

ORIGINAL ARTICLE

Dendritic cell distribution in lymphomas

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Abstract

Dendritic cells (DC) are specialized antigen presenting cells (APC) that have important roles in host defenses and in generating anti-tumour immune response. Altered frequency and maturation of DC have been reported in malignant tumours. We studied the distribution and maturation status of DC by immunohistochemistry, on the formalin-fixed, paraffin-embedded lymph node tissues of 32 histologically diagnosed lymphomas and 40 inflammatory conditions that were retrieved from the Department of Pathology, UKM Medical Centre, Kuala Lumpur. Our study showed a significant reduction in the total DC counts in the lymphoma tissues compared to the inflammatory conditions. The mature and immature DC counts were both significantly reduced ($p=0.008$ and 0.001 respectively), although a greater reduction was observed in mature DC numbers. We also observed compartmentalization of DC where the immature DC were seen within the tumour tissues and the mature DC were more in peri-tumoural areas. Our findings were similar to other reports, suggesting that reduced numbers of DC appears to be a factor contributing to lack of tumour surveillance in these cases.

Keywords: Dendritic cell, lymphoma, CD1a, CD38

INTRODUCTION

Dendritic cells (DC) that were first identified by Steinman and Cohn (1973)¹ are bone-marrow-derived professional antigen-presenting cells (APC) within the immune system, responsible for the initiation of primary, T-cell based immune responses.^{2,3} Dendritic cells are heterogeneous in humans. Myeloid and plasmacytoid DC have been identified by lineage and their ability to induce strong T-helper type 1 (Th1) and T-helper type 2 (Th2) responses respectively.⁴⁻⁶ Plasmacytoid DC are also crucial effectors in anti-viral immunity and induce Th1 development through secretion of interferon- α (IFN- α).⁷ DC are widely distributed in immature state in most tissues and are able to endocytose and process antigens, acting as sentinels for immune monitoring.^{2,8} DC undergo progressive maturation after stimulation and migrate to lymph nodes or secondary lymphoid tissues. Maturing DC lose endocytic activity, express increase MHC-peptide complexes, adhesion and co-stimulatory molecules and secrete inflammatory cytokines that are essential

for T-cell responses.^{9,10} Infiltration of tumours by DC thus reflects the host immune defense mechanisms.^{2,9} The clinical significance of tumour DC infiltration has been reported in a variety of malignant tumours and generally its presence is associated with favourable prognosis.¹¹⁻¹⁴ In light of the crucial role of DC in immune response of malignant tumours, it is of interest to analyze the DC count and its distribution in the lymphoma cases diagnosed in our hospital.

MATERIALS AND METHODS

Specimen

Archived lymph node tissue biopsies of 60 inflammatory conditions and 60 malignant lymphoma cases from UKM Medical Centre Kuala Lumpur, were initially retrieved for this study. Analysis could be performed only on lymph node tissue biopsies of 40 cases diagnosed as inflammatory conditions (control group) and 32 lymphoma cases due to inadequate tissue specimens. This study was carried out under a

laboratory protocol approved by the Institutional Review and Ethics Committee. The histology slides stained with haematoxylin and eosin (H&E) for all the cases studied were retrieved and reviewed by two of the authors, to re-confirm the diagnosis.

Antibodies

CD1a and CD83 purified mouse anti-human antibodies (BD Bioscience) against immature and mature DC respectively, were used in this analysis at a dilution 1:50. Secondary reagents, conjugated with peroxidase were obtained from DakoCytomation.

Immunohistochemistry

Immunohistochemical staining was performed principally on paraffin sections following a conventional antigen retrieval protocol.¹⁵ Four µm-thick sections were cut from each of the paraffin blocks of the cases and were mounted on to the slides, de-waxed and re-hydrated. Sections were then heated in a pressure cooker in 10 mmol/l citrate at PH 6.0. The slides were cooled and treated with peroxidase-blocking solution supplied for 10 minutes. Sections were then immunostained for immature and mature DC by two-stage peroxidase-based En Vision technique (DakoREAL™EnVision™Detection System, Peroxidase/DAB+, Rabbit/Mouse) and were then counterstained with haematoxylin.

