Dendritic cells in the tongue and oesophagus of laboratory guinea pig, rat, and rabbit: a light microscopic zinc iodide-osmium study

S. Koshy, I. Indrasingh and S. Vettivel

Department of Anatomy, Christian Medical College, Vellore, India

SUMMARY

Dendritic cells have been demonstrated in human and animal epithelium and subepithelial tissue. The present study used zinc iodide-osmium, which revealed dendritic cells in the epithelium and subepithelial tissue of tongue and oesophagus specimens of the laboratory guinea pig, rat, and rabbit. In guinea pig and rat tongue, basal and suprabasal dendritic cells were located in the epithelium of the dorsal surface. In rabbit tongue, suprabasal cells, without dendritic processes, were found in the epithelium of the dorsal surface. In the subepithelial tissue, dendritic cells were present; a few dendritic cells were found in the subepithelial tissue of the ventral surface. Dendritic cells were not found within the cornified layer of the epithelium. In guinea pig and rat oesophagus, basal columnar cells, without dendritic processes, were present in the epithelium. In rabbit oesophagus, basal cells, without dendritic processes, were seen in the epithelium; the subepithelial tissue showed dendritic cells. The cells, without evident processes, are probably not yet of typical, mature phenotype.

Key words: Dendritic cell – Guinea pig – Rabbit – Rat – Zinc iodide-osmium

INTRODUCTION

Inhaled and swallowed antigens must be identified and an immunoactivity initiated without interrupting the sensory function of the tongue. Dendritic cells (DCs) are bone marrow-derived and thymus-independent immunostimulatory cells (Steinman and Nussenzweig, 1980; Steinman, 1991); they can take up and present orally administered antigens to naïve T cells (Liu and MacPherson, 1991). Efficient capture and presentation of antigens by DCs is central to the induction of an immune response (Colaco, 1999).

Zinc iodide-osmium (ZIO) has been extensively used to identify DCs (Crocker and Hopkins, 1984; Dagdeviren et al., 1994; Breathnach and Goodwin, 1965; Niebauer et al., 1969; Rodriguez and Caorsi, 1978; Hart and Fabre, 1981; Sertl et al., 1986; Prickett et al., 1988; Steinman, 1991). DCs have been found in the mouth of the rhesus monkey (Hutchens et al., 1971), human oesophagus (Al Yassin and Toner, 1976), human tonsil (Crocker and Hopkins, 1984; Noble et al., 1996; Papadopoulos et al., 1999), respiratory tract of the infant rat (Nelson et al., 1994), stratified squamous epithelium of the rat (Muller, 1996), trachea and bronchi of the guinea pig

Submitted: October 17 2002
Accepted: December 20, 2002

Correspondence to:
Dr. Shajan Koshy. Department of Anatomy, Christian Medical College, Vellore 632 002, India
Fax: 0091 416 2262788/2262268/2232035.
E-mail: shajank@hotmail.com

(Lawrence et al., 1997), mucous membranes of the digestive and reproductive tracts in mice and rats (Bykov, 1997), epithelium of the respiratory tract of the rat (McWilliam and Holt, 1998), and in the human and animal respiratory tract (Holt and Stumbles, 2000).

Previous studies from this department, using ZIO, revealed the presence of DCs in human tonsil (Chandi et al., 1988; Chandi et al., 1989), human decidua (Abraham et al., 1996, 2000), and lip, tongue and oesophagus of the monkey (Indrasingh et al., 2001). Only a few light microscopic studies have been made on DCs in the tongue and oesophagus of animals. Therefore, the present study was carried out to demonstrate, using zinc iodide-osmium, the presence, location and morphology of the DCs in the tongue and oesophagus of the laboratory guinea pig, rat and rabbit.

Materials and methods

Fresh specimens of tongue and oesophagus were taken from laboratory guinea pig, rat and rabbit, which were sacrificed after routine practical classes for postgraduate students at the Department of Pharmacology of the Christian Medical College.

