[Frontiers in Bioscience 10, 3034-3041, September 1, 2005]

NEURO-IMMUNITY IN STRESS-RELATED ORAL ULCERATIONS: A FRACTAL ANALYSIS

Francesco Chiappelli [1,2], Joseph Alwan [3], Paolo Prolo [1,2], Russell Christensen [1], Milan Fiala [1,2], Olivia S. Cajulis [4], and George Bernard [1]

[1] University of California at Los Angeles, School of Dentistry, Division of Oral Biology & Medicine, [2] West Los Angeles Veterans Administration Medical Center, [3] Loma Linda University, School of Dentistry, [4] Dental Group of Sherman Oaks, Inc.

TABLE OF CONTENTS

1. ABSTRACT

By testing archival paraffinized biopsy blocks obtained from the oral pathology library with immunohistochemistry, we tested the hypothesis that substantial alterations are demonstrable in the cross-talk between sympathetic (VMAT) and para-sympathetic innervation (VAchT), and resident CD3+ T cells in the mucosa from oral lichen planus (OLP), compared to recurrent aphthous stomatitis (RAS) and control biopsies. We quantified fractal dimension and Euclidean dimension of CD3+ cells between the two pathologies, and across the set of CD3+ cells proximal to the vesicles of monoamines transport (VMAT)+ or the vesicles of acetylcholine transport (VAchT)+ innervation, compared to cells relatively distal to the nerve endings. The data show exquisite organization of the punctuate sympathetic and para-sympathetic staining about the resident CD3+ T cells in the OLP lesions, but not in the aphthous lesions or in control mucosa. Fractal analysis reveals that aphthous lesions are characterized by CD3+ T cells of larger size (Euclidean dimensional map), compared to control mucosa. CD3+ T cells in OLP lesions are also found to be significantly larger than those found in control lesions, when they are not proximal to sympathetic or para-sympathetic vesicles. The membrane of CD3+ T cells is overall more complex (fractal dimension) in aphthous lesions, compared to control sections. A similar trend is apparent, albeit not statistically significant, in CD3+ T cells resident in OLP lesions, whether or not they are located proximal to nerve endings. An overall decrease in the ratio of fractal dimension-over-topological dimension was also observed across the pathological lesions, compared to control. Taken together, these data indicate that as CD3+ T cells grow larger in the pathological conditions, they, in effect, stretch their plasma membrane, and that the cells may be at different stage of the cell cycle, relative to their position *vis a' vis* nerve endings. Because fractal analysis is performed on individual cells, it has the potential of being developed in a novel diagnostic test, as well as a prognostic tool for monitoring the etiology and the course of treatment at the individual cellular level. Our findings also open new frontiers of fundamental, clinical and translational biosciences of OLP.

2. INTRODUCTION

The term "stress-related oral ulcerations" refer to ulcers in the oral mucosa associated with depressed immune surveillance consequential to the experience of psycho-emotional stress. We have proposed that allostasis plays an integral role in the etiology and resolution of these pathologies (1,2).

Sterling and Eyer (3) defined "allostasis" as the set of psycho-physiological events that determine and regulate the process of recovery from stress. Heterostasis describes the situation where the demands upon the organism exceed its physiological capacity of recovery. Allostatic regulation refers to the recovery and maintenance of internal balance and viability amidst changing circumstances consequential to stress.

The allostatic response is thought to encompass the behavioral and physiological functions that direct the adaptive function of regulating homeostasis in response to stress challenges. The allostatic load pertains to the physiological, psychological, and pathological side effects associated with allostasis. By contrast, allostatic overload describes the psycho-biological collapse, which is invariably associated with a variety of systemic health outcomes (1-3).

Two allostatic responses have been identified, and we proposed that the lesions associated with recurrent aphthous stomatitis (aphthous canker sores, RAS), a stress-associated lymphocyte-dependent pathology of the gingival mucosa, pertain to a Type I allostatic response, which permits recovery by the patient. Aphthous lesions typically resolve in 7-10 days. Immunohistochemical techniques have established that aphthous lesions are characterized by invasion of CD3+ T cells (1,2,4), which is in fact associated with an increased TH1 pattern of cytokine at the molecular level (5).

By contrast, lesions associated with oral lichen planus (OLP), which are also characterized immunohistochemically by invasion of CD3+ T cells (4), pertain to a Type II allostatic response. Type II responses are typically chronic situations, from which the patient rarely recovers. Indeed, OLP is a chronic condition that afflicts the patient for a lifetime (1,2). Data also indicate that the resident infiltrated T cells in OLP lesions may be oligoclonally restricted to the Vβ2, Vβ6, and Vβ19 sub-families of the T cell receptor, perhaps as a consequence of *in situ* stimulation with a restricted epitope of either a nominal antigen on the major histocompatibility complex molecule for these Vβ sub-families, or of a common superantigen for the minority of the Vβ families (6). Another relevant observation from the perspective of the immunopathology of OLP is that the predominance of infiltrating CD3+ T cells express the CD8 moiety as well, and that they promote a significant increase in TH1 cytokine expression *in situ* (7).

