Oral Microbiology Immunology 2006: 21: 197–200
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY
AND IMMUNOLOGY

Short communication

Langerhans cells express matrix metalloproteinases 9 and 2 and tissue inhibitors of metalloproteinases 1 and 2 in healthy human gingival tissue and in periodontitis

A. Bodineau[1], G. Godeau[1], N. Brousse[2], B. Pellat[1], M. Folliguet[3], S. Séguier[1,2]

[1]Faculty of Dental Surgery (EA 2496), University René Descartes–Paris 5, Montrouge, [2]Department of Pathology, Necker-Enfants Malades Hospital (EA 219), Paris, [3]Department of Odontology, Louis Mourier Hospital, Colombes, France

Bodineau A, Godeau G, Brousse N, Pellat B, Folliguet M, Séguier S. Langerhans cells express matrix metalloproteinases 9 and 2 and tissue inhibitors of metalloproteinases 1 and 2 in healthy human gingival tissue and in periodontitis.
Oral Microbiol Immunol 2006: 21: 197–200. © Blackwell Munksgaard, 2006.

Background: As antigen-presenting cells, Langerhans cells may play an important role in the initiation and maintenance of periodontal disease. This study is the first report that extends our knowledge of the expression of matrix metalloproteinases and their endogenous tissue inhibitors by Langerhans cells in healthy and diseased gingival tissues.
Methods: Single and double immunolabeling procedures were carried out using monoclonal antibodies against CD1a, matrix metalloproteinases 2 and 9, and tissue inhibitors of matrix metalloproteinases 1 and 2, and analyzed by conventional and confocal microscopes.
Results: Langerhans cells expressed matrix metalloproteinases 2 and 9, and tissue inhibitors of matrix metalloproteinases 1 and 2 in healthy and diseased gingival tissues. The tissue inhibitors of matrix metalloproteinase-positive Langerhans cells were mainly observed in the upper epithelial layers. Matrix metalloproteinase 9-positive Langerhans cells were observed especially during periodontitis and in the basal epithelial layer or crossing the basement membrane.
Conclusion: During periodontal disease, changes in the expression of matrix metalloproteinases and their tissue inhibitors by gingival Langerhans cells could be implicated in the migration of the cells towards the connective tissue.

Key words: confocal; immunohistochemistry; Langerhans cells; matrix metalloproteinases; periodontitis; tissue inhibitors of matrix metalloproteinases

Dr. Sylvie Séguier, Faculté de Chirurgie Dentaire, Université René-Descartes–Paris 5, 1 rue Maurice Arnoux, 92120 Montrouge, France
Tel.: +33 1 58 076833;
fax: +33 1 58 076899;
e-mail: sylvie.seguier@univ-paris5.fr
Accepted for publication September 15, 2005

Langerhans cells are dendritic cells located in the epithelia of the oral mucosa where they are able to capture foreign antigens. As antigen-presenting cells, Langerhans cells travel to the regional lymph nodes (3, 5) and there initiate antigen-specific T-cell proliferation (2). They may play an important role in the initiation and maintenance of periodontal diseases (12). To reach the draining nodes, Langerhans cells must first cross the basement membrane, which contains molecules such as type IV collagen, laminin, fibronectin and heparan sulfate proteoglycans. In many cell types, local proteolysis of the basement membrane associated with cell migration depends on the activity of matrix metalloproteinases (MMPs) (15). MMPs are endopeptidases able to degrade extracellular matrix macromolecules, and MMP-2 and MMP-9 are particularly implicated in the degradation of the basement membrane (10). The activity of MMPs is regulated by their endogenous tissue inhibitors

(TIMPs); TIMP-1 forms high affinity complexes with the active forms of MMPs (4, 14) and TIMP-2 is able to bind to the proform of MMP-2 but not to MMP-9, which can bind to TIMP-1.

Previous studies in our laboratory have shown that during periodontitis the number of gingival Langerhans cells decreases according to the severity of the periodontal disease (11) and the important morphologic changes of Langerhans cells may reflect a cellular adaptation during the epithelial transmigration (13). The present study extends these quantitative studies to further examine the role that immunoexpression of MMPs/TIMPs by gingival Langerhans cells could play in the migration of Langerhans cells.