The tissue pieces were immersed in a solution of veronal-buffered zinc iodide-osmium tetroxide at pH 7.4 (Figueroa and Caorsi, 1980) for 48 hours at 4ºC in the dark, washed in distilled water, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Serial sections of seven-micron thickness were cut and the sections were transferred to glass slides, deparaffinised, mounted in Canada balsam without counter staining (Chandi et al., 1988; Abraham et al., 1996; Indrasingh et al., 2001), and viewed under a light microscope.

Results

Tongue of guinea-pig and rat

ZIO-positive DCs were located in the stratified squamous epithelium of the dorsal surface of the tongue (Figs. 1, 2). The cells were basal and supra basal and were located at different levels in the epithelium. Dendritic processes numbered 1 to 4 in the guinea pig and 1 to 3 in the rat. DCs were not found within the cornified layer of the epithelium.

Tongue of rabbit

ZIO-positive cells were found basally in the stratified squamous epithelium of the dorsal surface of the tongue. However, the cells had no processes (Fig. 3). In the subepithelial tissue, DCs

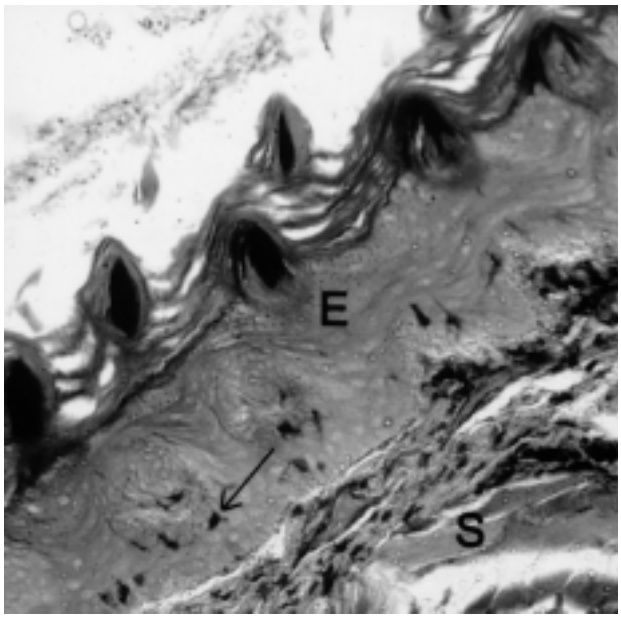

Figure 1.- Dorsal surface of tongue of guinea pig. E – epithelium; S – subepithelial tissue; dendritic cells (arrow) in basal and suprabasal region of the epithelium; processes numbered one to four. ZIO. x 55.

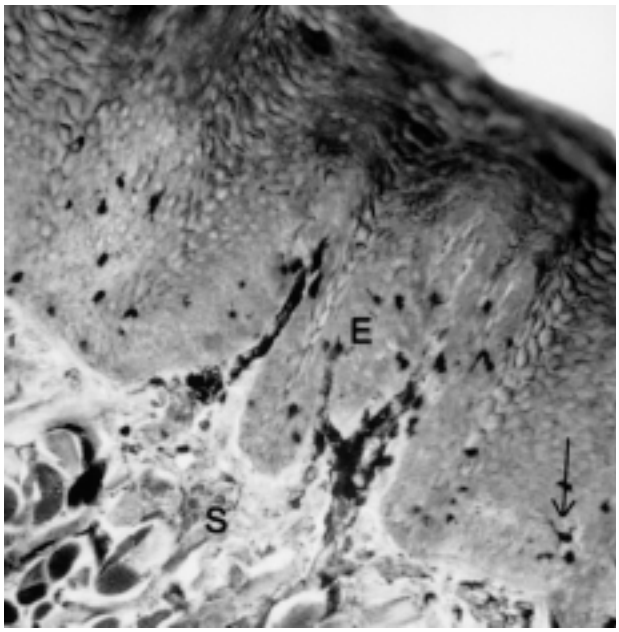

Figure 2.- Dorsal surface of tongue of rat. E – epithelium; S – subepithelium; dendritic cells (arrow) in basal and suprabasal region of the epithelium; processes numbered one to three. ZIO. x 255.

with a single process were present (Fig. 4). A few DCs with 2 to 3 typical processes were found in the epithelium or subepithelial tissue of the ventral surface of the tongue (Fig. 5).

