Lamina propria dendritic cells express activation markers and contact lymphocytes in chronic adult periodontitis

Lamina Propria Dendritic Cells Express Activation Markers and Contact Lymphocytes in Chronic Periodontitis

Claudio Cirrincione,* Nicola Pimpinelli,† Lorenzo Orlando,‡ and Paolo Romagnoli*

Background: Dendritic cells are characterized by shape, structure, and membrane molecule expression; they contact T lymphocytes to present antigens and stimulate plasma cell differentiation in vitro. Dendritic cells are known to be present in healthy human gingiva and to be altered in HIV-associated periodontitis. Here, we address the phenotype, location, and intercellular relationships of dendritic cells in chronic periodontitis.

Methods: Biopsies from patients with chronic periodontitis were analyzed by electron microscopy and indirect immunofluorescence for dendritic cells and lymphocyte markers.

Results: Langerhans' cells were spread in oral epithelium but restricted to the basal layer in pocket epithelium; they did not usually express major histocompatibility complex (MHC)-II antigens nor contact lymphocytes. Dendritic cells were abundant in the lamina propria of pocket epithelium; they were MHC-II positive, admixed with CD4-positive and CD8-positive T lymphocytes, and, they expressed CD54, CD80, and CD86. Dendritic cells often contacted lymphocytes and were also located within plasma cell aggregates.

Conclusions: The data suggest that prerequisites for mounting a T cell-mediated immune response exist in chronic periodontitis, although this response is limited to the lamina propria. These results suggest that T-cell responses offer limited protection and can contribute to tissue damage during periodontal disease. *J Periodontol 2002;73:45-52.*

KEY WORDS

Immune response; dendritic cells; lymphocyte mediators; periodontitis/immunology.

* Department of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy.
† Department of Dermatological Sciences.
‡ Department of Odontostomatology.

Dendritic cells are professional antigen-presenting cells and can stimulate primary and secondary responses in both lymphoid organs and peripheral tissues.[1,2] Dendritic cells can present antigens through major histocompatibility complex class I (MHC-I) and class II (MHC-II) molecules[3] to both CD4-positive[4] and CD8-positive T lymphocytes.[3,5] When presenting antigens to lymphocytes, dendritic cells increase the expression of MHC-II molecules and express adhesion molecules needed to stimulate lymphocyte responses; i.e., CD54/ICAM-1,[6] CD80/B7.1, and/or CD86/B7.2.[7-10] Upon electron microscopy, dendritic cells are characterized, in addition to shape, by an ovoid or indented nucleus with loose chromatin and by abundant cytoplasm with many smooth vesicles and tubules and usually few lysosomes.[11] However, dendritic cells in the connective tissue of non-lymphoid organs may contain valuable numbers of lysosomes.[12,13] Besides stimulating T-lymphocyte responses, dendritic cells can directly promote the differentiation of plasma cells in vitro.[14,15]

Human gingiva contains Langerhans'[16,17] and connective tissue dendritic cells.[18] These cells undergo modifications in HIV-associated periodontal disease, where Langerhans' cells show signs of hampered differentiation at electron microscopy; greatly reduce and even lose the expression of MHC-II; concentrate at the base of epithelium; and contact only CD8-positive lymphocytes.[18] Dendritic cells in the lamina propria,

however, increase in number, continue to express MHC-II, and form clusters with CD8-positive lymphocytes.[18] Much less is known about gingival dendritic cells in immunocompromised patients with periodontal disease. Langerhans' cells have been shown to increase in number with increasing bacterial plaque accumulation[19-21] and to decrease in infiltrated areas of periodontitis.[22,23] The role of dendritic cells in the lamina propria in these conditions is not well understood.

In this paper, we address the presence, immunophenotype, location, and intercellular relationships of dendritic cells in gingiva affected by chronic periodontitis.

MATERIALS AND METHODS

Patients and Biopsies

Biopsies were obtained from 10 patients with chronic periodontitis (5 males and 5 females, aged 45 to 74 years, mean 58.8 years). The patients had not taken any drugs or suffered from acute or chronic systemic disease in the last 3 months before biopsies, and had not undergone any periodontal treatment. Biopsies were harvested from buccal gingiva at sites that showed bleeding on probing and probing depths ranging between 5 and 10 mm (mean 6.8 mm). Individual sites corresponded to the following teeth: 4, 5, 13, 21, 21, 21, 25, 29, 31, 31. A section of each specimen was fixed in formalin and embedded in paraffin for diagnostic histology; another section was prepared for either immunohistochemistry or electron microscopy. All procedures were done in accordance with Italian law and the Helsinki declaration, upon informed consent, and were approved by the institutional review board.