The allostatic response is determined by the interaction and inter-regulation between the psychoneuroendocrine and the immune systems. We hypothesized that it may, therefore, be possible to demonstrate substantial alterations in the cross-talk between sympathetic and para-sympathetic innervation, and resident CD3+ T cells in the mucosa of aphthous compared to OLP biopsies.

Fractal analysis has emerged as a reliable procedure for quantifying the complexity of cellular or nuclear membranes (8-15). The fractal dimension of a cell is typically a reflection of its plasma membrane complexity. It is a non-integer measure of how "complicated" the cells, or any "self-similar" object is. Fractal dimension is generally larger than the integer derived from a topological dimension based on Euclidean geometry (the Euclidean dimension) by a factor of 5 up to 20%, depending on the cell growth and maturation stage. The fractal dimension allows the quantitative comparison of the objects (here, cells) under study, whereas the Euclidean dimension describes the number of coordinates required to specify the object. We have demonstrated elsewhere that fractal dimension analysis is sensitive enough to distinguish CD69+ from CD25+ activated CD3+ T cells (15).

Therefore, the purpose of this study was two-fold. Firstly, we sought to establish neural-immune interactions in aphthous lesion biopsies, compared to biopsies obtained from OLP lesions. We used double-label immunohistochemical techniques to monitor the proximal relationship between sympathetic (vesicles for monoamine transport, VMAT) or para-sympathetic nerve endings (vesicles for acetylcholine transport, VAchT), and CD3+ T cells. For control purposes, we verified that the identified CD3+ cells were actively dividing. Secondly, we sought to quantify the cell membrane complexity (fractal dimension) and topology (Euclidean dimension) of CD3+ cells between the two pathologies, and across the set of CD3+ cells proximal to VMAT+ or VAchT+ innervation, compared to cells relatively distal to these nerve endings.

3. MATERIALS & METHODS

3.1. Immunohistochemistry

Parafinized blocks of biopsies from normal control human oral mucosa (n=5), aphthous lesions (n=7), and OLP lesions (n=9) were obtained from the archival pathological specimen library (UCLA Oral Medicine). Diagnosis had been previously established and ascertained by two independent oral pathologists.

Blocks were microtome-sectioned (4 □m), and mounted on colorfrost - plus glass slide (Fisher Scientific, Pittsburgh, PA), dried at room temperature, and incubated at 58^{o} C for 30 minutes. Slides were deparafinized in batches by incubation in baths of progressively decreasing concentrations of xylene and alcohol, washed in double distilled water, and incubated in antigen retrieval buffer (100 mM Tris[7.4], 10% bovine serum albumin, 15 mM NaCl). Following inactivation of endogenous peroxidase, the slides were washed again thoroughly, and incubated with the primary antibody for 16 h at 4^{o} C. Primary antibody species were mouse monoclonal anti-human CD3

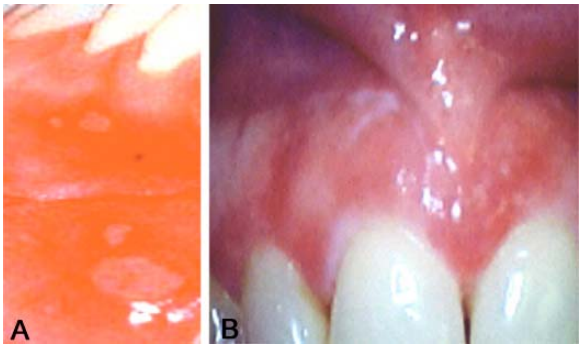

Figure 1. Pathological manifestation of stress-associated oral lesions – Panel A shows an oral photographs of a typical recurrent aphthous stomatitis lesion in the gingival and buccal aspects of the mucosa, with evident inflammation of the surrounding mucosa. Panel B shows a oral lichen planus lesion (reticular stage) in the gingival aspect, with clear white striations.

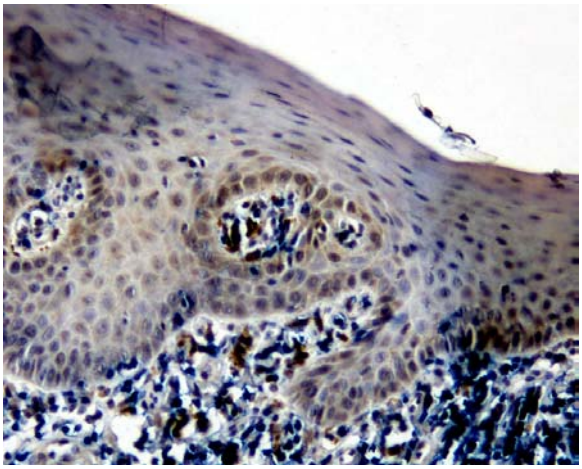

Figure 2. Morphological perspective of the oral mucosa – Methyl green counter-stained (20X objective) OLP section double-stained CD3-VAchT, showing the cleared demarcated organization of the cellular strata in the mucosa.

antibody (Becton Dickinson, San Jose, CA), rabbit polyclonal anti-rat VMAT-2 and VAchT antibody (SIGMA-Aldrich, St. Louis, MO), or rabbit polyclonal anti-human cyclin D3 antibody (Biosource, Camarillo, CA). Preliminary experiments had established their optimal dilutions.

