Journal of the Anatomical Society of India 66 (2017) 15–19

Contents lists available at ScienceDirect

Journal of the Anatomical Society of India

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

Original Article

The morphology and distribution of CD1a positive Langerhans cells in normal and squamous cell carcinoma of cervix

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ARTICLE INFO

Article history:
Received 8 December 2016
Accepted 11 May 2017
Available online 26 May 2017

ABSTRACT

Introduction: Langerhans cells (LCs), a type of dendritic cells are the professional antigen presenting cells present in the mucosa surfaces. They play an important role in antitumor immune response. The present study aims to find out the morphology and distribution of CD1a positive LCs in normal and squamous cell carcinoma of cervix.

Methods: Twenty two normal and eleven ectocervical specimens with squamous cell carcinoma were processed for immunohistochemistry and stained with monoclonal mouse anti-human CD1a (Dako, USA). The morphology of CD1a positive LCs was studied using Olympus BX43 microscope. Morphometric analysis was done using Cellsens imaging analysing software.

Results: There was a statistically significant difference in the number of LCs between normal (8 ± 2.76) and squamous cell carcinoma of cervix (5.36 ± 2.88). In the region of lymphatic infiltration both in epithelium and lamina propria, there were more number of LCs and most of the cells lost their dendritic processes in squamous cell carcinoma. 31.77% of the cells had no dendritic processes. The difference in the mean diameters of LCs was statistically significant ($p = 0.005$) between normal and squamous cell carcinoma of cervix.

Discussion: Fewer number of CD1a positive LCs and their loss of dendritic processes in the squamous cell carcinoma of cervix compared to normal cervix indicate that immune responses are suppressed in patients with cancer.

1. Introduction

Dendritic cells (DCs) are the most potnt antigen presenting cells present in peripheral tissues and secondary lymphatic organs.[1] They exert an important role in the induction of antitumor immune responses. Langerhans cells (LCs) are a type of dendritic cells present in the mucosal layer. They capture, process and present antigens to T-lymphocytes and play an important role in initiation and regulation of innate and adaptive immunity. These cells are known to be present in various organs including female reproductive organs. LCs have cell processes that increase the surface area for their contacts with other cells. In 1980, Figueroa and Caorsi classified LCs in the ectocervix into 5 types based on the number of processes they had as follows: type I – those having one process, type II – with one process which branched, type III – cells with two processes, type IV – those with three or more processes and type V – cells having three or more processes but with several collaterals.[2] Various studies reveal contradictory reports on the density of LCs in squamous cell carcinoma of cervix and their role in prognosis. The aim of the present study is to find out the morphology and distribution of CD1a positive LCs in normal and squamous cell carcinoma of cervix.

2. Materials and methods

Ethical approval from the Institutional Review Board was obtained for the study. Immunocompromised patients and patients

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http://dx.doi.org/10.1016/j.jasi.2017.05.002
0003-2778/

who have undergone chemotherapy and radiation were excluded from this study. Informed consent was obtained from all participants involved in the study. Normal cervical tissue was collected from 22 patients who underwent total abdominal hysterectomy for conditions like adenomyosis and endometriosis. Cancerous tissues were obtained form 11 patients who had squamous cell carcinoma of ectocervix. In carcinoma cervix, ectocervical tissue away from the tumour area with intact epithelium of about 1–2 cm was collected. Tissue collected were fixed in neutral formalin and processed for immunohistochemistry.

Five micron thick serial sections were taken and Immunostaining was done. The primary antibody used was the monoclonal mouse anti-human CD1a (DAKO, USA). A modification of standard avidin–biotin peroxidase technique known as polymer-HRP detection system was used in this study and for this the Universal HRP Detection system ready-to-use kit was purchased from SCYTEK Laboratories (USA).