Material and methods

With patient consent, healthy and diseased gingival tissue samples which would otherwise have been discarded were obtained from 12 patients: six from periodontitis patients (aged 46–66 (mean = 53), three males and three females) undergoing tooth extraction because of extreme tooth mobility due to the periodontal disease, and six from healthy patients (aged 25–61 (mean = 49), two males and four females) undergoing tooth extraction of the third molar or for orthodontic reasons. The diagnosis of chronic periodontitis was established on the basis of clinical and radiographic criteria (bone resorption) according to the classification system for periodontal diseases and conditions (1). All diseased samples were obtained during the extraction of a tooth showing at least one pocket depth = 5 mm and gingival index 2 and 3 from Löe & Silness (6). The surgical procedures were performed in the surgery room in one of Paris 5 University Dental Clinics and gingival samples were immediately placed in Tissue-Tek® O.C.T. Compound (Sakura Finetek Europe B.V., Zoeterwoude, Netherlands) and frozen in liquid nitrogen. Serial 6-µm-thick frozen sections were obtained, air-dried at room temperature for 2 h, then fixed in acetone for 10 min and kept at − 80°C until the day of the study.

Single and double immunolabeling observed by conventional microscope

For immunolabeling of Langerhans cells, sections were incubated with CD1a monoclonal mouse antihuman antibody (Beckman Coulter, Roissy, France) at 1 : 100 dilution for 45 min, then with peroxidase-conjugated rabbit antimouse immunoglobulin for 30 min (Dakopatts, Glostrup, Denmark) at 1 : 20 dilution in TBS bovine serum albumin (BSA) 1% with human serum, and with peroxidase-conjugated swine antirabbit antibody (Dakopatts) at 1 : 20 dilution for 30 min. The peroxidase reaction was developed with 3-amino-9-ethylcarbazole substrate-chromogen (Sigma Immunochemicals, Saint-Quentin Fallavier, France) for 5 min and the Langerhans cells were shown in red. For immunolabeling of MMPs and TIMPs, anti-MMP-2, anti-MMP-9, anti-TIMP-1 and anti-TIMP-2 monoclonal mouse antihuman antibodies were used at 1 : 50 dilution (Calbiochem, Meudon, France). Rehydrated sections were incubated with one of the primary antibodies mentioned above for 45 min, then with alkaline phosphatase-conjugated rabbit antimouse antibody (Dakopatts) for 30 min at 1 : 20 dilution in TBS BSA 1% with human serum, and with alkaline phosphatase-conjugated swine antirabbit antibody (Dakopatts) for 30 min at 1 : 20 dilution. The alkaline phosphatase reaction was visualized with fast blue substrate (Sigma Immunochemicals) for 25 min. MMP-2, MMP-9, TIMP-1, and TIMP-2 were then revealed in blue. All single immunolabelings were also performed using an avidin-biotin-immunoperoxidase technique with 3,3′-diaminobenzidine tetrahydrochloride with hydrogen peroxide (Dakopatts) and the immunolabelings shown in brown (these sections were counterstained with hematoxylin). Non-specific reactivity of the antibodies was checked by omitting the primary antibody or by using an irrelevant isotype matched