Following washing, the epitopes recognized by mouse primary antibody (i.e., CD3) were detected by the DAKO (Carpinteria, CA) diaminobenzidine (DAB) kit, which developed as a red-brown staining. The epitopes recognized by rabbit primary antibody (i.e., VMAT, VAchT, cyclin D3) were detected by the DAKO (5-Bromo-4-chloro-3-indolyl phosphate dipotassium/ nitrotetrazolium (BCIP/NBT) kit, which generated a dark blue staining.

Upon completion of staining development, the slides were washed, counter-stained with methylgreen and dehydrated by the reverse alcohol/xylene process. Fully dehydrated slides were permanently mounted using permanent mounting medium (Fisher Scientific), and stored at room temperature.

3.2. Data Analysis

Slides were analyzed with a Olympus DP11 microscope at 20X, 40X and 100X (oil immersion) magnification, with a 10X eyepiece. Digital pictures were processed by the Image-Pro Plus 4.1 (Media Cybernetics) software.

For fractal analysis, independent cells were isolated from the micrographs, and the contrast was equalized by means of computer-aided software (micrografx picture publisher). Euclidean estimates of size (i.e., relative estimates of cell radius) and fractal dimension obtained by the box-counting method (i.e., relative complexity of the staining pattern on the cell membrane) were computed with the FractalFox software (Qichang Li, Univ. Memphis).

Self-similarity was established by means of the evaluation of the fit of regression lines, taking higher values for the coefficient of determination as representative of better fit: the closer the coefficient of determination to 1.0, the larger the proportion of random error accounted for, and the greater the extent of self-similarity of the objects under study (12,15). Data were analyzed by parametric ANOVA followed by Dunnett post-hoc comparisons were carried out at a level of significance of 5% with Bonferroni correction.

4. RESULTS AND DISCUSSION

4.1. Immunohistochemistry

The typical manifestation of recurrent aphthous stomatitis (panel A) and of a reticular oral lichen planus lesion (panel B) is shown in Figure 1. Evident is the inflammation of the mucosa surrounding the aphthous lesion. Also evident is the striated nature of the OLP lesion.

Qualitative evaluation of the immunohistochemical staining revealed that at low magnification methyl counter-stained sections show a distinct histological pattern of stratified squamous epithelium, which is evident in the biopsies from normal human mucosa (not shown), and of aphthous lesions (not shown), and is preserved with complete integrity in the OLP lesions (Figure 2).

At higher magnification, under oil immersion, the double immunohistochemical stain reveals a particularly orderly pattern of apposition of the red-brown punctate stain, representing the sympathetic (VMAT, not shown) or the para-sympathetic (VAchT, Figure 3) nerve endings, with the cellular blue stain proper to the CD3+ T cell resident in the lesion. It is important to note that CD3+ T cells were verified to be cells actively engaged in cell activation and proliferation, as manifested by their expression of cyclin D3 (not shown). The peculiar

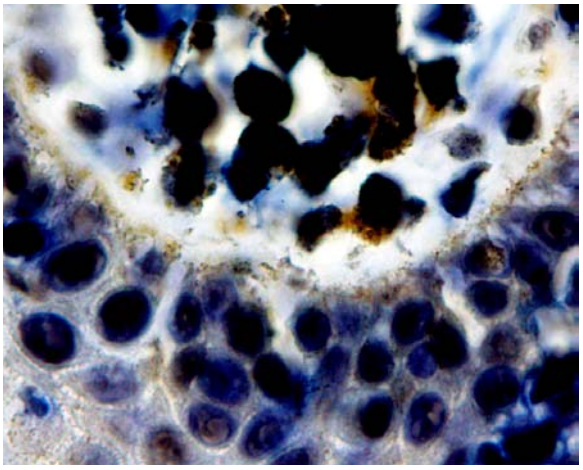

Figure 3. Neuro-immune Organization in OLP lesions – Same section as in Figure 2, at 100X magnification under oil immersion, showing the punctate brownish stain (VAchT) orderly aligned along the bluish stained membranous extensions of CD3+ T cells.