Oesophagus of guinea pig and rat

ZIO-positive columnar cells were present in the stratified squamous epithelium. The cells were located basally. However, processes were absent (Figs. 6, 7).

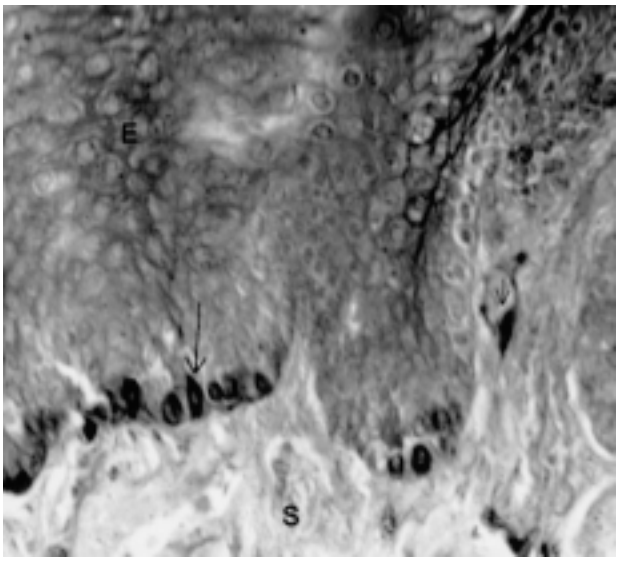

Figure 3.- Dorsal surface of tongue of rabbit. S – subepithelial tissue; cells without processes (long arrow) in basal region of epithelium; dendritic cell (short arrow) in subepithelial tissue. ZIO. x 370.

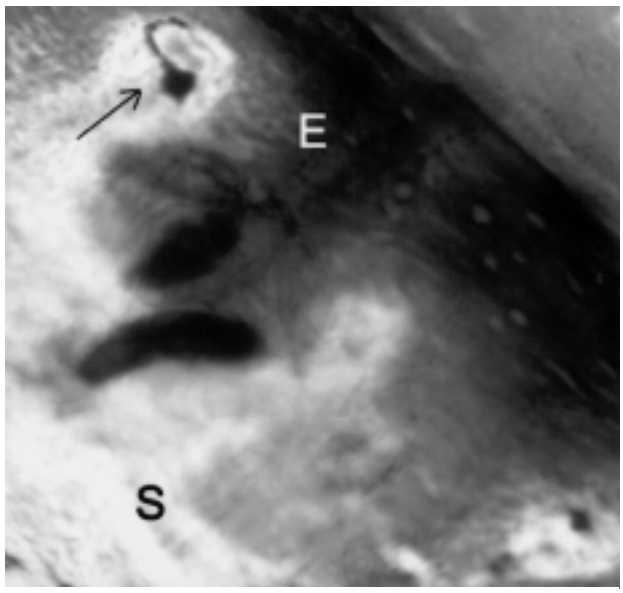

Figure 4.- Dorsal surface of tongue of rabbit. E – epithelium; S – subepithelial tissue; dendritic cells with a single process (thin arrow) in the subepithelial tissue. ZIO. x 520.