Controls: Tissue biopsies of skin and tonsils were used as positive controls for CD1a and CD83 immunohistochemistry studies respectively (Figures 1 & 2). The immature DC were particularly abundant in areas in contact with the external environment, mainly the skin and upon activation, the mature DC will migrate to lymphoid tissues such as the tonsils, lymph nodes and spleen.

Analysis of CD1a and CD83 immunostaining for immature and mature DC

All cell counts were performed using an Olympus (Tokyo, Japan) microscope at a magnification of 400(HPF) (x40 objectives and x10 eyepiece). Morphologically, the DC showed multiple cytoplasmic projections (dendrites) (Figures 3 & 4) and dendrites of immature DC were longer compared to the mature DC.¹³ Only cells with dendritic cytoplasmic processes were counted. For immunohistochemistry examination, cells displaying cytoplasmic staining and also with the appropriate morphology were included. For every section, 30 areas at magnification of 400 (HPF) were assessed and the DC counts were expressed as the mean number of cells per HPF. Each case was morphologically assessed and scored individually and blindly with respect to patient history, presentation and previous diagnosis by two of the authors.

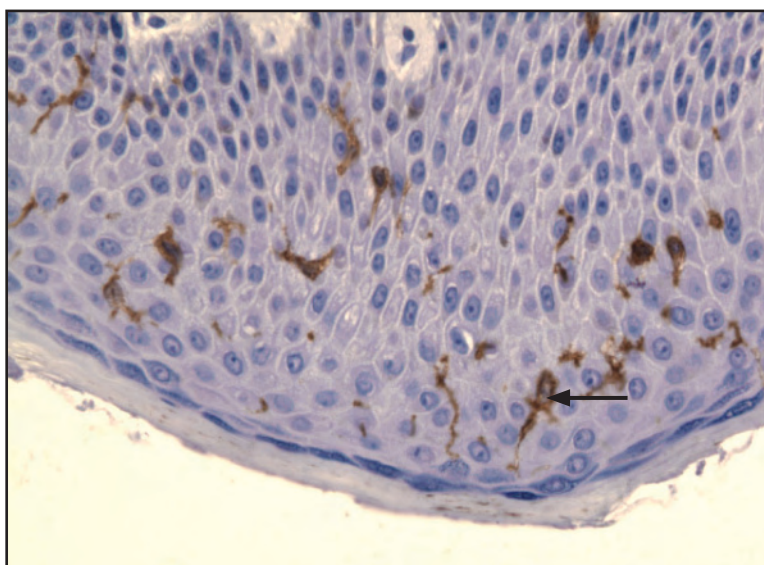


FIG 1: CD1a positive control cells from skin biopsy showing CD1a+ cells (→) with prominent long and slender dendrites.

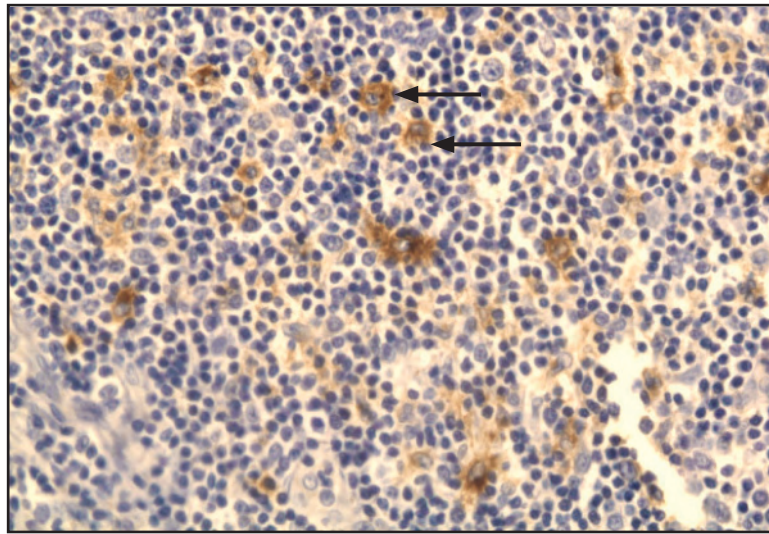


FIG 2: CD83 positive control cells from tonsils showing CD83+ cells (→) with relatively shorter dendrites.

