.7$  cells/hpf and  $0.4 \pm 0.5$  respectively;  $p = 0.001$ ). These findings correlated well with those observed in various studies by Sieling *et al.* [9], Miranda *et al.* [16] and Kelly *et al.* [17] Queresma *et al.* [18] who investigated the role of dendritic cells in the pathogenesis of leprosy, also showed similar results. CD1a + dermal dendrocytes were also found to be increased at tuberculoid pole as compared to lepromatous pole and difference was statistically significant. These findings were consistent with studies done by Miranda *et al.* [16] These studies showing increased number of CD1a positive

Langerhans cells and dermal dendrocytes in tuberculoid leprosy indicates the role of these cells in evolution of disease towards benign forms and vice versa. The current study also showed increased number of S-100 positive Langerhans cells and dermal dendrocytes in tuberculoid pole cases as compared to lepromatous pole. This difference between the two poles was statistically significant. The epidermal findings were in concordance with study done by Azadeh *et al.* [19] but the dermal dendrocytes were not increased in their study which was not in concordance with our findings.

Further work in this field is required for better understanding of role of each of these antigen-presenting cell populations. However, the quantitative differences observed in different histological forms indicate an effective participation of skin dendritic cells in the immune response to *M. leprae* and, consequently, in the evolution of the disease. Secondly, by targeting these nonpeptide antigens to LCs, vaccine could be made for the generation of immunity to *M. leprae*.

**Conflict of interest:** There is no conflict of interest to this study.

**Funding:** Cost to this study is nil.

**Ethical consideration:** The study was conducted on 30 clinically and histologically diagnosed cases of leprosy. Patients were not subjected to any additional procedure for the purpose of study. The study was conducted on ethical guidelines for biomedical research on human subject as given in "Declaration of Helsinki" and by Central Ethics Committee on Human Research (CECHR) of ICMR, New Delhi.

**Patient consent:** Patients were informed beforehand that biopsy is a routinely established procedure with no additional risk. Confidentiality will be maintained.

**Authorship and contributorship:** Amrita- data collection, analysis; RPS punia-interpretation of data, Deepak basia-helped in finalising draft.

**Data availability statement:** The supporting data is not shared

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