Immunohistochemistry

Several samples were immediately frozen in OCT;§ cryosections were acetone-fixed and immunolabeled. Other samples were fixed in paraformaldehyde-lysine-periodate in 0.1 mol/l phosphate buffer, pH 7.4 (PLP),[24] for 1 hour at room temperature; cryoprotected with 300 g/l sucrose in 0.1 mol/l phosphate buffer, pH 7.4; frozen and cryosectioned. The following primary monoclonal antibodies were applied for 90 minutes at 37°C (dilutions in parentheses): MHC class II beta chain antigen (1:150),‖ CD 54 (1:60),‖ CD 80 (1:200),‖ CD 86 (1:200),‖ CD 4,¶ CD 8,¶ (both 1:40) and CD 1a (1:50).# Fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibodies (1:50)¶ were applied for 60 minutes at 37°C. Omission of primary antibodies and use of irrelevant antibodies (mouse IgG1 from clone MOPC-31C‖ and IgG2a from clone RPC5,‖ both 1:100) gave negative results.

Cell Counts

Due to ethical and legal limitations in sampling, only 5 cases of periodontitis were available for counts. The number of labeled cells with dendritic shape per 0.1 mm^2 of epithelium and superficial lamina propria, respectively, was counted in photomicrographs of PLP-fixed biopsies with periodontitis at the epithelium-lamina propria border and containing both tissues to approximately equal extents.

The section surface (0.15 to 0.40 mm^2) was scanned for biopsy and location (i.e., oral side, sulcus, and pocket) at magnification ×40. Mean values and standard deviation (SD) were subjected to analysis of variance, with significance at $P < 0.05$. In sections immunostained for CD54, areas infiltrated with plasma cells were excluded from counts because of uncertainty in identifying dendritic cells admixed with CD54-positive plasma cells.

Electron Microscopy

Specimens were fixed with 2.5% formaldehyde and 2.0% glutaraldehyde in 0.1 mol/l cacodylate buffer, pH 7.4, osmicated and embedded in epoxy resin.** Sections were stained with uranyl acetate and lead citrate and observed in an electron microscope†† at 80 kV.

RESULTS

Immunohistochemistry

Since good morphology and simultaneous immunolabeling were obtained only from PLP-fixed, cryoprotected specimens, the following description is based on the results from these specimens. Many CD1a-positive Langerhans' cells were found in all biopsies; their numbers per 0.1 mm^2 of epithelial section surface area were 38 (SD ±2) in oral epithelium, 16 (±5) in sulcular epithelium, and 5 (±1) in the pocket ($P < 0.05$ among locations). In this last location, CD1a-positive cells were restricted to the basal layer, and a few were also found in the connective tissue (Fig. 1). Langerhans' cells were MHC-II negative in all cases of periodontitis (Fig. 2A).

Dendritic cells labeled for MHC-II were found in the lamina propria at all sites including the pocket (Fig. 2). They were significantly more numerous in pocket than in oral or sulcular lamina propria (Table 1). Highly dendritic, MHC-II positive cells were also located inside plasma cell aggregates in pocket lamina propria (Fig. 2C). Dendritic cells expressed CD54, CD80, and CD86 (Fig. 3). Few blood vessels and most, if not all, plasma cells were also labeled for CD54 (Fig. 3), thus making the identification of dendritic cells uncertain in areas infiltrated with plasma cells; these areas were therefore excluded from counts. CD4-positive and CD8-positive T lymphocytes were localized in pocket

§ Miles Scientific, Naperville, IL.
‖ Ancell, Bayport, MN.
¶ Sigma Chemical Co., St. Louis, MO.
# Sera-Lab, Crawley Down, U.K.
** Fluka, Buchs, Switzerland.
†† Model 1010, Jeol, Tokyo, Japan.