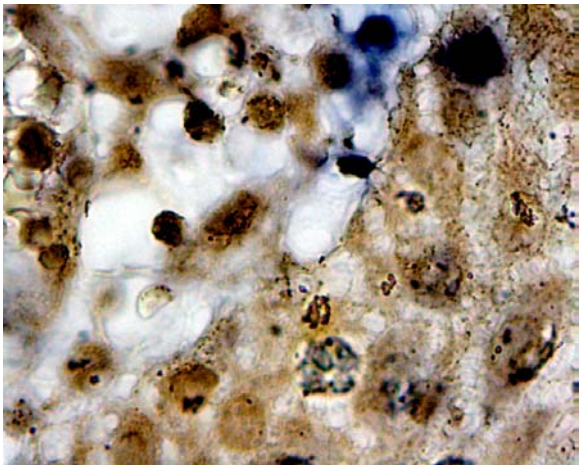

Figure 4. Neuro-immune Organization in aphthous lesions – Methyl green counter-stained aphthous lesion at 100X magnification under oil immersion as in Figure 3, showing the punctate brownish stain (VAchT) randomly distributed in the section independently from the bluish stained membranous extensions of CD3+ T cells.

organization of the neural markers is absent in aphthous lesions (Figure 4), where the distribution of the punctate staining appears to be random, relative to the position of CD3+ T cells. Estimation of the relative size of the punctate staining suggests that the neural vesicles are twice to three times as large in OLP lesions, compared to aphthous lesions. The punctate staining in aphthous and in control mucosa is indistinguishable, in terms of size or distribution. Residents CD3+ cells are 3-4 times more numerous in OLP, compared to aphthous lesions, and relatively rare in control biopsies.

4.2. Fractal Analysis

Quantitative assessment of these staining patterns are summarized in Table 1 and Figure 5. Panel A compares the Euclidean dimension map, roughly equivalent to the cell size, of CD3+ T cells in control (1.078±0.026) and aphthous biopsies (1.133±0.004, p=0.01 vs. control), and in CD3+ T cells that had invaded the OLP lesion but were not in direct contact with sympathetic or para-sympathetic nerve endings ("invading", 1.109±0.019, p=0.03 vs. control) and CD3+ cells that were directly interacting with VMAT or VAchT nerve endings in OLP lesions ("interacting", 1.097±0.014, NS vs. control). Panel B compares the fractal dimension, a measure of the complexity of the cell membrane, of CD3+ T cells in control (1.123±0.014) and aphthous biopsies (1.165±0.008, p=0.01 vs. control), and in invading CD3+ T cells in OLP lesions (1.134±0.017, NS vs. control) and interacting CD3+ cells in OLP lesions (1.123±0.017, NS vs. control).

In brief, these estimates indicate that CD3+ T cells are significantly larger in aphthous lesions, and in OLP lesions when the cells are not proximal to nerve endings, compare to control (panel A). These estimates also indicate that the overall complexity of the CD3 T cell membrane is significantly larger in T cell found in aphthous lesions, compared to control. CD3+ T cell membrane complexity shows a trend toward being also larger in OLP lesions, compared to control, but this difference does not reach statistical significance with the number of cells examined in this preliminary study. Further, these estimates indicate that, as expected on the basis of previous studies of fractal dimension in cellular biology, fractal dimension values are consistently larger by 5-15% than topological dimensions (e.g., Euclidean dimension). This study has further revealed is that this relation is decreased by close to two fold (1.68±0.16) in CD3+ T cells found in pathological lesions (aphthous and OLP), compared to control.

4.3. Immunohistochemistry Data Interpretation

Our findings confirm the literature (1,2,4,16) with respect to the invasion of aphthous and of OLP lesions by CD3+ T cells. Our results yield novel information in that they demonstrate an suggestive pattern of organization between resident CD3+ T cells and sympathetic and para-sympathetic nerve endings, which is evident in OLP but not in aphthous lesions (Figures 3 & 4). This juxtaposition of VMAT and VAchT neural vesicles along the edge of the T cell membrane is reminiscent of similar observations of sympathetic and para-sympathetic nerve endings forming synapse-like junctions with lymphoid cells in general and CD3+ T cells in particular (16,17). This pattern of neural-immune cross-talk is not apparent in control health mucosa.