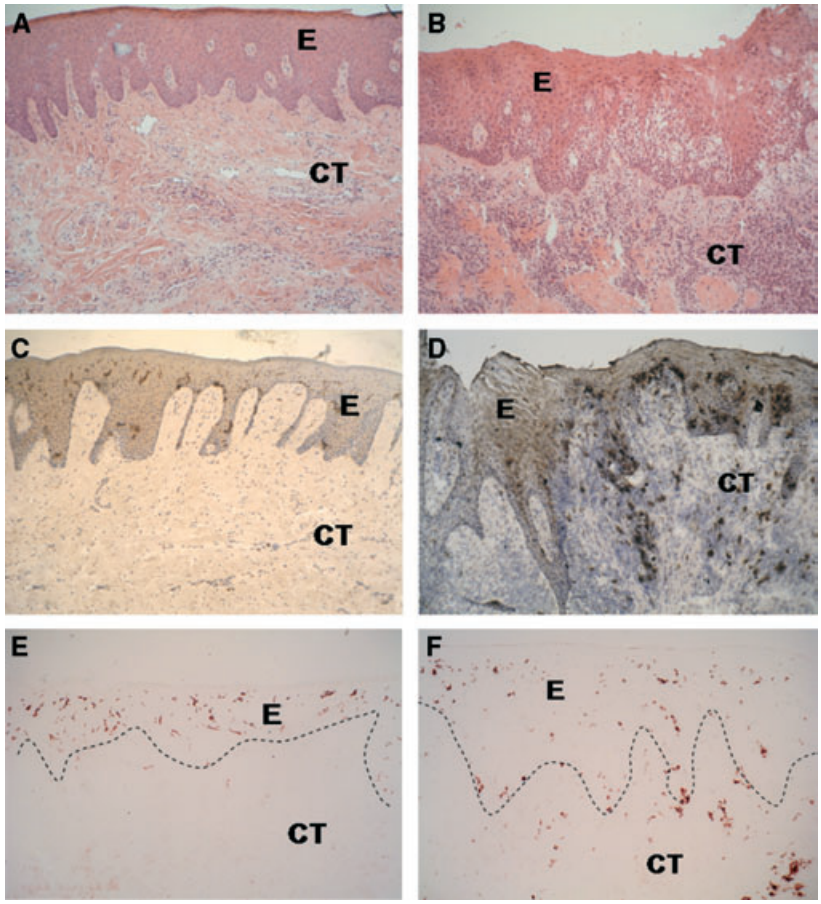

Fig. 1. Hematoxylin-eosin-saffron (HES) staining and immunolabeling of $CD1a^+$ Langerhans cells in healthy (A, C, E) and diseased (B, D, F) gingival sections. All the healthy and the diseased sections were from different subjects. During periodontitis (B), HES staining showed an infiltration of inflammatory cells into the connective tissue associated with an alteration of the epithelial structure. In healthy gingiva (C), immunolabeled cells are located in the epithelial tissue and none of them was seen in the connective tissue. During periodontitis (D) immunolabeled cells are located in both epithelium and connective tissue. Furthermore, single immunolabeling of $CD1a^+$ Langerhans cells shown in red using amino-9-ethylcarbazole as a substrate for peroxidase also showed the presence of $CD1a^+$ immunolabeled cells in the connective tissue of diseased samples (F) when compared with healthy gingival tissue (E). E, epithelium; CT, connective tissue. Magnification × 10.

