DEMONSTRATION OF LANGERHANS CELLS (LCS) IN THE INTRA-FOLLICULAR AND INTER-FOLLICULAR REGIONS OF THE HUMAN PALATINE TONSIL ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Dendritic cells (DCs) and macrophages function to capture and process antigen and present it to T lymphocytes, a critical step in early immune response. The dendritic Langerhans cells (LCs) belong to the system of antigen presenting cells and play a role in cutaneous immune responses. In our previous studies, we have ultrastructurally demonstrated the presence of LCs in the crypt epithelium and surface epithelium of the human palatine tonsil. In the present study our aim was to demonstrate the LCs in the intra-follicular and inter-follicular area of human palatine tonsil by conventional electron microscopy and immunocytochemistry. Here we report the presence of CD1a positive Langerhans Cells (LCs) in the intra-follicular and inter-follicular areas of the human palatine tonsil and confirm their presence ultrastructurally by demonstrating typical Birbeck granules (BGs) in their cytoplasm. Identification of LCs in the intra-follicular and inter-follicular areas of the palatine tonsil supports the migratory nature of the LCs. Apposition between the Langerhans cells and lymphocytes in the intra-follicular area further strengthens the function of Langerhans cells as antigen presenting cells in initiating immune responses in human palatine tonsil.

Key words: Langerhans cells, ultrastructure, inter and intra-follicular area, human palatine tonsil

INTRODUCTION:

Langerhans cells (LCs) were first observed in the human skin in 1868 by Paul Langerhans[1]. Birbeck et al in 1961[2] demonstrated the characteristic rod like granules in the cytoplasm of LCs ultrastructurally. These cells originate from bone marrow[3] and belong to the monocyte-macrophage-histiocyte lineage[4] LCs are now regarded as phagocytes, but belonging to the system of antigen presenting cells (APC), binding the antigen that penetrates the epidermis, presenting it to T lymphocytes, which in turn trigger a sequence of immune responses[5]. They express certain antigens like Ia antigen[6,7] and T6 (CD1a)[8]. Several authors had used CD1a as immunological marker to study and quantify the human LCs in different tissue[9,10,11,12,13,14] The location of the palatine tonsil in the aerodigestive tract suggests its functional role in generating an immune response to inhaled or swallowed antigen. In our earlier study, we have demonstrated ultrastructurally the presence of LCs in the surface and crypt epithelium of human palatine tonsil[15,16]. Langerhans cells have the ability to migrate[17], they are considered as a dynamic population which continually leave the epidermis, being replaced by circulating LCs precursors and having a mean epidermal stay of approximately 3 weeks[18]. Based on the above mentioned properties of the LCs, the present study was undertaken to demonstrate the presence of LCs in the intra and inter- follicular areas of human palatine tonsil, by conventional electron microscopy and immunohistochemistry.

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MATERIALS AND METHODS:

Tonsils were collected from 11 patients undergoing tonsillectomy for recurrent tonsillitis in Christian Medical College, Vellore after obtaining permission from Institutional review board. For immunohistochemical study each tonsil was cut and snaps frozen and stored at -70 ◦ C. Six micron cryo-sections were received in poly-L- lysine coated slides, fixed for twenty minutes in cold acetone, and stored at -20 ◦ C.

Staining: Avidin Biotin Peroxidase Method:

1. 6-Micron cryostat sections were fixed in cold acetone for twenty minutes. Then sections were kept in Tris buffered saline (TBS);
2. Endogenous peroxidase activity was blocked by incubating the sections with normal serum for fifteen minutes; (Dako Patts x 902) in a humidified chamber (1/5 dilution)
3. Drained of excess serum;
4. Incubated with primary antibody CD1a (1/40

dilution) for 30 minutes; (Dako CD1a Na1/34: Dako Patts, Denmark, code M721)
5. Rinsed thrice with TBS for five minutes;
6. Incubated with (1/200 dilution) secondary antibody - rabbit anti - mouse immunoglobulin biotinylated (RAM: Dako Patts E354) for 30 minutes;
7. Rinsed twice with TBS for five minutes each;
8. Blocked for 30 minutes in 100 ml methanol/1.5 cc 30% H2O2 (this step is to inhibit the endogenous peroxidase activity);
9. Washed in running tap water for 5 to 10 minutes;
10. Rinsed thrice with TBS for 5 minutes each;
11. Incubated in (1/200 dilution) of avidin-biotin (AB) complex (Dako patts K355) for 30 minutes;
12. Rinsed thrice with TBS for 5 minutes each;
13. Incubated for 5 minutes in 25mg/40ml diaminobenzidine (DAB) ("Sigma") in Tris-HCl buffer containing .5 ml of 1% H2O2 for visualization;
14. Washed in running water for 5 to 10 minutes;
15. Counterstained with Harris hematoxylin-15 seconds;
16. Washed in running water for 5 to 10 minutes;
17. Dipped in saturated lithium carbonate solution;
18. Dehydrated in ascending grades of alcohol;
19. Cleared in xylene and mounted in D.P.X.

The control sections were ones which were not treated with primary antibody CD1a (T6), but treated with secondary and AB complex.