Moreover, our study integrates into this immunohistochemical inquiry of neuro-immunity in aphthous and OLP lesions the domain of fractal analysis. To our knowledge, this is the first application of fractal inquiry in the evaluation of neural immune interactions in stress-related oral ulcers. Fractal analysis was successfully utilized to characterize the alterations in oral mucosal vascularization in the disease pathogenesis associated with bronchopulmonary dysplasia (18). Fractal dimension analysis was shown to be useful in the characterization of the bone healing process after mandibular orthognathic

Table 1. Analysis of Stress-Related Oral Ulcers, given in standard units of Euclidean Dimension vs. standard units Fractal Dimension

	Control	RAS	OLP, Invading	OLP, Interacting
Number of cells tested	4	2	5	3
Total number of cells tested	20	14	45	18
Euclidean Dimension, mean[1]	1.078	1.133	1.109	1.097
standard deviation	0.026	0.004	0.019	0.014
p value[2]		0.010	0.035	0.152
Fractal Dimension, mean[1]	1.123	1.165	1.134	1.123
standard deviation	0.014	0.008	0.017	0.017
p value[2]		0.011	0.165	0.499

[1] mean of values obtained per individual cells tested in each of n=5 control samples, n=7 recurrent aphthous stomatitis (RAS) samples, and n=9 oral lichen planus (OLP) samples, [2] comparison vs. control

surgery (19). In another study, the epithelial-connective tissue interface in control mucosa, epithelial dysplasia, and squamous cell carcinoma of the floor of the mouth was successfully compared by estimating the local connected fractal dimension using histological specimens in a manner similar to this study (20). However, the use of fractal analysis of infiltrating CD3+ T cells in recurrent aphthous stomatitis and oral lichen planus, as we report here, is novel.

4.4. Fractal Analysis Data Interpretation

The quantification of the observations presented in Figure 2-4 by fractal analysis reveals that aphthous lesions are characterized by CD3+ T cells of larger size (Euclidean dimensional map), compared to control mucosa. CD3+ T cells in OLP lesions are also found to be significantly larger than those found in control lesions, when they are not proximal to sympathetic or para-sympathetic vesicles ("invading") (Figure 5, panel A). The membrane of CD3+ T cells is overall more complex (fractal dimension) in aphthous lesions, compared to control sections. A similar trend is apparent, albeit not statistically significant, in CD3+ T cells resident in OLP lesions, whether or not they are located proximal to nerve endings (Figure 5, panel B). The larger size (Euclidean dimension) in "invading", compared to "interacting CD3+ T cells in OLP lesions suggests that cells proximal to nerve endings ("interacting") are in the G1 or S phases of the cell cycle, and that cells that are not proximal to nerve endings ("invading") tend to be preferentially in the G2 phase. This dichotomy is evidently associated with distinct functionalities of the T cells when they are interacting with sympathetic or para-sympathetic nerve endings, *vis a' vis* when they are not. Moreover, these differences may have a significance in the differential pathogenesis of OLP and aphthous, because one population only of resident CD3+ T cells is found in the latter condition, which is more comparable to the "invading" than the "interacting" sub-population.

Fractal dimension was quantified here only by the box-counting method, which is the common approach used for these assessments in the biological sciences. Related measurements, which ought to be assessed in future studies, include the Hurst exponent, which measures the smoothness of fractal time series and is based on the asymptotic behavior of the rescaled range of the process. Moreover, lacunarity, that is the size distribution of the holes that compose a fractal. High lacunarity pertains to a fractal that has large gaps or holes. A fractal that is translationally invariant has characteristically low lacunarity. Different objects may have the same fractal dimension, but appear largely different because they differ in lacunarity (8,15,21).

It is also important to note that limitations in fractal quantification arise because estimates are derived from pixel counts, a function of the number of pixels cut by the boundary of the object. Cellular contours with a fractal dimension greater than the planar Euclidean dimension of 1.0 appear more complex as the pixel size decreases and the resolution increases. By contrast, the more contorted boundaries have a characteristic higher fractal dimensions, with less error of estimate. Flawed interpretations of pixel-based quantification of fractal dimension therefore arise as a function of the distribution of the pixels, and a compact distribution yields better and more reliable results, whereas more widely dispersed, diffuse, or patchy pixel phenomenon engender more error in fractal quantification. This is why a correlation coefficient is generally generated as part of the fractal computation, whose value squared represents the percent of shared variance, the overlap as it were, in the pixel distribution (15). The determinations generated as part of this study produced good coefficients ($0.86 \pm .09$), that accounted for 74% of the variance across all the cells tested across the groups.

4.5. Clinical Implications

This research could break open new frontiers in the clinical biosciences in OLP along the fundamental, diagnostic and clinical-translational realm. From the viewpoint of fundamental biology, our findings indicate that a significant cross-talk exists between the autonomic nervous system and cellular immune processes involved in the OLP pathogenicity. This is important because the immunopathology of OLP is increasingly better characterized (7). Gaining a better understanding of the interface between neural and immune regulation in OLP, as we originally proposed (16), and of the role of the sympathetic and the para-sympathetic systems in the health and disease of the oral mucosa as we understand it to be systemically (17), will lead to novel modes of therapeutic interventions for OLP patients. OLP is a chronic painful condition that causes major discomfort for patients for a