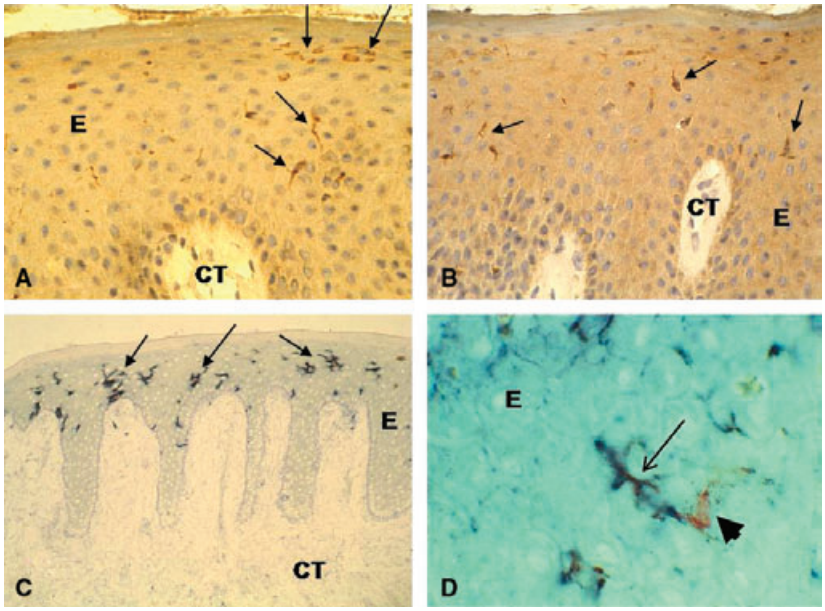

Fig. 2. Single immunolabeling of tissue inhibitors of matrix metalloproteinases (TIMP) 1 (A, B; magnification ×40) and double immunolabeling of Langerhans cells/TIMP-2 (C; magnification ×10) and Langerhans cells/TIMP-1 (D; magnification ×100) observed under conventional microscope in healthy (A, C) and diseased (B, D) gingival tissues . The double immunolabeling procedure showed $CD1a^+$ cells in red, TIMP expression in blue and $CD1a^+TIMP^+$ cells in purple. In healthy gingival samples (A), $TIMP\text{-}1^+$ Langerhans cells presenting their characteristic morphology were mostly observed in the upper epithelial layers, whereas during periodontitis samples (B) Langerhans cells appeared in the middle part of the epithelium and their morphology was less dendritic. As shown in both A and B, no $TIMP\text{-}1^+$ Langerhans cells were observed in the basal epithelial layer. In healthy gingival tissue (C), the double immunolabeling CD1a/TIMP-2 showed purple immunolabeled cells in the gingival epithelium, whereas in diseased gingival tissue (D) the double immunolabeling CD1a/TIMP-1 revealed that intraepithelial Langerhans cells correspond to a heterogeneous cell population with purple $TIMP\text{-}1^+$ Langerhans cells (arrow) in close contact with red $TIMP\text{-}1^-$ Langerhans cells (arrowhead). E, epithelium; CT, connective tissue.

primary antibody (MMP-3 monoclonal mouse antihuman antibody; Calbiochem). To reassess the data obtained from single immunolabeling, colocalization of molecules (CD1a/MMPs and CD1a/TIMPs) was analyzed by double immunolabeling. Staining with anti-CD1a antibody was performed using the same protocol as described above for a single immunolabeling procedure, peroxidase reaction excepted. After the two peroxidase-conjugated antibody incubations, the sections were incubated with one of the second primary antibodies, followed by incubations with alkaline phosphatase antibodies. The peroxidase reaction occurred at the end of the procedure, immediately before the alkaline phosphatase reaction. The double immunolabeling procedure used for identifying the molecules located on the same cells may give false negative results because visualization of the first molecule can preclude visualization of the second. Therefore, the application procedure of the first and second primary antibodies was reversed. Controls omitting the second primary antibody were also performed to avoid cross-reactions. With the double immunolabeling procedure the cells expressing both immunolabeled molecules were shown in purple.

Double immunolabeling procedure observed by confocal microscope

The sections were sequentially incubated with one of the monoclonal mouse antihuman antibodies directed against MMP or TIMP for 45 min and antimouse Cy3-conjugated secondary antibody (Biosource, Besançon, France) at 1 : 1000 dilution in TBS BSA 1% with human serum for 30 min. The sections were then incubated with fluorescein isothiocyanate (FITC)-conjugated CD1a antibody (Immunotech, Marseilles, France) at 1 : 20 dilution in TBS BSA 1% with human serum for 30 min. Mounted slides were analyzed using a confocal laser scanning microscope (Zeiss LSM 510, Gena, Germany). With respect to the use of color superimposition of confocal images, FITC in green and Cy3 in red provide clear colocalization information of the two immunolabeled molecules. Orange indicates that the molecules are colocalized.