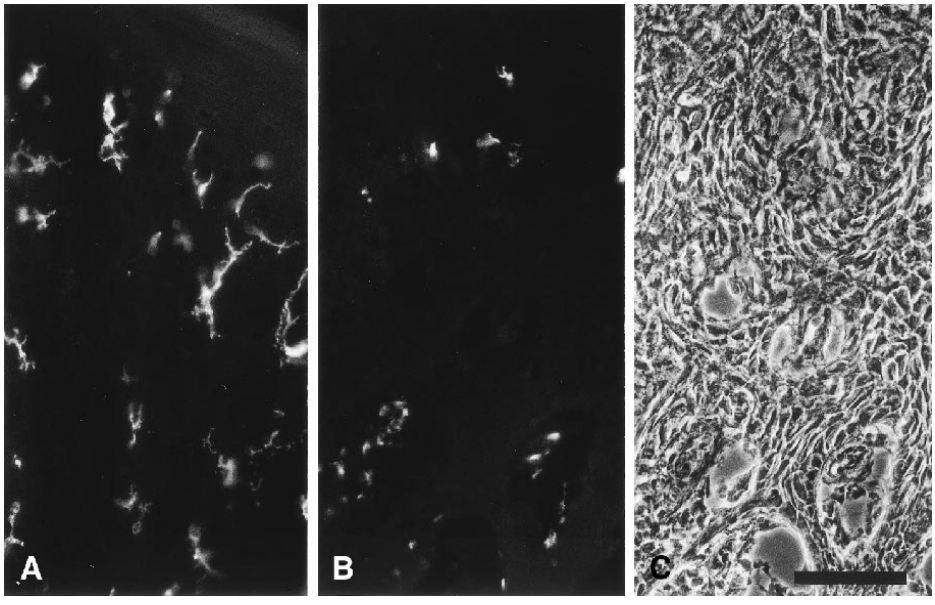

Figure 1.
*Localization of Langerhans' cells in periodontitis. CD1a-positive Langerhans' cells are spread in oral epithelium **(A)**, whereas in the pocket, they are restricted to the basal epithelial layer and lamina propria **(B,C)**. A and B: immunofluorescence for CD1a; C: phase contrast microscopy of the same field as middle panel (bar = 50 μm).*

and sulcular connective tissue and inside plasma cell aggregates; CD8-positive lymphocytes were significantly more numerous in pocket than in sulcular connective tissue (Table 1).

Electron Microscopy

Langerhans' cells and intraepithelial lymphocytes, usually not in contact with Langerhans' cells, were found in all biopsies (Fig. 4A).

Many cells in the superficial lamina propria had a shape and fine structure typical of connective tissue dendritic cells, including variable numbers of lysosomes. These dendritic cells contained intermediate filaments and microfilaments; the latter were inserted into the cell membrane at focal junctions with extracellular matrix (Fig. 4B). These cells corresponded to the MHC-II positive dendritic cells recognized by immunohistochemistry for shape, position, and approximate numbers.

Dendritic cells were located adjacent to lymphocytes in the sulcus and pocket lamina propria (Fig. 4C); sparse monocytes, plasma cells, granulocytes, and fibroblasts also were present. In addition, dendritic cells were found within aggregates of plasma cells (Fig. 4D).

DISCUSSION

In this study, we defined the distribution and immunophenotype of dendritic cells in chronic periodontitis. Many dendritic cells were found in the lamina propria, mainly near the epithelium. In the sulcular and pocket lamina propria, dendritic cells colocalized with lymphocytes and plasma cells and partly ex-pressed CD54, CD80, and CD86. Since the technique did not allow for double immunolabeling, it is impossible to state whether individual cells expressed one or more of these antigens. However, the quantitative data (Table 1) suggest that nearly all dendritic cells in the lamina propria express CD80 and CD86 and about half express CD54. The immunohistochemical data suggest that epithelial (Langerhans') and lamina propria dendritic cells behave dif- ferently in chronic periodontitis. Langerhans' cells express MHC-II weakly or not at all and usually do not come close to lymphocytes. On the contrary, dendritic cells in the lamina propria express all the molecules known to be necessary for T-cell stimulation[6–10] and contact CD4-positive and CD8-positive lymphocytes. These results suggest that if the T-lymphocyte system is activated during chronic periodontitis, it occurs in the lamina propria and does not involve Langerhans' cells. We observed very few CD1a-positive cells in the lamina propria, in accordance with the literature.[17,20,25] The labeling of blood vessels for CD54 in periodontal pockets confirms that this antigen is also involved in leukocyte recruitment into periodontally diseased tissues.[26-28]

Since fibroblasts do not express costimulatory molecules in periodontitis,[29] cells of the immune system, including dendritic cells, are probably the only antigen-presenting cells in this condition. However, fibroblasts might interfere with local immune responses through secretion of proinflammatory cytokines[30] or inhibition of professional antigen-presenting cell activity.[31] The similarities between the cells identified in previous papers as fibroblasts in contact with lymphocytes,[32-34] and those identified here as dendritic on the basis of shape, structure, and immunophenotype in parallel specimens, suggest that most cells contacting lymphocytes in periodontitis are dendritic, presumably antigen-presenting cells that were not recognized due to inadequate techniques at that time.

