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Mast Cell Profile and IL-4 Levels in Borderline Spectrum of Leprosy

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Abstract

Introduction: Mast cells are responsible for secreting cytokines and other chemical mediators, involved in immuno-inflammatory responses. However, their role has not been clearly defined in the immunopathogenesis of leprosy. *Aims and Objectives:* To count the number of mast cells in leprosy granulomas and to correlate their numbers with the serum levels of IL-4 in borderline spectrum of leprosy. *Materials and Methods:* Thirty cases of newly diagnosed, untreated patients of borderline tuberculoid (BT), borderline borderline (BB) and borderline lepromatous (BL) leprosy were included in the study. Skin biopsies were taken from the margins of the skin lesion of leprosy and stained with toluidine blue for quantification of mast cells. An average number of mast cells per granuloma was calculated after examining the granulomas in each skin biopsy. Concentration of serum IL-4 was measured quantitatively in the collected venous blood samples by ELISA method. *Results:* The mean of average number of mast cells increased from BT towards BL leprosy. Statistically significant difference was seen between BL and BT/BB leprosy. However, no significant difference was found between BB and BT Hansen's. A positive correlation was found between mast cell numbers and serum levels of IL-4 since both showed an increasing trend from BT through BL leprosy. *Conclusion:* We found a progressive rise in mast cell count and serum levels of IL-4 across the borderline spectrum of leprosy. This indicates that mast cells play a role in progression and dissemination of leprosy by proliferating at the site of inflammation and secreting an array of chemical mediators especially IL-4.

Keywords: Borderline Leprosy; Mast Cells; Granuloma; Interleukin 4; Tuberculoid; Lepromatous.

Introduction

Leprosy is a chronic inflammatory disease caused by Mycobacterium leprae, an obligate intracellular pathogen principally affecting peripheral nerves and skin [1].

It is diagnosed by the presence of one or more of the three cardinal signs of leprosy which include; definite loss of sensation in a hypopigmented or reddish skin patch, a thickened peripheral nerve, with loss of sensation and/ or weakness of the muscles supplied by that nerve and presence of acid-fast bacilli in slit skin smear [2]. T- lymphocytes are a major source of cytokines which play a key role in immunologic, inflammatory and reparative host responses in leprosy. Further, few previous studies suggest role of mast cells in the pathogenesis and progression of leprosy [3-8].

Mast cells are derived from the myeloid stem cells and are extensively distributed in the skin, gastrointestinal tract, upper and lower respiratory tracts. They are involved in allergic inflammation and immuno-inflammatory responses [9]. Mast cells are seen in small numbers in the granulomas of leprosy and are responsible for secreting cytokines, mainly tumour necrosis factor- α (TNF-α) and interleukin-4 (IL-4), and other mediators (tryptase, histamine, thromboxane, prostaglandin D2 etc) [10]. The close proximity of mast cells to the peripheral nerve fibres in the tissues alongwith the shortening of the distance between mast cells and nerve fibres during inflammatory events, suggest a functional interaction between these two components [11,12]. In the present study, we attempted to count the number of mast cells in the leprosy granulomas in borderline spectrum of leprosy and correlate it with serum levels of IL-4.

Materials and Methods

Thirty newly diagnosed, untreated patients of borderline leprosy including tuberculoid (BT), mid-borderline (BB) and borderline lepromatous (BL) as per the classification of Ridley and Jopling [13], attending the urban leprosy centre of our institute from November 2013 to February 2015 were included in the study. The study was approved by institutional ethics committee. Patients in lepra reactions, pregnant and lactating women, and patients already receiving or those who had received specific treatment for leprosy in the past were excluded. Equal number of consenting healthy, age and sex-matched volunteers were taken as controls. Informed and bilingual consent was taken from the patients and controls before inclusion in the study. A skin biopsy was taken from the margin of a representative skin lesion from the patients. The specimens were placed in 10% formalin solution and sent to the pathology department for haemotoxylin and eosin (H & E) staining for diagnosis of leprosy and toluidine blue staining for quantification of mast cells. Mast cells were visualized as violet to reddish purple cells against a blue background [Figure 1]. Mast cells were counted under 400 × magnification in ten sequentially observed granulomas, and an average number of mast cells per granuloma was calculated for each case. The mean of these values for subset cases of BT, BB and BL leprosy were also determined.