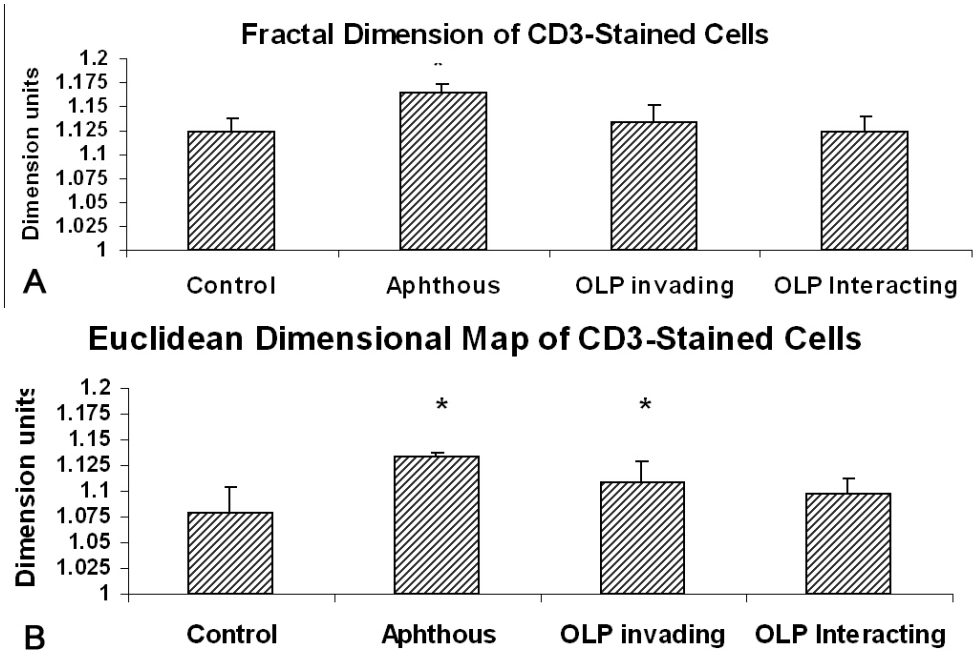

Figure 5. Fractal (panel A) and Euclidean dimensions (panel b) of CD3+ T cells in oral mucosa – CD3+ stained cells were isolated from the 100X micrographs from control normal human mucosa (n=7), from mucosa aphthous biopsies (n=7), and from OLP biopsies (n=15). The latter were dichotomized into CD3+ cells that had invaded the mucosa but were not in direct contact with sympathetic or para-sympathetic nerve endings ("invading", n=9), and into CD3+ cells that were directly interacting with VMAT or VAchT nerve endings, as shown in Figure 3 ("interacting", n=6). The individual cells were adjusted the brightness by means of computer-aided software (micrografx picture publisher). Euclidean estimates of size (i.e., relative estimates of cell radius) and fractal dimension were obtained by box-counting (i.e., relative complexity of the staining pattern on the cell membrane) with the FractalFox software (Qichang Li, Univ. Memphis; pers. comm.).

lifetime. Currently, the predominant treatment involves glucocorticoids, administered either locally in ointment form, or systemically. Treatment can be adjuvated by anti-fungals or anti-virals (22). New treatment approaches could be developed and tested in clinical trials that would take into account the involvement of the autonomic system in regulating the cellular immune arm of OLP pathology. For example, in order to counter the sympatho-inhibitory reflex induced by nitric oxide, an oral dose of 5 mg of the β-1-selective adrenergic receptor antagonist, Nebivolol, was administered. Nebivol attenuated the sympathetic tone in young adult healthy subjects, without affecting vagal tone or inducing cardiovascular side-effects (23). Current understanding of neuroendocrine-immune interactions would predict that by attenuating the sympathetic tone, Nebivolol treatment will enhance cellular immune surveillance (2,16,17). In a similar line of work, other receptor agonists or antagonists, or monoclonal antibodies targeted to the neurotransmitter vesicles identified in this research should now be tested for their ability to modulate autonomic-immune interface in OLP pathology.

Because fractal analysis is performed on individual cells, this approach has the potential of being developed in a novel diagnostic test, as well as a prognostic tool for monitoring the management of the pathology at the cellular level with small of amount of materials obtainable by simple scraping of the lesion. By this vary approach, oral mucosal smears for detection of cellular changes are often used to diagnose damaged and immature surface epithelial cells in cannabis smokers. For example, greater numbers of degenerate and atypical squamous cells are evident in oro-mucosal scrapings and smears obtained from cannabis smokers, compared to cigarette-smoking and non-smoking controls. Epithelial cells in smears taken from cannabis users and tobacco-smoking controls usually show koilocytic changes not seen in smears taken from non-smoking controls, an diagnostic indication of human papilloma virus infection (24). A similar approach could generate such scrapings and smears from OLP lesions, which could be processed by microscopy and fractal analysis to identify the overall state of the invading lymphocytes and their relative position *vis a' vis* autonomic innervation, and thus suggest the severity of the ongoing immune processes.