In gingival samples from patients presenting with chronic periodontitis, a local infiltration of inflammatory cells in the upper connective tissue associated with an important alteration of the epithelial structure was noted (Fig. 1B). Furthermore, single immunolabelings of Langerhans cells by peroxidase reaction developed with 3,3′-diaminobenzidine tetrahydrochloride (Fig. 1D) and with 3-amino-9-ethylcarbazole (Fig. 1F) showed that Langerhans cells cross the basement membrane and strongly migrate toward the connective tissue. These histologic findings were not observed in healthy gingival samples (Fig. 1C, E), in which Langerhans cells were mainly located in the epithelial compartment and no or very few Langerhans cells were noted in the gingival connective tissue. Thus, Langerhans cell migration was especially observed during periodontal disease. Our results concerning TIMP expression by Langerhans cells showed that they express TIMP-1 and TIMP-2 in healthy as well as in diseased gingival tissues. However, in healthy samples, dendritic $TIMP\text{-}1^+$ Langerhans cells often appeared in the upper epithelial layers (Fig. 2A) and Langerhans cells coexpressed TIMP-2 (Fig. 2C), whereas in periodontitis samples (Fig. 2B) $TIMP\text{-}1^+$ Langerhans cells with a decreased number of dendritic processes were located in the middle part of the epithelium and the double immunolabeling showed some $TIMP\text{-}1^-$ Langerhans cells (Fig. 2D). Indeed, in healthy and diseased samples, no $TIMP\text{-}1^+$ Langerhans cells were seen in the basal epithelial layer. Therefore, we suggest that TIMP expression may prevent migration of some Langerhans cells toward the connective tissue and a decrease in the expression of TIMPs could be implicated in migration and crossing of the basement membrane by Langerhans cells. Very few MMP^+ Langerhans cells were seen in healthy gingival tissues, whereas during periodontitis we observed some MMP^+ Langerhans cells in the middle of the epithelium. Some $MMP\text{-}2^+$ Langerhans cells were observed in the basal epithelial layer; however, numerous $MMP\text{-}9^+$ Langerhans cells were seen in the basal epithelial layer and in the vicinity of the basement membrane (Fig. 3). These MMPs, which most efficiently cleave basement membrane type IV collagen, could be considered key enzymes involved in the migration of Langerhans cells from the epithelium to the connective tissue and

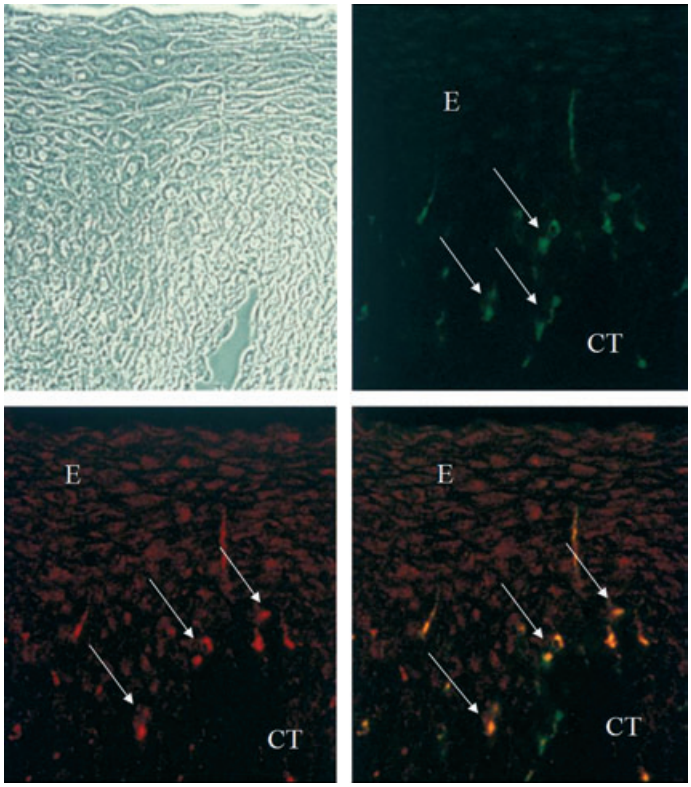

Fig. 3. Confocal microscopy analysis for CD1a and MMP-9 colocalization in diseased gingival tissue. The upper left image was obtained by transmission light microscopy of the confocal microscope. In the upper right image, Langerhans cells are revealed in green fluorescence by FITC-conjugated CD1a antibody (arrows). On the lower left image, MMP-9 was revealed in red fluorescence by Cy3-conjugated secondary antibody (arrows). When red and green staining overlap (lower right image) the Langerhans cells appear orange (arrows), located near the basement membrane (magnification ×40). E, epithelium; CT, connective tissue.