Ten ml venous blood samples of patients and controls were collected in plain vacutainer tubes and allowed to stand for 30 minutes at room temperature and then centrifuged at 300 g for 5 minutes. Sera were immediately separated and stored at -20^{0}C until the time of analysis for the cytokine levels. Concentration of serum IL-4 was measured quantitatively in the collected serum samples by the sandwich enzyme-linked immunosorbent assay (ELISA) method (Human IL-4 ELISA kit from Krishgen BioSystems).

Statistical Analysis

Quantitative data were presented as mean ± SD or median and interquartile range, as appropriate. Normality of data was checked by measures of Kolmogorov Smirnov tests of normality. For normally distributed data means of 3 groups were compared using One-Way ANOVA followed by Post Hoc Multiple Comparisons test. For skewed data Kruskal-Wallis test followed by Mann-Whitney test for two groups was applied. For categorical variables; number & percentages were calculated. Chi-square test or Fisher's exact (whichever appropriate) test was applied for categorical data. Spearman's correlation coefficient was applied to see relationship between different variables. All calculations were two-sided & were performed using SPSS version 17 (Statistical Packages for the Social Sciences, Chicago, IL). A "p" value of <0.05 was considered to indicate statistical significance.

Results

The study group comprised of 12 patients of BT, 8 patients of BB and 10 patients of BL leprosy. There were 21 (70%) males and 9 (30%) females. The mean age was 32.51±1.78 years among cases and 31.17±11.74 years among the controls. The highest number of mast cells was found in BL leprosy (mean of 9.45±2.26 cells per granuloma), intermediate in BB (3.50±1.07 cells per granuloma) and lowest in BT patients (mean of 2.81±0.94 cells per granuloma) [Figure 2].

On statistical evaluation [Table 1], significant difference was seen when the mean of average number of mast cells in BL was compared with those in BT and BB; however, the difference between BB and BT leprosy was statistically insignificant.

The mean serum levels of IL-4 were highest in patients with BL (76.29 ± 37.25 pg/ml) leprosy with intermediate levels in BB patients (22.99 ± 12.93 pg/ml) and lowest in patients with BT disease (4.12 ± 2.05 pg/ml), as shown in Table 2. On comparing mean serum levels of IL-4 of the three subsets of patients, a statistically significant difference was seen across the entire borderline spectrum (BT vs. BB, p = 0.001; BB vs. BL, p = 0.003; and BL vs. BT, p =0.000). Further, statistically significant difference was seen

Table 1: Comparison of mean of average number of mast cells between different groups of leprosy

Group	Mean ± SD	p value
BT vs. BB	2.81±0.94 vs. 3.50±1.07	**0.634**
BL vs. BB	9.45±2.26 vs. 3.50±1.07	**0.038**
BL vs. BT	9.45±2.26 vs. 2.81±0.94	**0.007**

BB – mid-borderline; BL – borderline lepromatous; BT – borderline tuberculoid; SD – standard deviation

Table 2: Comparison of serum levels of IL-4 between different groups of leprosy and healthy controls

Group	Serum IL-4 levels: Mean ± SD (pg/ml)	P value
BT vs. BB	4.12 ± 2.05 vs. 22.99 ± 12.93	0.001
BL vs. BB	76.29 ± 37.25 vs. 22.99 ± 12.93	0.003
BL vs. BT	76.29 ± 37.25 vs. 4.12 ± 2.05	0.000
BT vs. healthy controls	4.12 ± 2.05 vs. 4.48 ± 2.82	0.889
BB vs. healthy controls	22.99 ± 12.93 vs. 4.48 ± 2.82	0.000
BL vs. healthy controls	76.29 ± 37.25 vs. 4.48 ± 2.82	0.000

BB – mid-borderline; BL – borderline lepromatous; BT – borderline tuberculoid; IL – interleukin; SD – standard deviation

Table 3: Comparison of the findings of different published studies on mast cell count in subsets of leprosy