The stained slides were examined under the light microscope Olympus BX43 and photos were taken by using Olympus DP21 camera fitted on light microscope. The morphology of CD1a positive LCs present in the mucosa of the ectocervix was noted. Cellsens standard image analyzing software (version 1.4) was used for the morphometric analysis. The number of CD1a positive LCs per 25 mm length of ectocervical epithelium was counted for each specimen. Only the cells with well defined nuclei were counted. LCs were categorized into six types, the five types as mentioned in the Figueroa and Caorsi classification[2] and an additional type of cells without processes. The average number of each type of LC and total number were calculated per mm length of epithelium. The diameters of the LCs were taken in two perpendicular axes. The diameters of hundred LCs per specimen with well defined nuclei were measured in micrometers. The average diameters of the cells were calculated. The data was analysed using SPSS version 16. The descriptive statistics (range, mean and standard deviation) were obtained. Mann–Whitney *U*-test and Student independent *t*-test was used to compare the number and diameter of LCs by using SPSS version 16.

3. Results

Stratified squamous epithelium lining the normal ectocervix showed the presence of CD1a positive LCs in all the specimens. Regional variation was present in their distribution. LCs were present mainly in the suprabasal and midepithelial layers (Fig. 1a, all the five types of LCs as described by Figuroa and Caorsi[2] were present in the epithelium. In addition, CD1a positive cells without any dendritic process were also noted. In the normal cervix, type I cells were predominant (Table 1). Few LCs were also noted in the lamina propria of ectocervix. The mean number of CD1a positive LCs per mm length of normal ectocervical epithelium was 8 ± 2.76 and their diameter ranged from 8.57 ± 1.81 μm (Table 2).

In squamous cell carcinoma of cervix, CD1a positive LCs were randomly distributed in ectocervical epithelium. They were seen either in the entire thickness, or in the suprabasal layer or in the midepithelium or infrequently in the superficial layer. The number of CD1a positive cells in both moderately differentiated and poorly differentiated squamous cell carcinoma of cervix was less when compared with normal cervix. The mean number of CD1a positive LCs per mm length of ectocervical epithelium was 5.36 ± 2.88 (Table 2). The mean number of CD1a positive LCs per mm length of ectocervical epithelium was 6.82 ± 3.76 in moderately differentiated carcinoma and 4.14 ± 1.15 in poorly differentiated carcinoma. The cells without dendritic processes (31.77%) were more abundant in cancerous epithelium followed by cells with single process (29.26%) (Fig. 1b, Table 1). Though the number of CD1a positive cells was less in squamous cell carcinoma of cervix, two specimens of moderately differentiated carcinoma with nodal metastasis had more number of CD1a positive LCs, especially, in the region where lymphocytic infiltration was seen in the epithelium (Fig. 2). Though the number of CD1a positive LCs was sparse in the intact epithelium away from the tumour area, dense infiltrations of CD1a positive LCs was noted in the tumour area and its adjacent epithelium in poorly differentiated squamous cell carcinoma of cervix (Fig. 3).

The mean number of LCs was higher in the normal cervix (8 ± 2.76) compared to that of squamous cell carcinoma of cervix (5.36 ± 2.88). There was a statistically significant difference in the number of LCs between these two groups (p value = 0.006) (Table 2).

The diameter of the CD1a positive Langerhans cells in normal cervix was larger (8.57 ± 1.81 μm) than that of squamous cell carcinoma of cervix (8.28 ± 2.04 μm). The difference in the mean diameters of LCs was statistically significant ($p = 0.005$) between these two groups (Table 2).

In the lamina propria of both moderately and poorly differentiated squamous cell carcinoma, a few CD1a positive cells