Ultrastructural study:

Eleven human palatine tonsils were collected from patients who underwent tonsillectomy at the Christian Medical College Hospital, Vellore. The tonsils were finely divided under a dissecting microscope and fixed in phosphate buffered 2% glutaraldehyde-10% formalin mixture at pH 7.2-7.4 for 4 hrs. The material was then washed in phosphate buffer, post fixed in 1% osmic acid for 90 minutes, washed in phosphate buffer, dehydrated in a graded series of ethanol, cleared in propylene oxide, and finally embedded in araldite. Ultrathin sections were cut with a glass knife, stained with uranyl acetate and lead citrate and viewed using a Philips E.M 201 at 60kV.

RESULTS:

CD1a positive Langerhans cells (LCs) were identified in the lymphoid follicles and inter-follicular areas of human palatine tonsil (Fig.1). Ultrastructural study also revealed the presence of LCs in the intra-follicular and inter-follicular areas in the human palatine tonsil. In the inter-follicular area, cells with dendritic processes were identified. The cytoplasm contains well developed organelles like mitochondria, rough endoplasmic reticulum, and electron dense lysosome like granules, Golgi bodies and characteristic lobulated nucleus. Most significant was the presence of electron dense bodies called Birbeck granules, which exhibit different morphological features in different planes of section, resembling rods, tennis-racket and horse-shoe (Fig 2a, 2b). In longitudinal section the Birbeck granules are characterised by two limiting membranes. Between these two limiting membranes and parallel to them is a lamellar structure which may appear homogenous or exhibit a distinct linear periodicity depending on the plane of section (Fig 2c). Within the lymphoid follicle, a pale stained cell with indented nucleus, whose dendritic processes enclose the wall of capillary, was noted in our study. The cytoplasm contains very few small electron dense granules. The dendritic process in close approximity to the blood vessel wall shows horse-shoe shaped Birbeck granules in its dendritic process. The dendritic processes were found to be in close contact with surrounding lymphocytes (Fig 3).

DISCUSSION:

Langerhans cells are not a permanent inhabitant of the epidermis, they migrate from the skin to the lymph nodes under normal conditions. The migration may be accelerated in an immune reaction[19]. Langerhans cells capture the antigens[20,21,22,23,24], and then migrate via the lymphatics and home to the T cell rich area of lymph nodes, where they are called interdigitaing cells (IDCs)[26,27,28,29,30]. At this site, they present the processed antigen to naïve T cells and generate an antigen specific primary T cell response[31,32,33,34]. During their migration from peripheral tissue to lymphoid organs, dendritic cells (DCs) undergo a maturation process encompassing dramatic changes in phenotype and functions[27,35,36]. Birbeck granules are typical elements which distinguish LCs from other antigen presenting cells[37]. The present study demonstrates CD1a positive Langerhans cells in the intra-follicular and inter-follicular areas, which is confirmed ultrastructurally by the presence of typical Birbeck granules. The above finding supports the migratory nature of the Langerhans cells in generating primary immune response in the human palatine tonsil.

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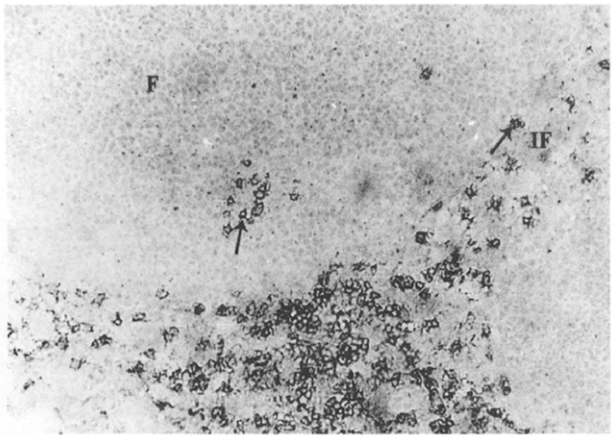

Fig. 1 CD1a positive Langerhans Cells in the inter-follicular(IF) and intra-follicular(F) areas of the human palatine tonsil. F- Follicle, IF- inter-follicular area, Arrows indicate CD1a positive Langerhans cells. Counter stained with Harris haematoxylin (X, 234).

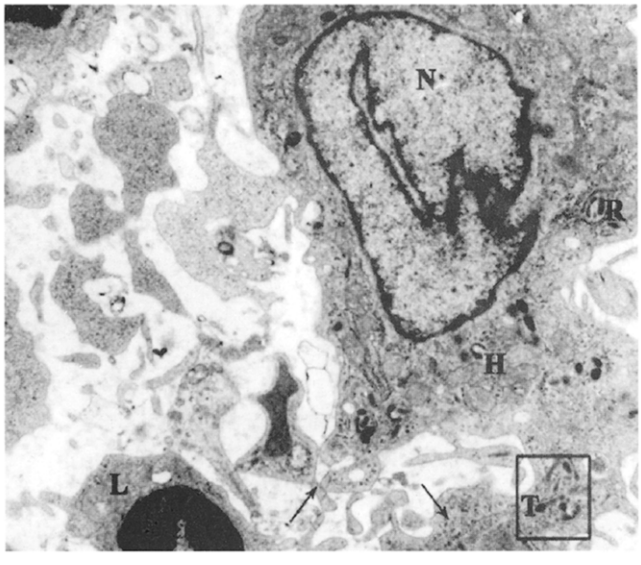

Fig. 2a Langerhans cell in the inter-follicular area showing the LC granules or the Birbeck granules in the polymorphic form (R-Rod, T-Tennis-racket, H horse-shoe shaped) Arrows indicate the dendritic processes (X, 6969)