Author(s)	Number of Patients (n), Controls, and profile of patients	Mast cell staining technique	Relative mast cell density
Current study	n=30 BT-12, BB-8, BL-10	Toluidine blue	Progressive increase along the spectrum from BT to BL
Rav *et al* [3]	n=250 TT-50, BT-22, BB-12, BL-20, LL-36, IL-110	Toluidine blue	Progressive rise from TT to LL
Aroni *et al* [4]	n=28 TT-2, BT-7, BL-4, LL-11, HL-4	Chloroacetate esterase	Lower in tuberculoid than lepromatous group
Mysorekar *et al* [5]	n=118; controls=20 IL-25, TT-21, BT-20, BB-14, BL-20, LL-18	Toluidine blue	Progressive increase from TT to LL
Bagwan *et al* [6]	n=53; controls=7 IL-6, TT-9, BT-24, BB-1, BL-6, LL-7	Toluidine blue	Progressive increase from TT to LL
Sherif ES [7]	n=60 IL-1, TT-22, BT-13, BB-13, BL-5, LL-6	Giemsa	Progressive increase from TT to LL
Magalhaes *et al* [8]	n=51 BT-17, BB-17, BL+LL-17	Anti-tryptase antibody	BB and BT had higher density than BL+LL
Naik *et al* [14]	n=60	Toluidine blue	Progressive increase from IL to both polar TT and LL
Chatura *et al* [18]	n=50 TT-5, BT-24, BB-3, BL-10, LL-8	Fite-Faraco	Highest counts in BT and BL vs. their polar counterparts
Joshi *et al* [19]	n=37 IL-6, TT-3, BT-18, BB-2, BL-3, LL-5	Toluidine blue	BT>BL

BB – mid-borderline; BL – borderline lepromatous; BT – borderline tuberculoid; HL – histoid leprosy; IL – indeterminate leprosy; LL – lepromatous leprosy; TT – polar tuberculoid (leprosy)

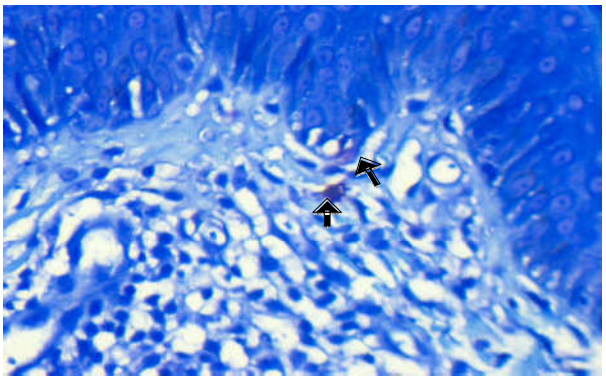

Fig. 1: Mast cells on toluidine blue staining, highlighted by black arrows (400 ×)

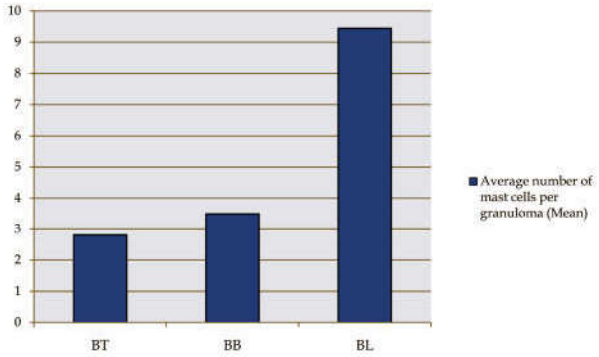

Fig. 2: Mean of average number of mast cells in each subset of leprosy

between mean serum levels of IL-4 of healthy controls and patients with BB and BL leprosy ($p<0.05$); but not with the BT patients ($p=0.889$). In our study, a trend of progressive increase in number of mast cells as well as serum levels of IL-4 was seen from BT to BL leprosy. On statistical evaluation, a positive correlation was found between mast cell count and mean serum levels of IL-4 ($r=0.664$, $p<0.05$).

Discussion

Mast cells are 'dynamic cells' with a central role

in allergic inflammation, protective immune response and other inflammatory responses. They are found at all strata of the skin including the dermis, around blood vessels, nerves, appendages, at dermoepidermal junction (DEJ) and also in subcutaneous tissue [9,14,15]. Mast cells can be stimulated to degranulate by direct injury, chemical agents like opioids and alcohol, certain antibiotics such as polymyxins or by cross-linking of immunoglobulin E (IgE) receptors and complement proteins [16]. Mast cells also produce IL-4, which induces naïve CD4+ T cells to differentiate into Th_2 cells while suppressing the development of Th_1 cells. They also suppress macrophage cytotoxic activity, parasite killing, and macrophage-derived nitric oxide production [17].