The results of this study indicate that inhibited expression of MHC-II molecules by Langerhans' cells is not exclusive to HIV-infected subjects, but that an important divergence exists in the overall picture of the gingival immune system between HIV-infected[18] and immunocompromised patients with periodontitis.

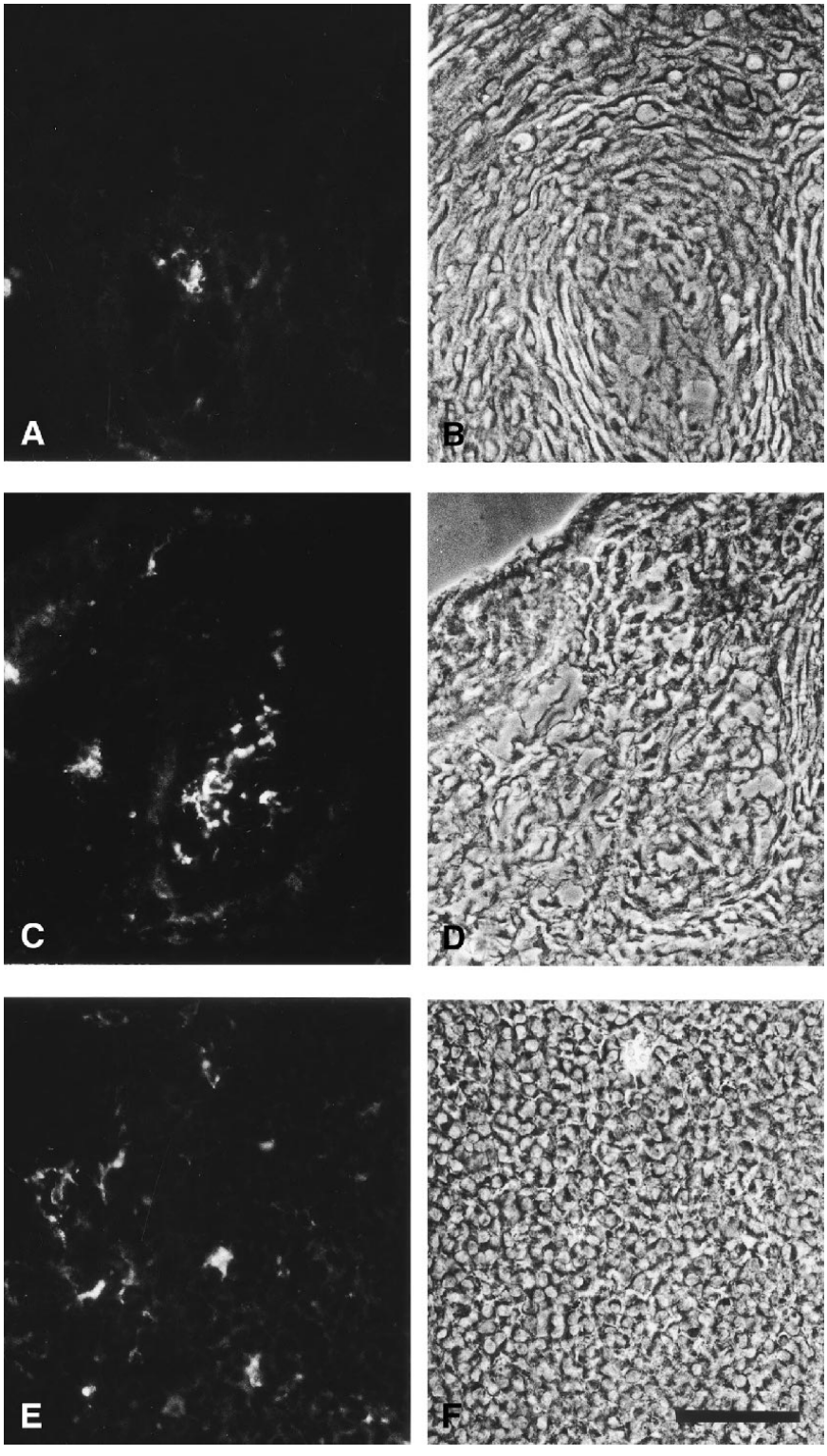

Figure 2.
Location of lamina propria dendritic cells in periodontitis. MHC-II positive, mostly dendritic cells were found in the superficial lamina propria on the oral side ***(A, B)*** *and in higher numbers within pocket infiltrate* ***(C, D)****, as well as within plasma cell aggregate* ***(E, F)****. Langerhans' cells in the epithelium did not label for MHC-II (compare A with A in Figure 1). A, C, and E: immunofluorescence for MHC-II; B, D, and F: phase contrast microscopy of the same fields in A, C, and E, respectively (bar = 50 μm).*

Contrary to HIV-associated periodontitis, in the latter case, epithelial Langerhans' cells appear to be as well differentiated as in controls. In addition, dendritic cells in the lamina propria are associated with both CD4-positive and CD8-positive lymphocytes and with plasma cells. Data on the presence of both classes of T lymphocytes in chronic periodontitis have been available since 1990[35] and have been expanded here by data on dendritic cells. These findings indicate that prerequisites for mounting an efficient, T helper cell-mediated immune response exist in chronic periodontitis, but are lacking in HIV-associated periodontitis.

The lack of MHC-II expression by Langerhans' cells in periodontitis suggests that these cells do not help stimulate immune responses against periodontal pathogens. This might depend on a specific cytokine mixture in the periodontal tissues of these patients, possibly dominated by interleukin-10,[36] which is known to inhibit the antigen-presenting function of Langerhans' cells.[37] Cytokines in periodontitis are secreted partly by immune system cells and partly by other epithelial and connective tissue cell types, and their mixture depends on factors inherent to both bacteria and host.[38] Selective impairment of Langerhans cell function might also be caused by excessively high antigen load, a condition that damages Langerhans' cells but leaves the antigen-presenting potential of lamina propria dendritic cells unaltered.[39]