in their crossing of the basement membrane (7, 9). In a previous study we showed a significant decrease in the number of Langerhans cells (11) during severe periodontitis compared with healthy gingival tissue. Thus, in response to the bacterial elements (5) that invade the gingival epithelium, Langerhans cells migrate towards the connective tissue, their migration being regulated by tumor necrosis factor α and interleukin 1β (3). The migratory capacity of Langerhans cells could be closely linked to the balance between MMP and TIMP expression, as was suggested by other investigators (8, 9) and the presence of MMP-2^{+} and, especially, MMP-9^{+} Langerhans cells in periodontitis samples supports the hypothesis that MMPs could participate in cell migration through the basement membrane and towards the connective tissue.

References

1. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999: **4**: 1–6.
2. Barrett AW, Cruchley AT, Williams DM. Oral mucosal Langerhans' cells. Crit Rev Oral Biol Med 1996: **7**: 36–58.
3. Cumberbatch M, Griffiths CE, Tucker SC, Dearman RJ, Kimber I. Tumour necrosis factor-alpha induces Langerhans cell migration in humans. Br J Dermatol 1999: **141**: 192–200.
4. Docherty AJP, O'Connell J, Crabbe T, Angal S, Murphy G. The matrix metalloproteinases and their natural inhibitors: prospects for treating degenerative tissue diseases. Trends Biotechnol 1992: **10**: 200–207.
5. Jotwani R, Palucka KA, Al Quotub M, Nouri-Shirazi M, Kim J, Bell D, et al. Mature dendritic cells infiltrate the T-cell-rich region of oral mucosa in chronic periodontitis: *in situ, in vivo*, and *in vitro* studies. J Immunol 2001: **167**: 4693–4700.
6. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963: **21**: 533–551.
7. Noirey N, Staquet MJ, Gariazzo MJ, Serres M, Andre C, Schmitt D, et al. Relationship between expression of matrix metalloproteinases and migration of epidermal and *in vitro* generated Langerhans cells. Eur J Cell Biol 2002: **81**: 383–389.
8. Osman M, Tortorella M, Londei M, Quaratino S. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases define the migratory characteristics of human monocyte-derived dendritic cells. Immunology 2002: **105**: 73–82.
9. Ratzinger G, Stoitzner P, Ebner S, Lutz MB, Layton GT, Rainer C, et al. Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin. J Immunol 2002: **168**: 4361–4371.
10. Reynolds JJ, Meikle MC. Mechanisms of connective tissue matrix destruction in periodontitis. Periodontol 2000 1997: **14**: 144–157.
11. Seguier S, Godeau G, Brousse N. Immunohistological and morphometric analysis of intraepithelial lymphocytes and Langerhans cells in healthy and diseased human gingival tissues. Arch Oral Biol 2000: **45**: 441–452.
12. Seguier S, Godeau G, Leborgne M, Pivert G, Brousse N. Immunohistologic and morphometric analysis of cytotoxic T lymphocytes in gingivitis. J Periodontol 1999: **70**: 1383–1391.
13. Seguier S, Godeau G, Leborgne M, Pivert G, Brousse N. Quantitative morphological analysis of Langerhans cells in healthy and diseased human gingiva. Arch Oral Biol 2000: **45**: 1073–1081.
14. Willenbrock F, Murphy G. Structure-function relationships in the tissue inhibitors of metalloproteinases. Am J Respir Crit Care Med 1994: **150**: S165–S170.
15. Xia M, Leppert D, Hauser SL, Sreedharan SP, Nelson PJ, Krensky AM, et al. Stimulus specific of matrix metalloproteinase dependence of human T cell migration through a model basement membrane. J Immunol 1996: **156**: 160–167.