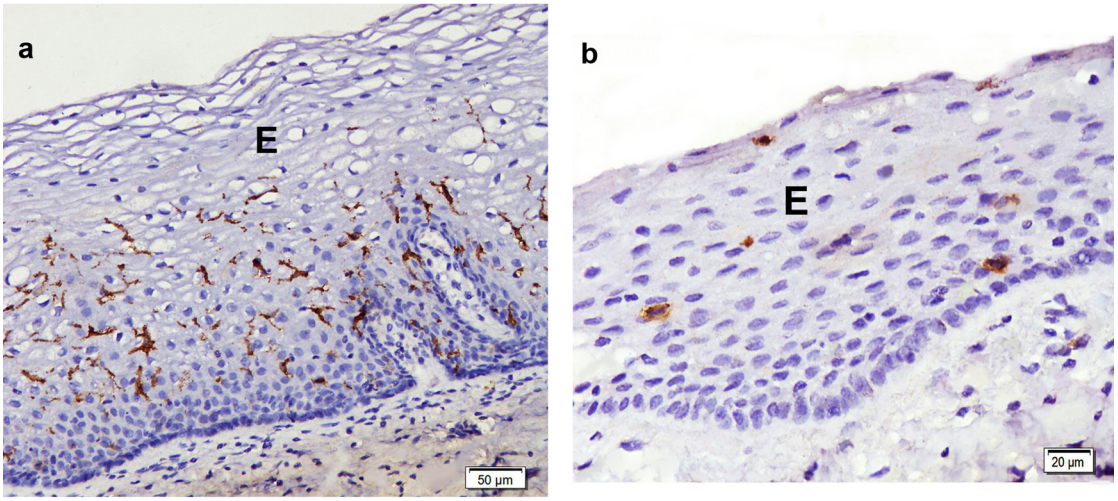

Fig. 1. (a) Normal human ectocervical epithelium (E) showing CD1a positive Langerhans cells (brown cells). Note the cells are predominantly present in the suprabasal and mid-epithelial layers. (b) A few rounded CD1a positive LCs (brown cells) with no process in the ectocervical epithelium (E) in a case of poorly differentiated squamous cell carcinoma of cervix.

Table 1
Distribution of different types of CD1a positive LCs per mm length of normal (N) and squamous cell carcinoma (C) of ectocervix epithelium.

Types of cells	Mean		Standard deviation		Minimum		Maximum		Percentage	
	N	C	N	C	N	C	N	C	N	C
Type I	2.65	1.57	0.99	0.79	1.32	0.40	4.68	3.28	33.17	29.26
Type II	0.78	0.32	0.65	0.30	0.12	0.00	2.40	0.88	9.79	6.04
Type III	1.85	1.15	0.98	0.94	0.48	0.04	4.56	3.08	23.06	21.52
Type IV	0.64	0.37	0.45	0.32	0.16	0.00	1.80	1.00	8.04	6.92
Type V	0.23	0.24	0.42	0.32	0.00	0.00	1.68	1.00	2.91	4.48
Cells without process	1.84	1.70	1.01	0.78	0.12	1.00	3.76	3.12	23.02	31.77

Table 2
Comparison of the number of CD1a positive LCs per mm length of the ectocervical epithelium and their diameter between normal and squamous cell carcinoma (SCC) of cervix using the Mann–Whitney *U* test.

		Mean	Standard deviation	Minimum	Maximum	*p* value
Number of CD1a positive LCs	Normal cervix	8.00	2.76	3.84	16.04	0.006
	SCC of cervix	5.36	2.88	1.64	11.48	
Diameter of CD1a positive LCs	Normal cervix	8.57	1.81	4.08	15.31	0.005
	SCC of cervix	8.28	2.04	3.50	17.03	

were present. Large numbers of LCs was seen in the lamina propria where there were lymphocytic aggregations. In addition, High endothelial venules (HEVs) with lymphocytes in their lumen were seen in the lamina propria. CD1a positive LCs were seen in close contact with the walls of capillaries and HEVs as well as the lymphocytes in the lumen of HEVs (Fig. 4).

4. Discussion

Dendritic cells are the professional antigen presenting cells with the characteristic ability to initiate a primary immune response. They are considered as the principal inducer of antitumor T-cell mediated immunity.[3,4] They are derived from bone marrow progenitors. They circulate in the peripheral blood and reach peripheral tissues, where the immature DCs capture invading antigens.[5] Thus DCs continuously monitor their microenvironment and when they come across danger signals, they undergo a differentiation and maturation process. Maturing DCs usually migrate to lymphoid tissues where they present antigens to T lymphocyte to commence a primary immune response.[6] LCs, a type of DCs are MHC class II expressing bone marrow derived dendritic cells. They mainly present as immature cells on different epithelial tissues to detect invading pathogens or foreign antigens.[7] After antigen uptake these cells undergo a maturation process and are capable of initiating primary T lymphocyte mediated immune responses. It has been noted that inadequate presentation of tumour antigens by LCs, is one possible mechanism for the escape of tumours from the host immune system.[8]

