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# Inducible nitric oxide synthase in pityriasis lichenoides lesions

**Background:** Pityriasis lichenoides (PL) is an inflammatory skin disease of unknown etiology. Nitric oxide (NO) has emerged as an important mediator of many physiological functions. The importance of NO-mediated signaling in skin diseases has been reported by several studies.
**Methods:** A review of clinical records and histopathological slides of 34 patients diagnosed with PL was performed. Three different groups of skin biopsies including PL chronica (24 patients), PL et varioliformis acuta (10 patients) and 15 normal skin samples were subjected to the immunohistochemistry technique for inducible nitric oxide synthase (iNOS) detection.
**Results:** Normal skin group exhibited a few number of iNOS-positive cells in the dermis and rare positive cells in the upper epidermis, unlike abundant epidermal and dermal iNOS expression observed in both PL groups.
**Conclusion:** According to our results, we hypothesize that NO produced by iNOS could participate in PL pathogenesis. Abnormal and persistent responses to unknown antigens, probably a pathogen, associated with NO immunoregulatory functions could contribute to the relapsing course observed in PL. NO anti-apoptotic effect on T-cell lymphocytes could play a role on maintenance of reactive T cells, leading to a T-cell lymphoid dyscrasia.

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Pityriasis lichenoides (PL) is an inflammatory dermatosis of unknown etiology, with autoregressive course. The incidence and prevalence of PL are still unclear.[1,2] PL frequently affects children and young adults. Because of their clinicopathological similarities, PL et varioliformis acuta (PLEVA) and PL chronica (PLC) are now considered to represent a spectrum of the same disease. Both conditions usually exhibit a relapsing course, with continuous crops of lesions that can recur for years.[1]

The tissue reaction of PLC is characterized by a superficially band-like CD4+ T lymphocytic infiltrate with sparse perivascular involvement, while PLEVA exhibits a wedge-shaped infiltrate with predominance of CD8+ T lymphocytes, obscuring the dermoepidermal junction with erythrocytes extravasation.[2–4]

PL has been regarded as a hypersensitivity reaction to an infectious agent. Young age at onset, presence of acute eruptive lesions, seasonality and reports of familial outbreaks favor the hypothesis of infectious etiology.[5,6] Several microorganisms have been related to PL mainly including bacteria and viruses.[6–12]

Recently, PL has been defined as a T-cell lymphocyte disease including in the field of cutaneous T-cell lymphoid dyscrasias, which may progress toward development of cutaneous T-cell lymphoma.[13,14] T-cell clonality in the skin lesions is a common finding reported in both PL variants.[15,16]

Nitric oxide (NO) has emerged as an important mediator of many physiological functions. NO is both water and lipid soluble and is able to pass freely within and between cells, functioning as a highly efficient

local signaling molecule.[17] It is synthesized in the skin by reduction of sweat nitrate[18] or oxidation of L-arginine by a family of NO synthases (NOS). NOS are constitutive: endothelial NOS and neuronal NOS or inducible NOS (iNOS). The constitutive forms of NOS are calcium-dependent and produce low levels of NO for homeostatic functions.

On the other hand, iNOS generates higher levels of NO in a calcium-independent manner and is involved in inflammatory and pathological conditions.[17–30] In 1992, Heck et al. reported the NO synthesis by normal human skin.[31] Since then, this molecule has been regarded as a key factor in several skin reactions, modulating the skin response to external stimuli such as infections, heat, ultraviolet light and wound healing.[17–30] Actually, all three NOS isoforms are expressed in the skin. Disturbances in the signaling cascade of NO and reactive nitrogen intermediates have been shown to be associated with skin diseases and disorders.[17–30]

Despite its clinical relevance on pediatric patients, its association with some infectious diseases and the risk of cutaneous T-cell lymphoma, little is known about the PL pathogenesis. Thus, in the present work, we intended to study the iNOS expression in both PLC and PLEVA skin lesions and compare it with normal skin samples.

## Methods

Paraffin-embedded skin biopsies of 34 PL patients were retrieved from the files of Dermatopathology Laboratory of the University of Sao Paulo Medical School Hospital, comprising in the period of 1994–2006. The PL diagnosis was confirmed by clinical features of patients, histopathology and evolving course of the disease. The PL groups were classified based on histopathological criteria.[1] Fifteen normal skin samples from mammoplasty and abdominoplasty composed the control group.