The fact that lamina propria dendritic cells, instead of Langerhans' cells, behave as antigen-presenting cells and, perhaps, the occurrence of a peculiar cytokine mixture in periodontitis can lead to inappropriate stimulation of T cells, originating responses mediated by Th2 and cytotoxic lymphocytes that can be harmful to tissue.[38,40-42] Elicitation by dendritic cells of a Th2, instead of Th1, lymphocyte-mediated response depends in part also on the ratio of stimulator to responder cell numbers.[43] Immune responses driven by Th2 lymphocytes lead to secretion of antibodies, a fact that is known to occur in periodontitis, and antibodies

can be protective to tissues, or not, depending on the antigens recognized[44,45] and the immunoglobulin type secreted.[46-48] Antibodies may even contribute to tissue damage, in cases where autoreactive idiotypes against tissue cells[49-51] and extracellular matrix components[52] are generated. Dendritic cells are responsible for selecting epitopes to be presented to helper T lymphocytes in the context of MHC-II molecules; therefore, they can influence which antigens will be recognized by antibodies secreted by plasma cells upon T-cell help in periodontitis tissues. A hint to such a role of dendritic cells is given by the association of a definite MHC-II haplotype (HLA-DR4) with enhanced susceptibility to disease and to generation of anticollagen antibodies.[53] Also, dendritic cells in vitro have proved to be capable of stimulating B cell differentiation to plasma cells independent of T lymphocytes.[14,15] The presence of dendritic cells expressing costimulatory molecules inside plasma cell aggregates observed in this study lends support to the hypothesis that in vitro findings might be applied in vivo and that dendritic cells play a role in plasma cell differentiation during chronic periodontitis. It may be speculated that dendritic cells instruct lymphocytes to secrete specific types of immunoglobulins, thus contributing to regulate this aspect of local immune response. Since dendritic cells can present antigens to CD8-positive T lymphocytes in the context of MHC-I molecules, they may also be involved in generating cytotoxic lymphocytes directed against epithelial or connective tissue cells and contributing to tissue damage. A hint to such a role of dendritic cells is given by the association of definite MHC-I haplotypes (HLA-A2 and HLA-B5) with partial protection against chronic periodontitis.[53]

Table 1.