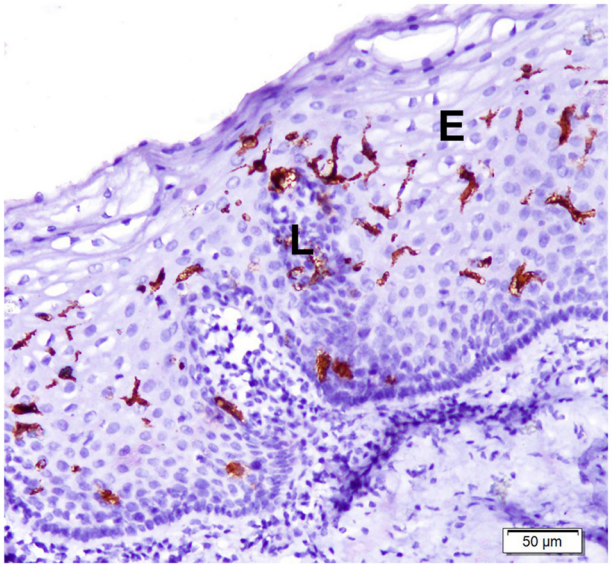

Fig. 2. Moderately differentiated squamous cell carcinoma of cervix with nodal metastasis showing increased number of CD1a positive LCs (brown cells) around a lymphocytic infiltration (L) in the ectocervical epithelium (E).

Many studies have proved the presence of LCs in normal and pathological cervical tissue.[9–13] Previous studies have demonstrated LCs mainly in the basal and suprabasal layers of the stratified squamous epithelium of the normal cervix[14–16] and some in the midzone[17] which is in accordance with the present study. One report demonstrated LCs at all levels of the ectocervical epithelium, although preferentially in the intermediate and superficial layers.[2] In other tissues which are lined by stratified squamous epithelium like normal oral mucosa, LCs were confined mainly in the suprabasal region. In the present study, in carcinoma cervix, they were present either in the entire thickness of the stratified squamous epithelium, or in the suprabasal and intermediate layers or infrequently in the superficial layer.

LCs play a vital role in up-regulation of T-cell reactions against cancer cells. Yet, tumour develops different mechanisms to modulate LC number and functions thereby escape immune recognition and elimination.[18] In this study, the mean number of LCs is less in cervix with squamous cell carcinoma. This is in accordance with Hubert et al., who noted that the density of CD1a positive LCs were low in the epithelium of squamous intraepithelial lesion (SIL) and squamous cell carcinoma biopsy specimens as compared with normal epithelium of exocervix[19] and also with Conners et al., who reported a significant reduction in LCs in cervical dysplasia.[20] It has been observed that both suppression of dendritic cell differentiation and dendritic cell apoptosis contribute to the reduction of dendritic cell numbers in cancer, thus associated with tumour progression.[18] Reduction in the number of LCs has been reported in other malignancies like

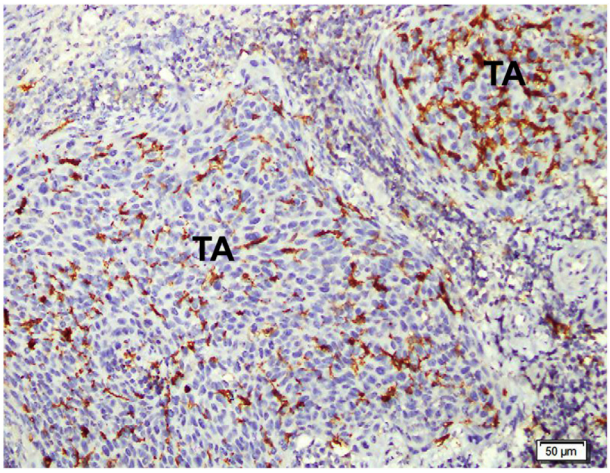

Fig. 3. Note dense infiltration of CD1a positive LCs (brown cells) in the tumour area (TA) in a case of poorly differentiated squamous cell carcinoma of cervix.

skin melanoma.[21] But in oral malignancies an increase in the number of S-100 positive LCs have been reported compared to normal.[22] The reduction in number of LCs in cervical cancer can be attributed to the higher concentration of pro-inflammatory markers associated with innate immune response while the oral cavity has a higher concentration of T-cell associated markers.[23]

The different types of CD1a positive LCs found in the human ectocervix represent different degrees of antigenic stimulation of the cells. Those with the largest number of processes have more surface receptors and display a high antigen-binding activity.[2] Figueroa and Caorsi reported type I and type III cells were the most numerous whereas those of Type V were the least frequent in the normal ectocervical epithelium. The present study showed that most of the LCs have lost their dendritic processes in cancer cervix as indicated by the increased number of LCs without no processes. This could be due to the fact that tumour derived factors alter dendritic cell maturation and function.