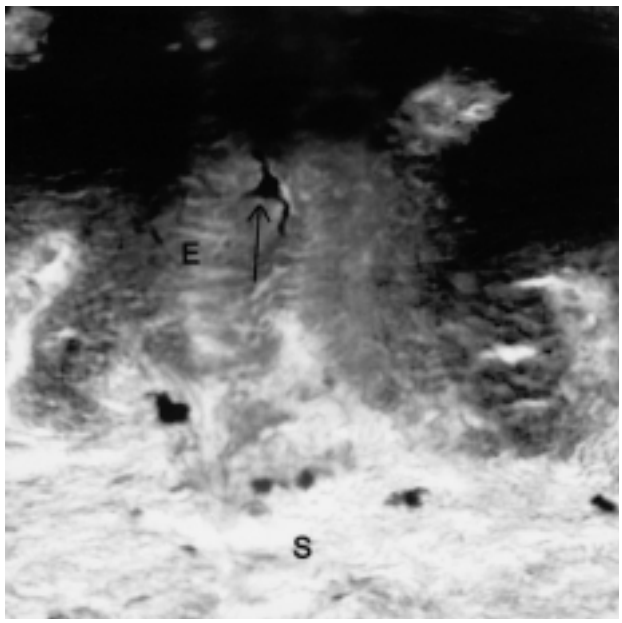

Figure 5.- Ventral surface of tongue of rabbit. E – epithelium; S – subepithelial tissue; dendritic cells with one to three typical processes (arrow) in the epithelium or subepithelium. ZIO. x 550.

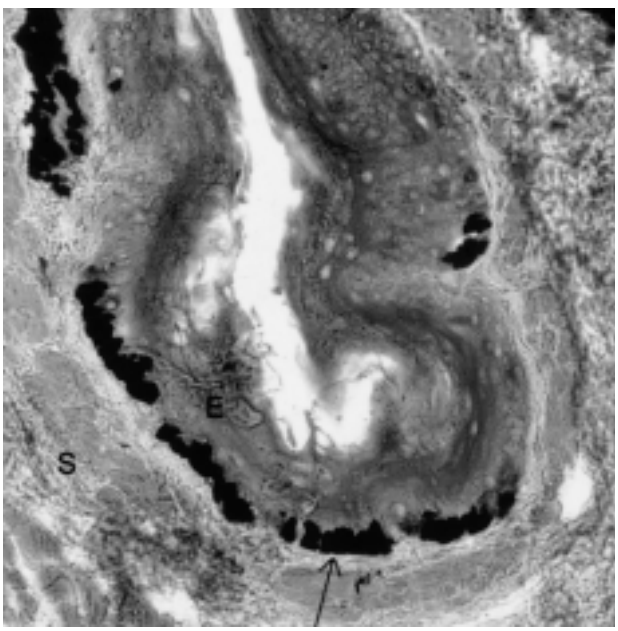

Figure 6.- Oesophagus of guinea-pig. S – subepithelial tissue; columnar cells without processes (arrow) in the basal region of the epithelium. ZIO. x 265.

Oesophagus of rabbit

ZIO-positive cells were found in the stratified squamous epithelium. The cells were located basally. The subepithelial tissue showed dendritic cells with processes (Fig. 8).

Discussion

ZIO has been extensively used to identify the DCs. Cellular reactivity to ZIO is attributed to certain reducing substances such as catecholamines and ascorbic acid (Stockinger and Graf, 1965) and to lipid moieties unmasked from lipoprotein (Niebauer et al., 1969). The results of the ZIO technique, with marked deposition of reaction product in the mitochondrial granules, probably, indicate the presence of lipids and/or precursor proteins (Taffarel et al., 1984).

DCs, which belong to the mononuclear phagocyte family, initiate immune reactions in lympho-

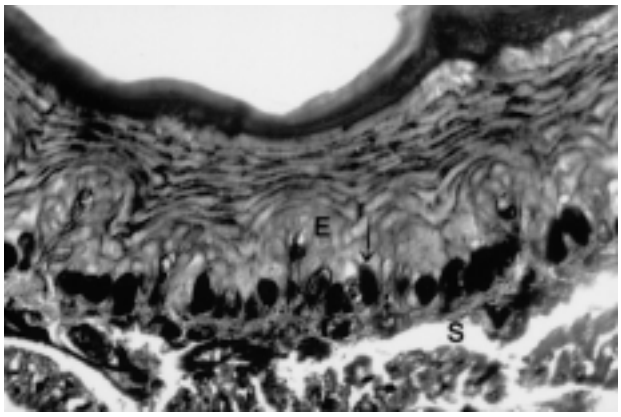

Figure 7.- Oesophagus of rat. E – epithelium; S – subepithelial tissue; Columnar cells without processes (arrow) in the basal region of the epithelium. ZIO. x 345.