Immunolabeled Cells (N) in the Superficial Lamina Propria*

	Oral†	Sulcular†	Pocket†	*P*
MHC-II	10 ± 5	9 ± 2	32 ± 10	<0.05
CD54	5 ± 1	11 ± 3	15 ± 10	n.s.‡
CD80	7 ± 5	16 ± 3	30 ± 3	<0.05
CD86	12 ± 18	13 ± 7	35 ± 17	n.s.
CD4	8 ± 5	26 ± 23	16 ± 12	n.s.
CD8	n.d.§	12 ± 5	30 ± 10	<0.05

* Periodontitis biopsies.
† Mean values and SD of labeled cells per 0.1 mm^2 of section surface area.
‡ n.s. = not significant.
§ n.d. = not determined.

In conclusion, although this morphological study does not directly address the mechanism of action of dendritic cells in chronic periodontitis, it shows that there are prerequisites for Langerhans' cell unresponsiveness and lamina propria dendritic cell activity which shape the local immune response during disease and perhaps ultimately steer that re-sponse toward success or failure.

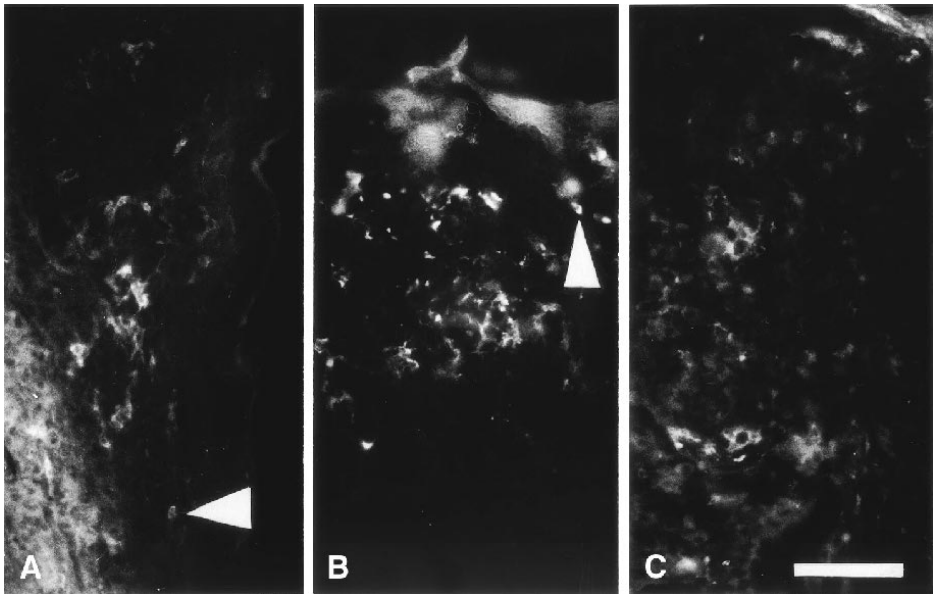

Figure 3.
Expression of marker molecules by dendritic cells in the lamina propria. As shown by immunofluorescence, at least a few dendritic cells in the lamina propria express CD54 ***(A)****, CD 80 (****B****; the limited labeling of epithelium is an artifact), and CD 86* ***(C)****. All pictures are taken from infiltrated areas in the sulcus (A and B) and pocket (C). Plasma cells labeled for CD54 are shown in the lower left corner of A; areas like this were excluded from counts. Roundish cells like those indicated by arrow heads in A and B were also excluded from counts (bar = 50 μm).*