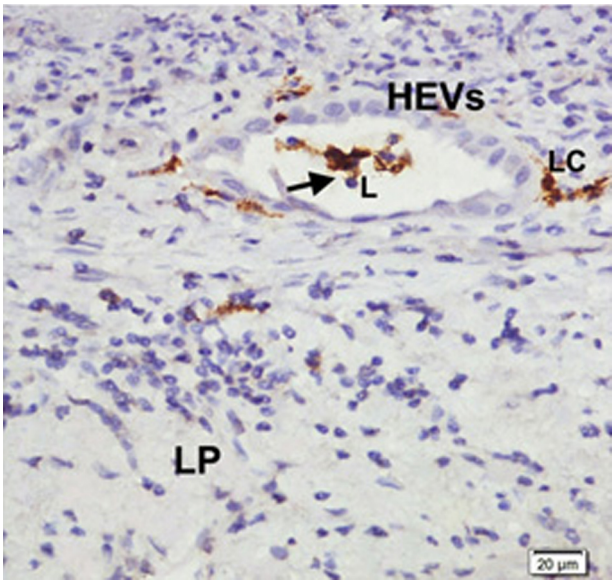

Fig. 4. Poorly differentiated squamous cell carcinoma of cervix showing a High Endothelial Venule (HEV) in the lamina propria (LP). Note the presence of Langerhans cell (LC) adjacent to the wall of HEV. Arrow indicates the close apposition of CD1a positive LC with lymphocytes (L) in the lumen of the HEV.

In the present study, large numbers of CD1a positive LCs were noted in regions of lymphocytic infiltration both in the epithelium and lamina propria. This is in accordance with an earlier study demonstrating lymphocytic infiltration with large numbers of LCs both in epithelium and lamina propria of the normal ectocervix.[24] In addition to the LCs, intraepithelial lymphocytes have been demonstrated in the ectocervical epithelium. The presence of intraepithelial lymphocytes and the accumulation of LCs in the regions of lymphocytic infiltration indicate that the antigen presentation to T-lymphocyte happens not only in the secondary lymphoid organs but also in peripheral tissue where T cells are available.

In addition, dense infiltration of CD1a positive LCs was noted in the tumour area of a poorly differentiated squamous cell carcinoma. Infiltration of DCs is a common feature of most tumours.[1] It has been often interpreted that the presence of DC in the tumour bed elicits immune response. DCs present tumour antigens not only on class II MHC but also on class I MHC, termed cross-presentation.[25] Yet tumours evade immune responses, progress metastasize and eventually result in death of host.[26]

The tumour microenvironment has been characterized as immunosuppressive that impairs both innate and adaptive arms of the immune system.[27] Accumulation of CD1a positive LCs in tumour area in poorly differentiated squamous cell carcinoma cervix may indicate that the tumour microenvironment alters DC maturation and function leading to tumour escape.

High endothelial venules (HEVs) are the sites of migration of lymphocytes from blood vascular system to lymph nodal parenchyma. In the present study, HEVs were observed in the region of lymphatic aggregation. LCs have been shown to migrate not only via lymph vessels but also through the veins.[28] Close apposition of CD1a positive LCs with the walls of HEVs as well as with the lymphocytes in the lumen of HEVs were demonstrated in squamous cell carcinoma of cervix. This suggests the possible role of vascular DCs in immunosurveillance and the presentation of antigens to the lymphocytes.

5. Conclusion

In conclusion, fewer number of CD1a positive LCs and their loss of dendritic processes in the squamous cell carcinoma of cervix compared with normal indicate that immune responses are suppressed in patients with cancer. It should be also noted that the clinical prognosis not only depends on the number of LCs but also on their maturation and functional status.

Conflict of interest

The authors have none to declare.
All authors declares that there is no conflicts of interest.

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