Statistical analysis

The data obtained from the immunohistochemical study of the lymphoma cases and inflammatory conditions were expressed as median values. Comparison of medians of all the parameters between the lymphoma cases and controls was made. The non parametric tests (Mann-Whitney Test and Kruskal-Wallis test) were used as the variables were not normally distributed. A p value of less than 0.05 was regarded as significant.

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 12.00 software (Chicago, USA)

RESULTS

Clinicopathological characteristics of patients

The demographic and clinicopathological characteristics of the cases are summarized in Table 1. Majority of the lymphoma cases were Malays (82.5%) followed by Indians (12.5%).

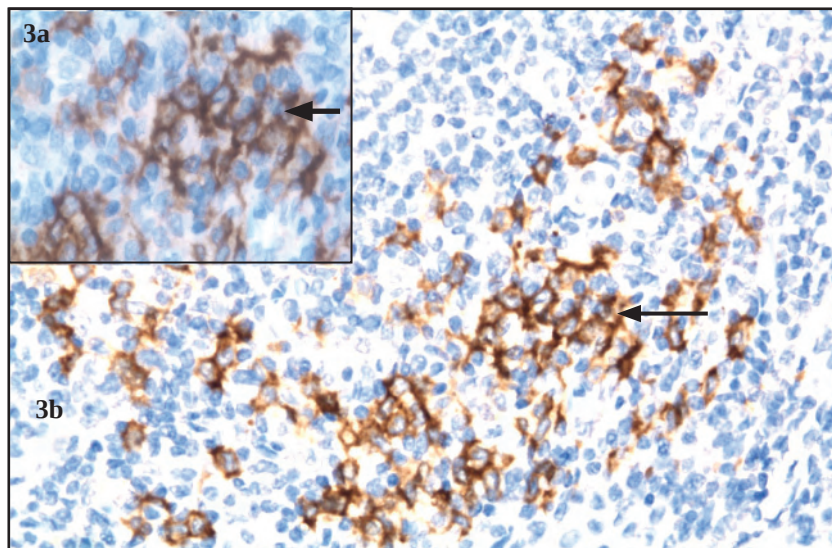


FIG 3a and 3b: CD1a staining of a lymph node with diagnosis of diffuse large B-cell lymphoma (DLBCL) (3a x 200 and 3b x 400). CD1a is expressed by clusters of dendritic cells (→)

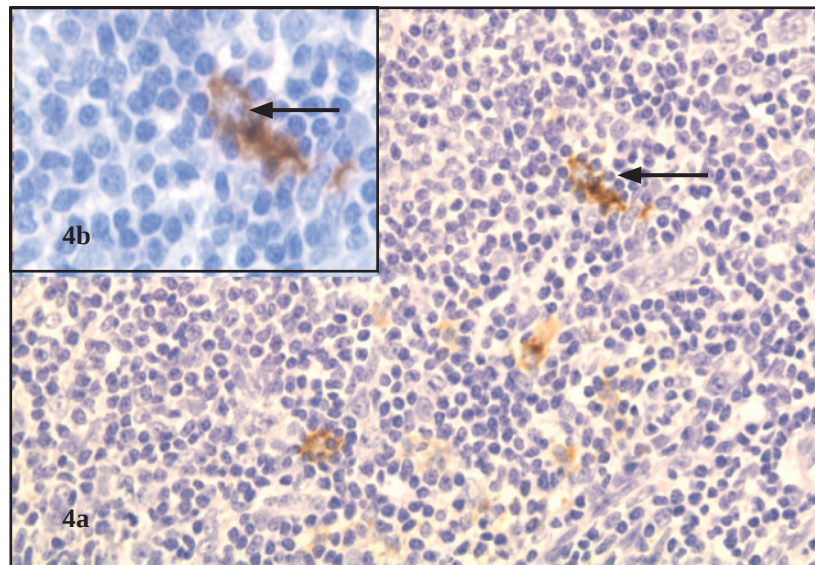


FIG 4a & 4b: CD83 staining of a lymph node with diagnosis of diffuse large B-cell lymphoma (DLBCL) (4a x 200 and 4b x 400). Occasional dendritic cell (DC) showing CD83 expression (→)