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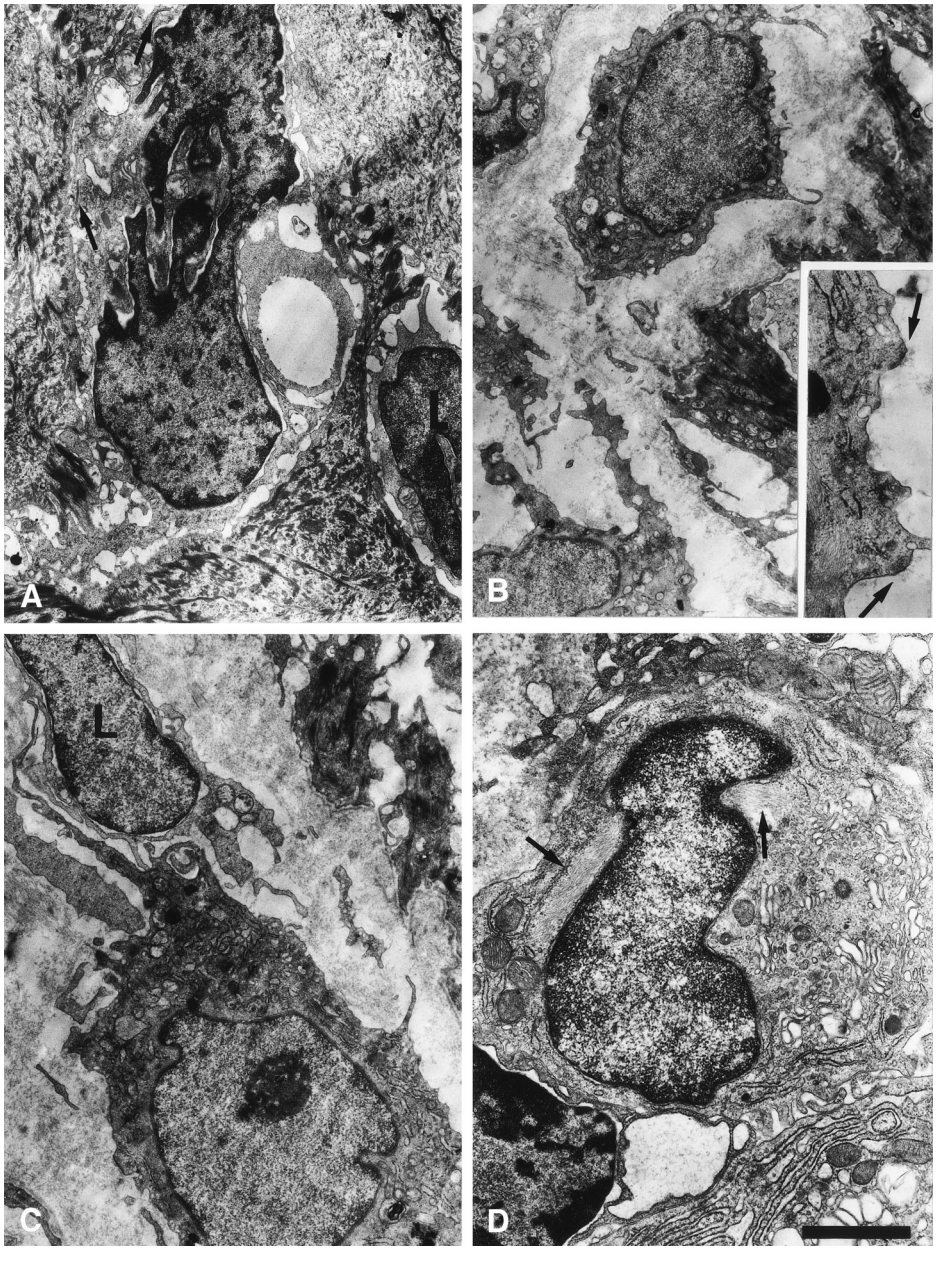

Figure 4.
Structure and intercellular relationships of dendritic cells as shown by electron microscopy. ***A.*** *A Langerhans' cell (arrows indicate Birbeck granules) and a lymphocyte (L) do not contact each other in the sulcular epithelium of a periodontitis case.* ***B.*** *Dendritic cells in the lamina propria of clinically healthy gingiva are located between a capillary (top left) and epithelium (right) and contain a few lysosomes; inset shows focal junctions to the extracellular matrix (arrows) and intermediate filaments in the cytoplasm of lamina propria dendritic cells in periodontitis.* ***C.*** *A lamina propria dendritic cell is in contact with a lymphocyte (L) in periodontitis.* ***D.*** *A dendritic cell is located among plasma cells in periodontitis; the arrows indicate intermediate filaments. Electron microscopy; bar = 2 µm, and 1 µm for inset in B.*

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Send reprint requests to: Dr. Paolo Romagnoli, Department of Anatomy, Histology and Forensic Medicine, Sect. E. Allara-Viale Pieraccini 6, I-50139 Firenze, Italy. Fax: 39 055 4271385; e-mail: paolo.romagnoli@unifi.it.

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