Fig. 2b Higher magnification of the large square inset in fig 2a. M- Mitochondria, RER- Rough endoplasmic reticulum, R-Rod-shaped Birbeck granule, H- Horse-shoe shaped Birbeck granule, N nucleus (X, 41700)

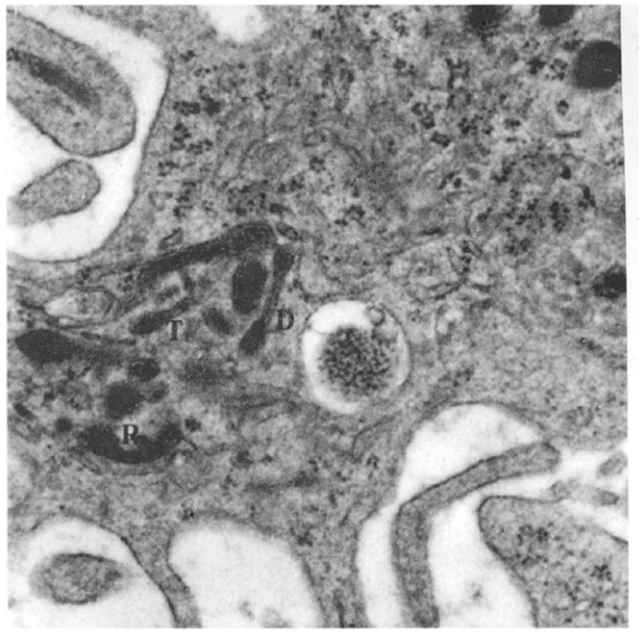

Fig. 2c Higher magnification of small square inset in 2a. R Rod shaped, D Dumb bell shaped and T tennis racket shaped Birbeck granules with dense linear central axial core within the lucent zone, enclosed by a dense limiting membrane (X, 41700)

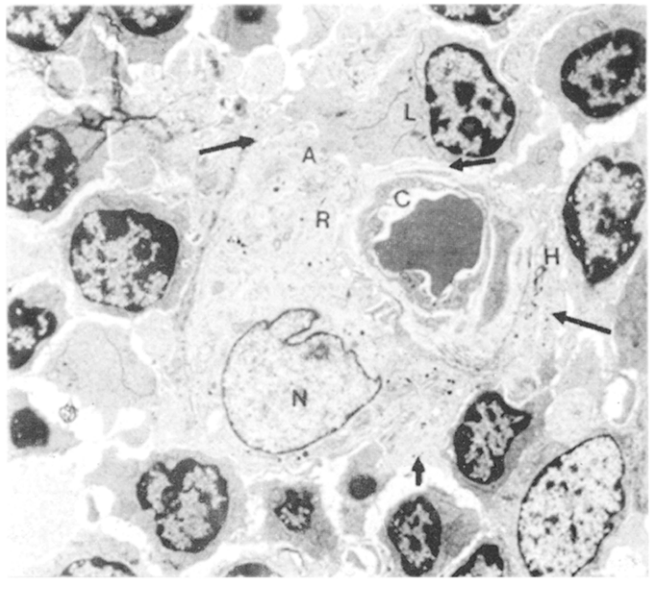

Fig. 3 A Langerhans Cell in the intra-follicular area enclosing the capillary wall showing apposition of lymphocytes. C Capillary, L lymphocyte, N- Nucleus, Birbeck granules in the polymorphic form (R-Rod, H horse-shoe shaped) Arrows indicate the dendritic processes (X, 4680)

apposition of lymphocytes to LCs in cellular hypersensitivity in contact dermatitis[5]. Pharyngeal tonsils contained several type of antigen presenting cells; only dendritic cells were in close contact with immunocompetent cells and other antigen

presenting cells[38]. Immuno-electron microscopy and acid-phosphatase histochemistry revealed the interaction between the antigen presenting cells and the lymphocytes in the human palatine tonsil 39. Ultrastructural study indicates the apposition of Langerhans cells and the lymphocytes in human tonsillar epithelium[40]. In the present study also we could demonstrate the apposition of the Langerhans cells and lymphocytes in the intra follicular area (Fig.3) which further strengthens the function of Langerhans cells as antigen presenting cells in initiating immune responses.

CONCLUSION:

The LCs were observed in the intra-follicular and inter-follicular areas of the human palatine tonsil, proving their migratory nature. Apposition between the Langerhans cells and lymphocytes in the intra-follicular area further strengthens the function of Langerhans cells as antigen presenting cells in initiating immune responses in human palatine tonsil.

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