The prevalence of malignant lymphoma in our study was higher in the older age group (53.1% in patients more than 45 years) than that of the younger age group (21.9 % in patients less than 25 years). Diagnosis of malignant lymphoma in this study was based on the WHO classification. Of the 32 cases, 15 samples (46.9%) were diffuse large B-cell lymphoma (DLBCL), 9 cases (28.1%) were of Hodgkin's disease, 4 cases (12.5%) were follicular lymphoma and 4 cases (12.5%) were of anaplastic large cell lymphoma (ALCL). The majority of the cases were at the advanced stage of the disease and none was in stage I. (Table 1).

Dendritic cells count and distribution

The total DC count as well as the immature and mature DC counts were lower in lymphoma cases compared to controls (Table 2). The number of immature DC (CD1a+) in the malignant lymphoma tissues was observed to be much higher within the tumour areas whereas the mature DC (CD83+) were seen in the peritumoural areas.

DISCUSSION

Defective dendritic cell (DC) differentiation, maturation, and functionality are possible mechanisms underlying impaired anti-tumour immunity in cancer patients. Studies on altered DC numbers and distribution have been reported in patients with malignant disorders and these

findings were clinically relevant, as reports have shown association with poorer prognosis.^{11-14,16} Lymphoma is among the ten most common cancers in Malaysians.¹⁷ Diffuse large B-cell lymphoma (DLBCL) is the most common sub-type of non-Hodgkin's lymphoma (NHL) reported locally.^{18,19} Majority of the lymphoma tissues analyzed in this study were of DLBCL subtype. Reduction in DC numbers was reported in a recent study on non-Hodgkin's lymphomas and this reduction had potentially contributed to the loss of tumour control.²⁰ We observed that both NHL and Hodgkin's lymphoma (HL) showed a marked reduction in the total DC counts compared to the lymph node tissues diagnosed with inflammatory conditions (Figure 5, Table 2). To the best of our knowledge there are no reports of similar studies in HL. The reduction in the frequency of DC observed in our study could therefore also be a possible contributing factor to the lack of anti-tumour immune response in our cases.

Presence of mature DC within a malignant area is important to initiate the anti-tumour effect and was reported that their presence in solid tumours are associated with a better outcome.²¹ Although our results showed that both the mature and immature DC were reduced in the lymphoma tissues compared to the tissues in the inflammatory conditions, the reduction in mature DC was more prominent (Figure 6, Table 2).

Compartmentalization of immature DC

TABLE 1: Demographic and clinicopathological characteristics of patients

	Control	Lymphomas
<u>Age in years</u>		
<25	14 (35%)	7 (22%)
25 – 45	20 (50%)	8 (25%)
>45	6 (15%)	17 (53%)
<u>Sex</u>		
Male	16 (40.0%)	16 (5.0%)
Female	24 (60.0%)	16 (50.0%)
<u>Race</u>		
Malay	33 (82.5%)	15 (46.9%)
Chinese	1 (2.5%)	11 (34.4%)
Indian	5 (12.5%)	5 (15.6%)
Others	1 (2.5%)	1 (3.1%)
<u>Lymph node site</u>		
Tonsils	29 (72.5%)	2 (6.3%)
Cervical	6 (15.0%)	17 (53.1%)
Supraclavicular	–	2 (6.3%)
Submandibular	–	1 (3.1%)
Preauricular	1 (2.5%)	–
Axillary	2 (5.0%)	2 (6.3%)
Paratracheal	–	1 (3.1%)
Para-aortic	–	1 (3.1%)
Inguinal	1 (2.5%)	5 (15.6%)
Unknown	1 (2.5%)	1 (3.1%)
<u>Diagnosis</u>		
Reactive hyperplasia	36 (90%)	–
TB lymphadenitis	4 (10%)	–
DLBCL*	–	15 (46.9%)
Hodgkin's lymphoma	–	9 (28.1%)
Follicular lymphoma	–	4 (12.5%)
ALCL**	–	4 (12.5%)
<u>Stage (Ann Arbor)</u>		
Stage I	–	0 (0%)
Stage II	–	5 (15.6%)
Stage III	–	12 (37.5%)
Stage IV	–	15 (46.9%)