Five micrometer histological sections were obtained from each paraffin-embedded specimen and placed on glass slides coated with 3-amino-propyl-thriethoxy5 silane. After dewaxed in xylene and rehydrated by a descending alcohol series, the slides were incubated overnight with 1 : 500 rabbit polyclonal anti-iNOS antibody (Calbiochem-Novabiochem Corp, San Diego, CA, USA). After phosphate buffer wash, the slides were incubated for 30 min with undiluted alkaline phosphatase-labeled secondary antibody amplification polymer (EnVision™ G|2-PA, Rabbit/Mouse (Dako, Carpinteria, CA, USA). This reaction was visualized by Permanent Red Chromogen (Dako). Slides were counterstained with Harris hematoxylin. The presence of red precipitate indicated positive specific binding of the primary antibody. The negative staining controls received the same treatment, omitting the primary antibody.

Digital images were acquired by Sight DS-2Mv digital camera connected to a light microscope Eclipse – 80i (Nikon, Japan) and the software NSI-elements (Nikon). Then, images were analyzed by computer-assisted image analysis program (CHPTool Cyclops, Brazil). Photomicrographs of 640 × 480 pixels were obtained from noncoincident consecutive fields, throughout all epidermal layers at a magnification of ×200. Microscopic fields with folds or not well-preserved tissue components were excluded. Area fraction of iNOS-positive expression was determined based on selected pixels for each epidermal field. The weighed mean of iNOS immunostaining was calculated to all epidermal fields for each slide. Inflammatory cell density in the dermis was estimated by counting labeled cells in 12 noncoincident, ×400 magnifications, random fields with an eyepiece reticule of known area (0.0625 $mm^2$).

Computer-based statistical analysis was performed by MINITAB software (version 14). Intergroup comparison (PLEVA, PLC and skin controls) was performed by the Mann-Whitney test; p values below 0.05 were regarded as statistically significant.

## Results

According to histopathological criteria,[1] 24 biopsies were classified as PLC and 10 as PLEVA. The mean age of the PL patients was 21.85 (range 2–73 years) and 38.8 years in normal skin control group. Skin involvement was limited to trunk and extremities in 64.7% of the patients, while universal involvement was observed in 23.5% and the remainder patients had generalized lesions sparing only the face. The disease was recurrent in 17.6% of the patients.

All 34 PL skin samples showed variable positive immunostaining for iNOS in keratinocytes, Langerhans cells, macrophages, lymphocytes, dermal dendrocytes, pericytes and endothelial cells. Epidermal iNOS expression was more intense in terminally differentiated keratinocytes (Fig. 1A). Three cases of PLC, which exhibited prominent vacuolar degeneration of basal layer, displayed intense and homogeneous iNOS expression throughout all epidermis layers (Fig. 1B). The iNOS immunostaining pattern was cytoplasmic, although some samples also exhibited weak nuclear iNOS expression. Intense iNOS expression was observed in the superficial and deep infiltrate, and in the components of dermal microvascular unit (Fig. 2A–E). Additionally, variable iNOS immunostaining was also observed in fibroblasts, dermal dendritic cells and eccrine sweat glands (Fig. 2F). Although three normal skin control samples displayed moderate iNOS expression at the upper