In the clinical-translational, the findings presented here indicate that OLP lesions are characterized by complex multi-cellular interactions, such that intervention at the level of one cellular system may not be sufficient to control and cure the pathology – case in point, the recurrence of OLP following cessation of glucocorticoid treatment (25,26). We infer that a more promising approach to treating OLP lesions might have to involve a total or partial replacement of the basal layer, such as which could be obtained following stem cell

intervention. Cutting-edge research could take full advantage of the observation that the intestinal epithelium follows the paradigms of stem cell biology that pertain to all self-renewing tissues. Its particular two-dimensional structure folded into valleys and hills offers the proliferative population of cells in the crypts, in contrast to the differentiated cells found in the *villi* (27). Colon biopsies, which are relatively non-invasive and commonly performed in middle-aged and aging adults as a diagnostic tool to monitor early signs of colon carcinoma, could be used to isolate adult colon epithelial stem cells of either of these two types. These cell populations, following organ culture under certain conditions, could be made to grow on a semi-porous membrane. OLP lesions from the same patients could be surgically excised, and the semi-porous membranes grafted along the edges of the lesion. Expectations are that the stem cells would continue to proliferate, now invading the oral mucosa, and forming, a healthy basal layer, during which process normal autonomic innervation would be restored. Such "mucosal implants" grafts would not be subject to rejection, since they would carry the identical major histocompatibility complex of the patient, and could hold promise of successful relief for the patient, and perhaps complete cure.

5. SUMMARY

This preliminary study has shown ordered juxtaposition of sympathetic (VMAT) and para-sympathetic nerve endings (VAchT) along the borders of CD3+ T cells in OLP biopsies, a relationship not observed in aphthous or control oral mucosa biopsies. This research has also demonstrated the feasibility of quantifying the infiltrating CD3+T cells by fractal and Euclidean topological dimension. An overall decrease in the ratio of fractal dimension-over-topological dimension is observed across the pathological lesions, compared to control, suggesting that as CD3+ T cells grow larger in the pathological conditions, they, in effect, stretch their plasma membrane. The net effect is that, while both the Euclidean and the fractal measure increase, the latter increases more slowly than the former, thus relaxing the overall extent of convolutions and complexity of the cell's plasma membrane relative to its size.

6. ACKNOWLEDGMENTS

Biopsy blocks were obtained from the UCLA Oral Pathology & Medicine archival library. Euclidean estimates of size (i.e., relative estimates of cell radius) and fractal dimension were obtained by box-counting (i.e., relative complexity of the staining pattern on the cell membrane) using the FractalFox software developed and distributed by Qichang Li, Univ. Memphis. The research was supported in part by the UCLA School of Dentistry, Faculty Pilot Grant Program.

7. REFERENCES

1. Chiappelli, F. & O.S. Cajulis: Psychobiological views on "stress-related oral ulcers". *Quintessence Intern* 35, 223-7 (2004)

2. Prolo, P. & F. Chiappelli: Immune Suppression. In S. Vanstone, G. Chrousos, I. Craig, R. de Kloet, G. Feuerstein, B. McEwen, N. Rose, R. Rubin, & A. Steptoe (Eds) *Encyclopeadia of Stress II*. London. Elsevier (2005 , in press)

3. Sterling, P. & J. Eyer: Allostasis: A new paradigm to explain arousal pathology. In: S. Fisher & J. Reason (Eds) *Handbook of Life Stress, Cognition, and Health*, Wiley, New York (1988)

4. Eversole, L.R: Immunopathogenesis of oral lichen planus and recurrent aphthous stomatitis. *Semin Cutan Med Surg* 16, 284-94 (1997)

5. Borra, R.C., P.M. Andrade, I.D. Silva, A. Morgun, L.L Weckx, A.S. Smirnova & M. Franco: The Th1 /Th2 immune-type response of the recurrent aphthous ulceration analyzed by cDNA microarray. *J Oral Pathol Med* 33, 140-6 (2004)

6. Kawamura, E., S. Nakamura, M. Sasaki, Y. Ohyama, T. Kadena, W. Kumamaru & K. Shirasuna: Accumulation of oligoclonal T cells in the infiltrating lymphocytes in oral lichen planus. *J Oral Pathol Med* 32, 282-9 (2003)

7. Khan, A., C.S. Farah, N.W. Savage, L.J. Walsh, D.J. Harbrow & P.B. Sugerman: Th1 cytokines in oral lichen planus. *J Oral Pathol Med* 32, 77-83 (2003)

8. Glenny, R.W., H.T. Robertson, S. Yamashiro & J.B. Bassingthwaighte: Applications of fractal analysis to physiology. *J Appl Physiol* 70, 2351-2367 (1991)

9. Keough, K.M., P. Hyam, D.A. Pink & B. Quinn. Cell surfaces and fractal dimensions. *J Microsc* 163, 95-99 (1991)

Losa, G.A., G. Baumann & T.F. Nonnenmacher: Fractal dimension of pericellular membranes in human lymphocytes and lymphoblastic leukemia cells. *Pathol Res Pract* 188, 680-6 (1992)