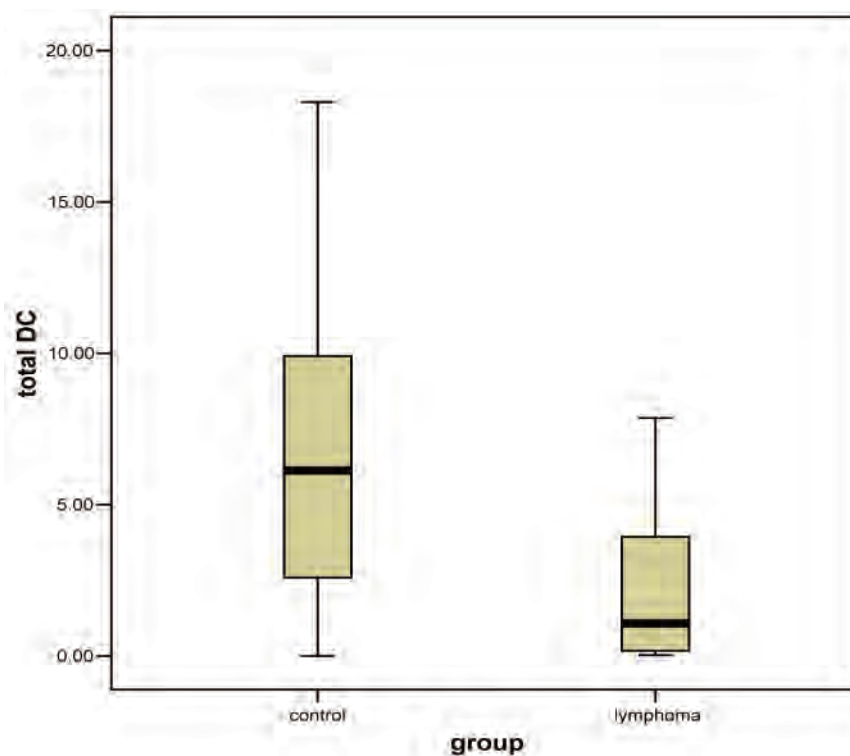
*DLBCL – diffuse large B-cell lymphoma, **ALCL – anaplastic large cell lymphoma

(CD1a+) within the tumour tissues and mature DC (CD83+) in the peri-tumoural tissues which was reported previously on malignant breast tissues²² was also observed in our lymphoma cases. In patients with breast carcinoma, melanoma, renal cell and prostate carcinoma, similar findings were seen indicating that various malignancies could actively recruit

immature DC to the tumour site but subsequently impede their differentiation into fully functional antigen-presenting cell (APC).^{13,22} Immature DC cannot induce anti-tumour immune responses, but most importantly immature DC can induce T-cell tolerance or anergy.²³ The high numbers of immature DC in the tumours were reported to be due to increased migration. Tumour

TABLE 2: Dendritic cell counts in lymph node sections of inflammatory conditions (controls) and lymphomas

Inflammatory conditions (controls)		Lymphomas
No. of DC Count/HPF		
Range	0 - 18.3	0 -19.2
Mean	6.6	2.5
Median	6.1	1.1 *(p = 0.0001)
No. of Immature DC (CD 1a+)/HPF		
Range	0 - 18.3	0-18.2
Mean	5.0	2.25
Median	4.73	0.78 *(p = 0.001)
No. of Mature DC (CD83+)/ HPF		
Range	0 - 13	0-1.73
Mean	1.63	0.29
Median	0.90	0.08 *(p = 0.008)