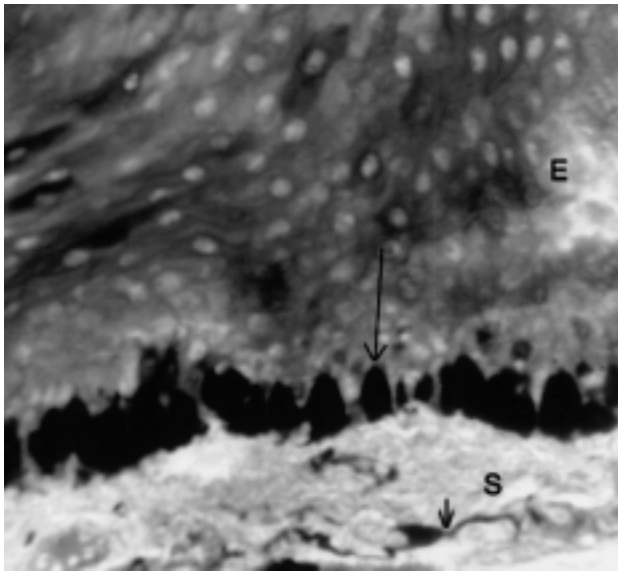

Figure 8.- Oesophagus of rabbit. E – epithelium; S – subepithelial tissue; cells without processes (long arrow) in the basal region of the epithelium; dendritic cells (short arrow) in subepithelial tissue. ZIO. x 420.

cytes and play a critical role in antigen handling (Nossal et al., 1968; Veerman and van Rooijen, 1975). DCs are the most important and the only type of antigen-presenting cells capable of presenting peptides to virgin T cells, thereby initiating cell-mediated immunity to newly encountered antigens.

DCs reside in the interstitium of many tissues and mucosal epithelia, where they take up and process both soluble and particulate antigens. Following exposure to antigens, DCs mature and develop potent immunostimulatory activity while migrating to draining lymph nodes; there, they initiate T cell responses (Pavli et al., 1996).

Basal and suprabasal dendritic cells were present with the epithelium of tongue of the rhesus monkey; suprabasal cells were abundant in keratinised tissue; in no case were dendritic cells found within the epithelial cornified layer (Hutchens et al., 1971). In rat tongue lesions in graft-versus-host disease, dendritic cells were present and were increased in number in the lamina propria (Fujiwara et al., 1997). In monkey tongue, DCs were found to be basal and suprabasal in the epithelium and the processes numbered one to five (Indrasingh et al., 2001). Also in guinea pig and rat of the present study, the DCs were basal and suprabasal and the processes numbered one to three or four. In the rabbit tissue of this study, ZIO-positive cells were basal in the epithelium but the cells had no processes; in the subepithelial tissue, DCs with a single process were found; a few DCs with two to three typical processes were found in the epithelium or subepithelial tissue of the ventral surface. In the monkey oesophagus, DCs were suprabasal and occupied the middle part of the epithelium; the processes were thin and numbered two to three (Indrasingh et al., 2001). In the guinea pig and rat tissue studied here, the ZIO-positive cells were basal and processes were absent. In the rabbit tissue, DCs were basal; the subepithelial tissue had DCs with processes. An absence of processes in DCs indicates that the cells are not yet of mature phenotype; on exposure to antigens, DCs mature (Pavli et al., 2001). The presence of DCs in subepithelial tissue indicates their migratory nature.

DCs can stimulate protective antitumor responses (Morse and Lyerly, 1998). Immunotherapy, using autologous DCs, is playing an emerging role in novel cancer therapies (Hermans et al., 1998). DCs have been applied in cancer vaccines (Timmerman and Levy, 1999). They have the capacity to induce responses and are used as a potent adjuvant for the treatment of human cancer (Nestle and Burge, 1999). DC tumour vaccines for cancer immunotherapy reverse T cell energy and result in tumour rejection (Avigan, 1999).

The location of the palatine tonsil at the gateway to the respiratory and digestive tracts suggests a functional role in generating an immune response to inhaled or swallowed antigens. Similarly, to respond to inhaled or swallowed antigens, DCs need to be present in the tongue and oesophagus. The distribution of DCs in the tongue and oesophagus is important because of the antigens that enter through the mouth and nose. The distribution of DCs in mucosal epithelium and subepithelial tissue suggests that these DCs act as an immune adjuvant by recruiting T cell responses when foreign luminal antigens enter the mucosa.