In this study, we found a difference in the number of mast cells in granulomas of different subsets of leprosy, with maximum number of mast cells in BL patients, lesser in BB cases and lowest in BT cases. Our findings are in agreement with findings of the study by Rav *et al* in 1990 who included 250 patients of leprosy and reported a decrease in mast cell density from lepromatous to the tuberculoid end of the spectrum [3]. They additionally reported mast cells in 100% cases of indeterminate leprosy, thereby suggesting the role of mast cells in evolution and progression of the disease [3]. Aroni *et al* in 1993 also reported a significantly lower mast cell count in tuberculoid group than the lepromatous group ($p<0.001$) [4]. They suggested that the larger number of mast cells in lepromatous group may be due to increased vascularity and changes in the endothelial cells. Mast cell mediators are also known to be mitogenic for fibroblasts and endothelial cells. Thus, mast cells may also have a role in fibroblast proliferation that occurs after a lepra reaction [4]. The above findings were corroborated by Mysorekar *et al* in 2001, Bagwan *et al* in 2004, and Sherif ES in 2008 [5-7].

However, many workers have reported contradictory findings. Magalhaes *et al* found maximum density of mast cells in BB patients (73.64/mm^2) followed by BT (65.06/mm^2) and BL (50.43/mm^2) [8]. Further, Naik *et al* in 2003 reported lower density of mast cells in BT (37/mm^2) and BL (46/mm^2) leprosy than their polar counterparts (TT-51/mm^2, LL-49/mm^2) [14]. They suggested that periodic follow up of indeterminate and borderline lesions for mast cells might help in predicting the stability of lesions [14]. Similarly, Chatura *et al* in 2012 reported a progressive decrease in mast cell density along the borderline spectrum of leprosy [18]. Recently, Joshi *et al* and reported higher number of mast cells in BT leprosy than in BL leprosy (BT-105.56/mm^2, BL-100/mm^2) [19]. The reason for contradictory findings of these studies and the current study may be explained on the basis of the assertion put forward by Magalhaes *et al*. They suggested that such discrepancies might stem from the difference in the mast cell staining methods and mast cell counting technique adopted in different studies [8]. A summary of these studies has been tabulated [Table 3].

We also found a progressive rise in IL-4 levels from BT to BL leprosy, suggesting the role of IL-4 in disease progression with Th_2 activation. In our study, a trend of progressive increase in number of mast cells as well as the serum levels of IL-4 was seen from BT to BL leprosy. On statistical evaluation, a positive correlation was found between mast cell numbers and IL-4 ($r=0.664$, $p<0.05$). A few previous studies have also reported progressive rise in serum levels of IL-4 along the borderline sprectum of leprosy [7,20,21].

It is known that high levels of IL-4 in a Th_2 predominant milieu promotes further mast cell proliferation, thereby resulting in positive feedback for further IL-4 secretion. To summarise the results from our study and the discussed literature, it seems certain that mast cells play a role in leprosy by proliferating at the site of inflammation and secreting cytokines like IL-4 leading to Th_2 response, cell damage, inflammation, fibrosis, culminating into progression and downgrading of the disease.

Conclusion

In the current study, a progressive rise in mast cell count across the borderline spectrum of leprosy with highest counts in BL leprosy was seen. This suggests a probable role of mast cells in downgrading of leprosy. The high levels of IL-4 down the spectrum of borderline leprosy suggest its role in dissemination of disease while promoting a Th2 response.

The correlation between IL-4 levels and mast cell numbers may suggest that mast cells are a major source of this cytokine in leprosy. Although the findings of this study suggest that mast cell stabilizers and anti-IL-4 therapy may have a therapeutic role in prevention of disease worsening, this hypothesis has not yet been studied. Further studies are mandated to understand the complex mechanisms involved in downgrading of leprosy and should take into account other immune cells and cytokine mediators.