**FIG 5: Comparison of the total number of DC counts per HPF in inflammatory conditions (control) and lymphoma cases**

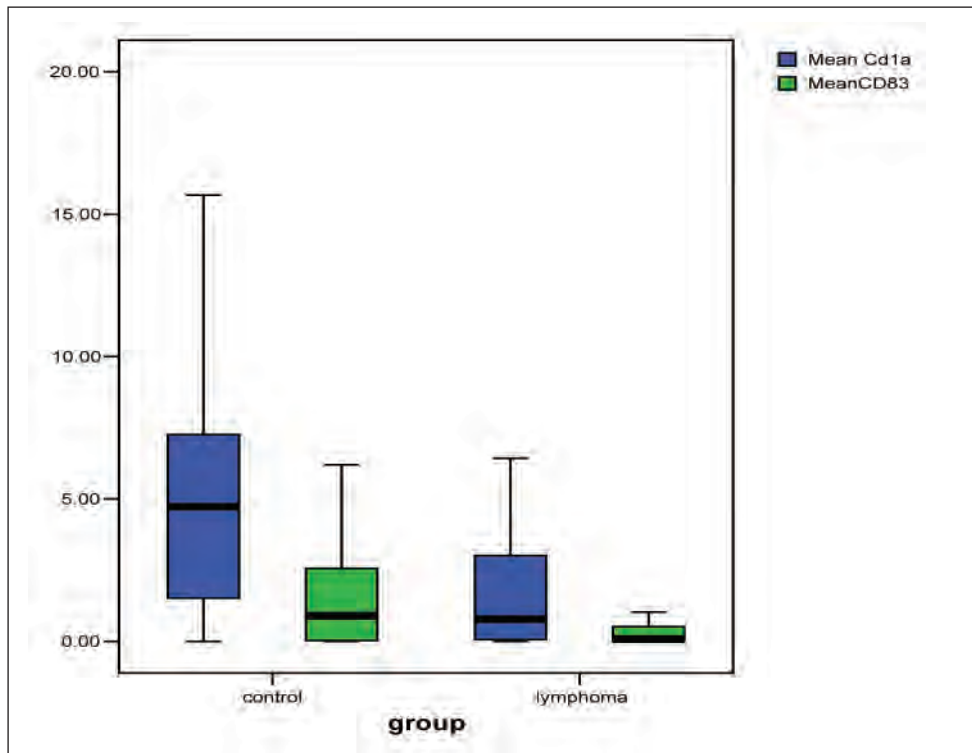


FIG 6: Comparison of the median of immature (CD1a+) and mature DC (CD 83+) counts in the inflammatory conditions (control) (C) and lymphomas.

derived cytokines and DC chemo-attractant factors controlling DC infiltration were shown to modulate DC recruitment.²⁴ It was also indicated that the various malignancies could actively recruit immature DC to the tumour site and impede their differentiation.²² Tumour-induced inhibition of differentiation and function is mediated by tumour-derived soluble factors such as IL-10, IL6, M-CSF, prostaglandins and vascular endothelial growth factor (VEGF).^{25–27} In fact DC dysfunction was suggested to be a systemic process and supported by soluble factors derived from tumours, as similar findings were reported in peripheral blood of breast cancer and multiple myeloma cases.^{28,29} Tumour cells are also capable of inducing cell death thus impairing the function of DC.³⁰ This may also be another mechanism to explain why tumour cells escape immune surveillance. Although previous reports^{16,28} have shown that tumours prevent DC maturation and an increase in immature DC in tumours was associated with poor outcome, a report by Chang KC showed that in a subset of (DLBCL), the presence of CD1a+ DC within tumours with rimming of CD45+ T-cells were correlated with a favorable outcome.³¹ This is an

important finding in view of another prognostic factor in lymphomas and future studies with a larger number of cases may be useful. Our study had shown no significant difference in the number of mature ($p=0.300$) and immature ($p=0.017$) DC, because of the small sample size of the four histological types of lymphomas, but the DC maturation were reduced in all the cases. There was also no significant difference in the number of mature ($p=0.159$) and immature ($p=0.455$) DC between different stages of malignant lymphoma studied.

In conclusion, we have shown in this study, though small in sample size, that there were reductions of total DC in lymphoma as compared to inflammatory conditions. There was also a greater reduction of mature DC than that of immature DC, implying lymphoma not only dampen DC migration to malignant lymph nodes, but also interfere with the maturation of DC. Whether lymphoma render the mature DC defective could be further studied in the recruitment of T-lymphocytes in the peritumoural areas.

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