Acknowledgements

The authors thank Dr. Kalpana Earnest, Professor and Head, Department of Pharmacology, for

permitting us to collect the specimens from the animals used for routine practical classes in the department and the Fluid Research Committee of Christian Medical College for funding this study.

REFERENCES

ABRAHAM S, INDRASINGH I, VETTIVEL S and CHANDI G (1996). Zinc iodide-osmium-positive decidual and dendritic cells in normal human decidua. *J Anat Soc India,* 45: 97-100.

ABRAHAM S, INDRASINGH I, VETTIVEL S and CHANDI G (2000). Gross morphology and ultrastructure of dendritic cells in the normal human decidua. *Clin Anat,* 13: 177-180.

AL YASSIN T and TONER PG (1976). Langerhans cells in the human oesophagus. *J Anat,* 122: 435-445.

AVIGAN D (1999). Dendritic cells: Development, function and potential use for cancer immunotherapy. *Blood Rev,* 13: 53-64.

BYKOV VL (1997). Langerhans cells of the mucous membranes of the digestive and reproductive tracts in laboratory mice and rats. *Morfologia,* 12: 73-77.

BREATHNACH AS and GOODWIN D (1965). Electron microscopy of guinea-pig epidermis stained by the osmium-iodide technique. *J Anat,* 100: 159-172.

CHANDI G, INDRASINGH I and SRIDHARAN G (1988). Dendritic cells in tonsillar epithelium. *J Anat Soc India,* 37: 181-185.

CHANDI G, INDRASINGH I and CHANDI SM (1989). Electron microscopic demonstration of Langerhans cells in human tonsillar epithelium. *Clin Anat,* 2: 271-276.

COLACO CA (1999). Why are dendritic cells central to cancer immunotherapy? *Mol Med Today,* 5: 14-17.

CROCKER J and HOPKINS M (1984). Histiocytes and dendritic reticulum cells shown by a zinc iodide-osmium technique. *J Clin Pathol,* 37: 620-627.

DAGDEVIREN A, ALPM H and ORS U (1994). New application for the zinc iodide-osmium tetroxide technique. *J Anat,* 184: 83-91.

FIGUEROA CD and CAORSI I (1980). Ultrastuctural and morphometric study of the Langerhans cells in the normal human exocervix. *J Anat,* 131: 669-682.

FUJIWARA K, SUGIURA J and SAKAI Y (1997). Electron microscopic studies of the early cellular events occurring in oral mucosa of rats affected by graft-versus-host disease (GVHD). *J Oral Pathol Med,* 26: 90-92.

HART DNJ and FABRE JW (1981). Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissue of the rat heart and other issues, but not brain. *J Exp Med,* 153: 347-361.

HERMANS IF, RAWSON MP, RONCHESE F and RITCHIE DS (1998). The emerging role of the dendritic cell in novel cancer therapies. *NZ Med J,* 111: 111-113.

HOLT PG and STUMBLES PA (2000). Characterization of dendritic cell populations in the respiratory tract. *J Aerosol Med,* 13: 361-367.

HUTCHENS LH, SAGEBIEL RW and CLARKE MA (1971). Oral epithelial dendritic cells of the rhesus monkey – histologic demonstration, fine structure and quantitative distribution. *J Invest Dermatol,* 56: 325-336.

INDRASINGH I, ABRAHAM S and VETTIVEL S (2001). Zinc iodide osmium-positive cells and dendritic cells in stratified squamus epithelium of lip, tongue, and oesophagus of Bonnet monkey (*Macaca radiata*). *J Anat Soc India,* 50: 34-36.

LAWRENCE TE, MILLECCHIA LL, FRAZER DG and FEDAN JS (1997). Pulmonary dendritic cells distribution and prevalence in guinea pig airways: effect of ovalbumin sensitization and challenge. *J Pharmocol Exp Therapeutics,* 282: 995-1004.