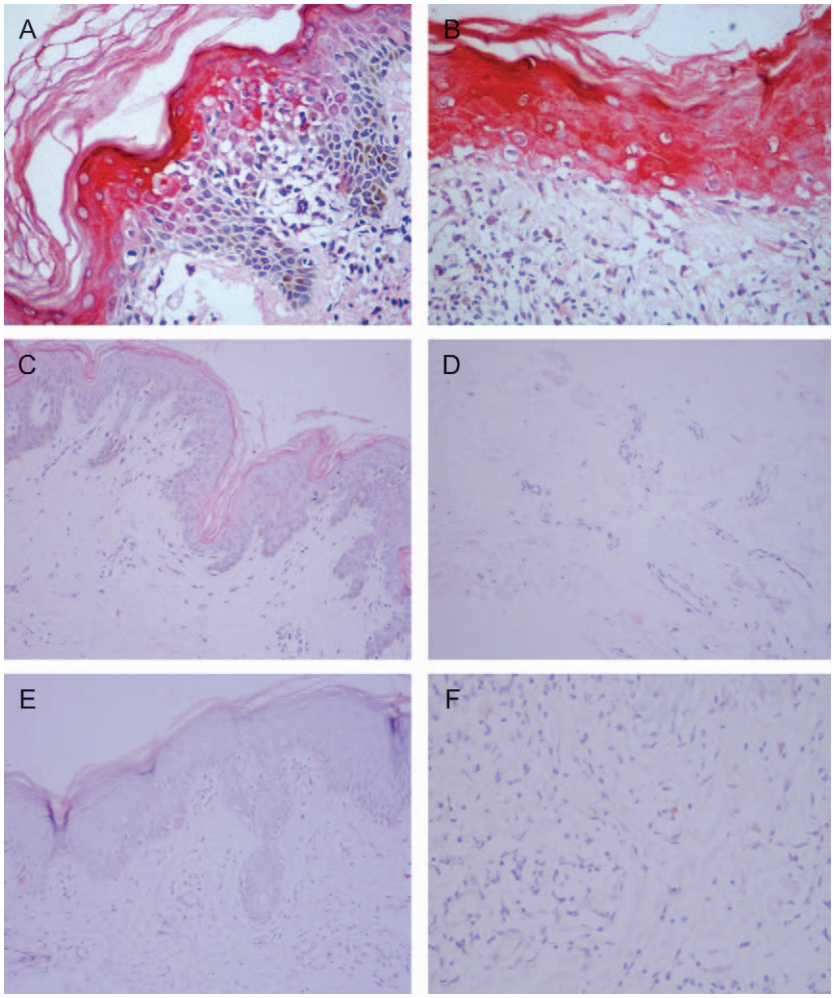


*Fig. 1.* Epidermal iNOS expression in pityriasis lichenoides lesions. A - iNOS expression in upper spinous and granulose layers of the epidermis; B - iNOS expression in all epidermal layers (A and B ×400 original magnification). C and D - Control normal skin without iNOS immunolabeling (C - ×200 original magnification; D ×400 original magnification). (EnVision alkaline phosphatase system with polyclonal rabbit anti iNOS antibody and permanent red substrate-chromogen). E and F - Pityriasis lichenoides lesions subjected to immunolabeling technique without the primary antibody (E and F - ×400 original magnification).

epidermal layers, most of the samples exhibited only a few positive cells in the epidermis and superficial dermis (Fig. 1C,D).

When the primary antibody was omitted from the immunostaining reaction, neither epidermal nor dermal labeling was found (Fig. 1E,F). There was no significant difference in epidermal and dermal iNOS expression between PLEVA and PLC groups.

The PL biopsies showed a higher epidermal and dermal iNOS expression when compared with normal skin control group ($p = 0.0005$ and $p = 0.0002$, respectively).

## Discussion

We analyzed 34 biopsy specimens of PL and classified them into the acute variant, i.e. PLEVA, or the chronic variant, i.e. PLC, according to standard histopathological criteria.[1] A discrepancy between the clinical and the histopathological diagnosis was observed in seven cases, probably because the histopathological diagnosis does not strictly traduce the entire clinical picture and evolving course of individualized patients. In fact, PL is a skin disease that has PLEVA and PLC at the polar ends. Furthermore, its wide clinicopathological spectrum includes intermediate or overlapping forms of disease.[1,11]

We showed strong iNOS expression in epidermal and dermal cells in both PLC and PLEVA lesions when compared with normal skin control group. iNOS expression was also observed in three normal skin samples at the upper epidermal layers. This pattern of iNOS expression in healthy epidermis was already reported by Tsukazaki et al.[21] Such difference between PL and normal skin groups on epidermal iNOS expression was probably related to the inflammatory process of PL, and not because of epidemiological features of patients. The mean age of the PL group was smaller (21.85 years) than the control group (38.8 years), although there is some evidence that aging process is accompanied by a spontaneous induction of iNOS mRNA.[23]

The epidermal iNOS expression in PL group was predominant at the upper epidermal layers, although it was observed throughout all epidermis layers in