10. Churilla, A.M., W.A. Gottschalke, L.S. Liebovitch, L.Y. Selector, A.T. Todorov & S. Yeandle S. Membrane potential fluctuations of human T-lymphocytes have fractal characteristics of fractional Brownian motion. *Ann Biomed Eng* 4, 99-108 (1996)

11. Kerenji, A.S., Z.L. Bozovic, M.M. Tasic, Z.M. Budimlija, I.A. Klem & A.F. Polozovic: Fractal dimension of hepatocytes' nuclei in normal liver vs. hepatocellular carcinoma (HCC) in human subjects – preliminary results. *Arch Oncol* 8, 47-50 (2000)

12. Bernard, F., J.L. Bossu & S. Gaillard: Identification of living oligodendrocyte developmental stages by fractal analysis of cell morphology. *J Neurosci Res* 65, 439-45 (2001)

13. Naumov, Y.N., E.N. Naumova, K.T. Hogan, L.K. Selin & J. Gorski: A fractal clonotype distribution in the CD8+ memory T cell repertoire could optimize potential for immune responses. *J Immunol* 170, 3994-4001 (2003)

14. Chiappelli, F. & E. Cajulis: Fractal Characterization of T Lymphocytes: Implications for Fundamental and Clinical Immunology. *J Theor Biol*. (2005, in press)

15. Prolo, P., F. Chiappelli, E. Cajulis, J. Bauer, S, Spackman, H. Romeo, M. Carrozzo, S. Gandolfo & R. Christensen: Psyconeuroimmunology in Oral Biology and Medecine: The Model of Oral Lichen Planus. *Ann NY Acad Scien* 966, 429-40 (2002)

16. Elenkov, I., R. Wilder, G. Chrousos & S. Vizi: The Sympathetic Nerve—An Integrative Interface between Two

Supersystems: The Brain and the Immune System. *Pharmacol Rev* 52, 595-638 (2000)
17. de Felice, C., G. Latini, S. Parrini, G. Bianciardi, P. Toti, R.J. Kopotic & D.M. Null: Oral mucosal microvascular abnormalities: an early marker of bronchopulmonary dysplasia. *Pediatr Res* 56, 927-31 (2004)
18. Heo, M.S., K.S. Park, S.S. Lee, S.C. Choi, J.Y. Koak, S.J. Heo, C.H. Han & J.D. Kim: Fractal analysis of mandibular bony healing after orthognathic surgery. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 94, 763-7 (2002)
19. Landini, G. & J.W. Rippin: How important is tumour shape? Quantification of the epithelial-connective tissue interface in oral lesions using local connected fractal dimension analysis. *J Pathol* 179, 210-7 (1996)
20. Mandelbrot ,B: A Fractal's Lacunarity, and how it can be Tuned and Measured. In T.F. Nonnenmacher, G.A. Losa & E.R. Weibel (Eds) Fractals in biology and medicine. Basel & Boston: Birkhäuser Verlag (1994)
21. Carrozzo, M. & S. Gandolfo: Oral diseases possibly associated with hepatitis C virus. *Crit Rev Oral Biol Med* 14, 115-27 (2003)
22. Chiladakis, J.A., E. Georgiopoulou & D. Alexopoulos: Autonomic Effects of Nebivolol versus Atenolol in Healthy Subjects. *Cardiovasc Drugs Ther* 18, 469-73 (2004)
23. Darling, M.R., G.M. Learmonth & T.M. Arendorf: Oral cytology in cannabis smokers. *SADJ* 57, 132-5 (2002)
24. Lozada-Nur, F. & C. Miranda: Oral lichen planus: topical and systemic therapy. *Semin Cutan Med Surg* 16, 295-300 (1997)
25. Carbone, M., E. Goss, M. Carrozzo, S. Castellano, D. Conrotto, R. Broccoletti & S. Gandolfo: Systemic and topical corticosteroid treatment of oral lichen planus: a comparative study with long-term follow-up. *J Oral Pathol Med* 32, 323-9 (2003)
26. Radtke, F. & H. Clevers: Self-renewal and cancer of the gut: two sides of a coin. *Science* 307, 1904-9 (2005)

Key Words: Oral Lichen Planus (OLP), Recurrent Aphthous Stomatitis (RAS), Oral Mucosa CD3+ T Cells, Vesicles of Monoamines Transport, Vesicles Of Acetylcholine Transport, Fractal Dimension, Euclidean Dimension

Send correspondence to: Dr Francesco Chiappelli, CHS 63-090, UCLA, Los Angeles, CA 90095-1668, Tel: 310-794-6624, Fax: 310-794-7109, E-mail: chiappelli@dent.ucla.edu

http://www.bioscience.org/current/vol10.htm