LIU LM and MACPHERSON GG (1991). Lymph-borne (veiled) dendritic cells can acquire and present intestinally administered antigens. *Immunology,* 73: 281-286.

MCWILLIAM AS and HOLT PG (1998). Immunobiology of dendritic cells in the respiratory tract: steady state and inflammatory sentinels? *Toxicology Lett,* 102-103: 323-329.

MORSE MA and LYERLY HK (1998). Immunotherapy of cancer using dendritic cells. *Cytokines Cell Mol Therapy,* 4: 35-44.

MULLER T (1996). The different morphological types of dendritic cells and their innervation in stratified squamous epithelia of the rat as revealed by methylene blue staining. *Biomed Res,* 7: 149-157.

NELSON DJ, MCMENAMIN C, MCWILLIAM AS, BRENAN M and HOLT PG (1994). Development of the airway intraepithelial dendritic cell network in the rat from class II histocompatibility (Ia)-negative precursors: differential regulations of Ia expression at different levels of the respiratory tract. *J Exp Med,* 179: 203-212.

NESTLE FO and BURGE G (1999). Dendritic cells: Role in skin diseases and therapeutic applications. *Clin Exp Dermatol,* 24: 204-207.

NIEBAUER G, KRAWCZYK WS, KIDD RL and WILGRAM GF (1969). Osmium zinc iodide reactive sites in the epidermal Langerhans cell. *J Cell Biol,* 43: 80-89.

NOBLE B, GORFIEN J, FRANKEL S, ROSSMAN J and BRODSKY L (1996). Microanatomical distribution of dendritic cells in normal tonsils. *Acta Otolaryngol Suppl Stockh,* 523: 94-97.

NOSSAL GJV, ABBOT A, MITCHELL J and LUMMUS Z (1968). Antigens in immunity. XV. Ultrastructural features of antigen capture in primary and secondary lymphoid follicles. *J Exp Med,* 127: 277-290.

PAPADOPOULAS EJ, SASSETTI C, SAEKI H, YAMADA N, KAWAMURA T, FITZHUGH DJ, SARAF MA, PAVLI P, HUME DA, VAN DE POL E and DOE WF (1993). Dendritic cells, the major antigen-presenting cells of the human colonic lamina propria. *Immunology*, 78: 132-141.

PAVLI P, MAXWELL L, VAN DE POL E and DOE WF (1996). Distribution of human colonic dendritic cells and macrophages. *Clin Exp Immunol,* 104: 124-132.

PRICKETT TCR, MCKENZIE JL and HART DNJ (1988). Characterization of interstitial dendritic cells in human liver. *Transplantation,* 46: 754-761.

RODRIGUEZ EM and CAORSI I (1978). A second look at the ultrastructure of the Langerhans cell of the human epidermis. *J Ultrastruct Res,* 65: 279-295.

SERTL K, TAKEMURA T, TSCHACHLER E, FERRANS VJ, KALINER M.A and SHEVACH EM (1986). Dendritic cells with antigen-presenting capability reside in airway epithelium, lung parenchyma, and visceral pleura. *J Exp Med,* 163: 436-451.

STEINMAN RM (1991). The dendritic cell system and its role in immunogenicity. *Ann Rev Immunol,* 9: 271-296.

STEINMAN RM and NUSSENZWEIG MC (1980). Dendritic cells: Features and functions. *Immunol Rev,* 53: 127-147.

STOCKINGER I and GRAF J (1965). Electronenmikroskopische Analyse der Osmium-Zinkiodid Methode. *Mikroskopie*, 20: 16-35.

TAFFAREL M, ATTLAS M and MACHADO RD (1984). Mitochondrial granules in electrophorus electricus: a cytochemical approach. *J Submicroscopic Cytology,* 16: 487-494.

TIMMERMAN JM and LEVY R (1999). Dendritic cell vaccines for cancer immunotherapy. *Ann Rev Med,* 50: 507-529.

VEERMAN AJP and VAN ROOIJEN N (1975). Lymphocyte capping and lymphocyte migration as associated events in the in vivo antigen tapping process. An electron microscopic autoradiographic study in the spleen of mice. *Cell Tissue Res,* 161: 211-217.