References

1. Sasaki S, Takeshita F, Okuda K, Ishii N. Mycobacterium leprae and leprosy: a compendium. Microbial Immunol 2001;45:729-36.
2. World Health Organization Regional Office for South-East Asia New Delhi. Enhanced Global strategy for further reducing the leprosy burden and sustaining leprosy control activities 2006-2010. Operational guidelines. Lepr Rev 2009;78:17-8.
3. Rav SD, Pratap VK, Sharma NK, Dayal SS. Mast cell in leprosy. Indian J Lepr 1990;62:467-72.
4. Aroni K, Kontochristopoulus G, Liossi M, Panteleos D. An investigation of mast cells in two basic leprosy groups. Int J Lepr 1993;61:634-5.
5. Mysorekar VV, Dandekar CP, Rao SG. Mast cells in leprosy skin lesions. Lep Rev 2001;72:29-34.
6. Bagwan IN, Khandekar MM, Khadana P, Jadhav MV, Deshmukh SD. A study of mast cells in granulomatous lesions of the skin, with special emphasis on leprosy. India J Lepr 2004;76:31-7.
7. Sherif ES. Role of mast cells and cytokine profile [TNF-α, IFN- α, IL-4 and IL-4 mRNA] in different types of leprosy. Available from: https://elsadany66.wordpress.com/article/role-of-mast-cells-and-cytokine-profile-i2p6c6e8rrui-3/ [Last accessed on August 16, 2016].
8. Magalhaes GO, Valentim VC, Pereira MJS, Nery JAC, Illarramendi X, Antunes SLG. A quantitative and morphometric study of tryptase-positive mast cells in cutaneous leprosy lesions. Acta Tropica 2008; 105:62-6.
9. Metcalfe DD, Baram D, Yoseph AM. Mast cells. Physiol Rev 1997; 77:1033-79.
10. Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. Intestinal mucosal mast cells in normal and nematode infected rat intestines are in intimate contact with peptidergic nerves. Proc Natl Acad Sci USA 1987;84:2975-9.
11. Antunes LG, Liang Y, Costa Neri JA, Sarno EN, Frendscho MH, Johansson O. Mast cell subsets and neuropeptides in leprosy reactions. Arq Neuropsiquiatr 2003;61:208-19.
12. Longley J, Duffy TP, Kohn S. The mast cell and mast cell disease. J Am Acad Dermatol 1995;32:545-61.
13. Ridley DS, Jopling WH. Classification of leprosy according to immunity; A five group system. Int J Lepr 1966;34:255-73.
14. Naik R, Pai MR, Bantwal PB, Narayan S, Nayak KS, Gadhi A. Study of mast cells in non neoplatic skin lesions. Indian J Pathol Microbiol 2003;46:173-5.
15. Galli SJ, Metcalfe DD, Arber DA, Dvorak AM. Basophils, Mast Cells, and Related Disorders. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Oliver W. Press OW, Burns LJ, Caligiur M, editors. Williams Hematology, 9th ed. New York: Mc Graw Hill 2015.p.965-82.
16. Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2003;111(2 Suppl):486-94.
17. Wang P, Wu P, Siegel ML, Egan RW, Billah MM. Interleukin (IL)-10 inhibits nuclear factor kB (NF-kB) activation in human monocytes: IL-10 and IL- 4 suppress cytokine synthesis by different mechanisms. J Biol Chem 1995:9558–63.
18. Chatura KR, Sangeetha S. Utility of Fite-Faraco stain for both mast cell count and bacillary index in skin biopsies of leprosy patients. Indian J Lepr 2012;84:209-15.
19. Joshi MM, Panicker NK, Buch AC, Chandenwale SS. Mast cells in non-neoplastic skin lesions. Indian Med Gazette 2013;145:8-13.
20. Yamamura M, Uyemura K, Deans RJ, Weinberg K, Rea TH, Bloom BR, *et al*. Defining protective responses to pathogens: cytokine profiles in leprosy patients. Science 1991;254:277-9.
21. Misra N, Murtaza A, Walker B, Narayan NPS, Misra SR, Ramesh V, *et al*. Cytokine profile of circulating T cells of leprosy patients reflects both indiscriminate and polarized T-helper subsets: T-helper phenotype is stable and uninfluenced by related antigens of Mycobacterium leprae. Immunology 1995;86:97-103.