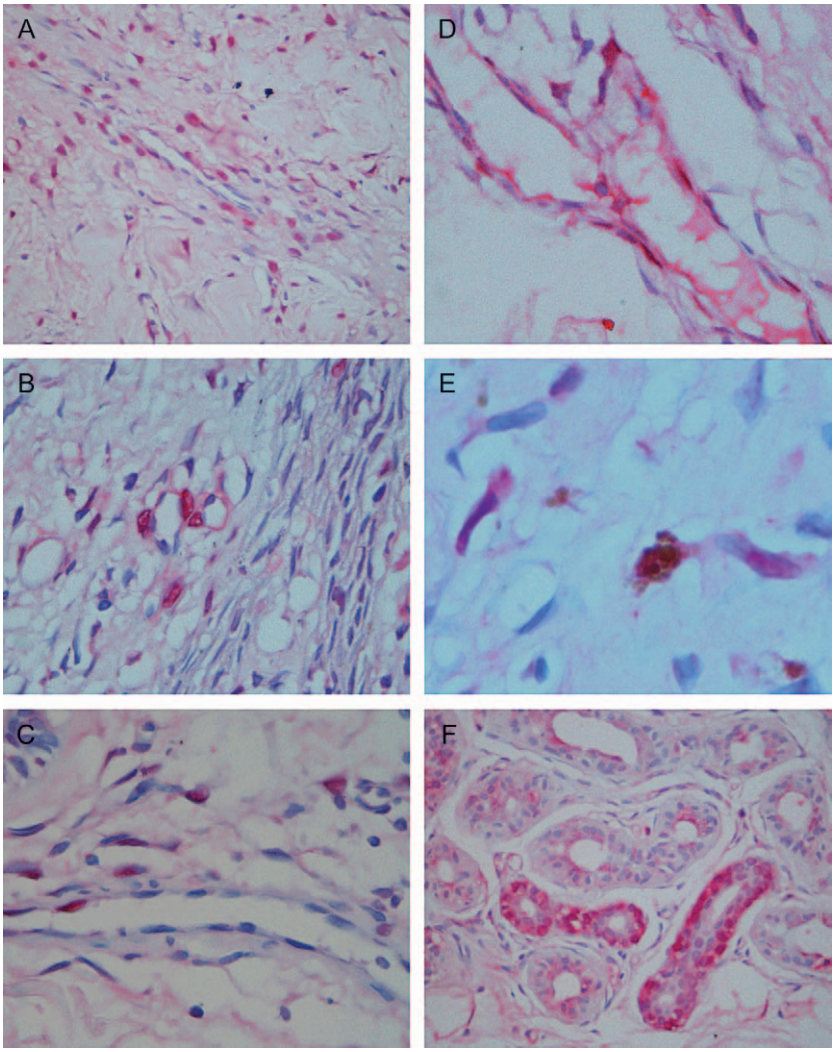


*Fig. 2*. Inducible nitric oxide synthase (iNOS) expression in the dermis of pityriasis lichenoides lesions. A) iNOS expression in dendrocytes, fibroblasts and endothelial cells (×400 original magnification). B and C) iNOS expression by the dermal microvascular unit elements (×1000 original magnification). D) iNOS expression by endothelial cells and pericytes (×1000 original magnification). E) iNOS expression in macrophages (×1000 original magnification). F) Eccrine sweat epithelium strongly expressing iNOS (×400 original magnification) (EnVision alkaline phosphatase system with polyclonal rabbit anti-iNOS antibody and permanent red substrate-chromogen).

three PL samples (Fig. 1D). Variable patterns of epidermal iNOS expression were showed in other skin diseases.[21,22,25–27,29] Ultraviolet B irradiation induced iNOS expression by keratinocytes in all epidermal layers of lupus erythematosus and Sjögren's syndrome lesions.[21] Nevertheless, experimental photo-induced lupus erythematosus lesions exhibited impaired epidermal iNOS expression and increased keratinocytes apoptosis.[25] Strong iNOS expression in lower epidermal layers was described in toxic epidermal necrolysis and Stevens-Johnson lesions.[26]

iNOS expression have also been described in cutaneous infectious diseases, such as leprosy[27] and American cutaneous leishmaniasis.[28] In the later disease, strong iNOS expression correlated to localized lesions with few parasites. Epidermal and dermal iNOS expression was also described in herpes zoster skin lesions.[29]

The strong epidermal iNOS expression observed in PL lesions could be related to the action of an unknown pathogen/infectious agent. It could result in the activation of keratinocytes and Langerhans cells, leading to iNOS expression, similarly to that previously described for skin infectious processes.[20]

NO is a pleiotrophic biomodulator, highly diffusible and reactive, which acts as both intracellular and extracellular messenger in non-specific host defense process and also in cytotoxic, proliferation, differentiation and immunoregulatory activities.[17–30]

NO production by keratinocytes can control cell proliferation and differentiation,[22] apoptosis[21,25,26,29] and inflammatory responses.[24,27,29] Probably, the NO, which is produced by iNOS, may play a role in the development of the PL skin lesions.

Black et al. suggested that sudden release of a vasoactive material in a high concentration would be responsible for the presence of focal damage, hemorrhage and dilation of small blood vessels observed in PL lesions, in the absence of marked vascular damage.[32] In the present work, we showed high iNOS expression by endothelial cells in both PLC and PLEVA. This observation suggests that NO,

via iNOS release, could be such 'vasoactive material'. The rationale for this possibility lies in the understanding that cytokines can modulate the endothelial cell functions during the inflammatory process. Therefore, one of the prominent effects that cytokines can exert on endothelial cells is the induction of iNOS expression, leading to a high NO release, contributing to the erythema formation.[21] Endothelial iNOS expression was showed in allergic contact dermatitis and atopic dermatitis skin lesions,[20] solar erythema,[21] leprosy[27] and herpes zoster.[32] According to these studies, NO produced by endothelial iNOS could play a regulatory role on the inflammatory process observed in these skin diseases.

PL lesions are characterized by prominent keratinocyte apoptosis, observed mainly in PLEVA lesions. NO is known to have different effects on apoptosis depending on the cell type, concentration and the biological milieu.[33] The iNOS expression, observed in both type of PL lesions, could be related to NO-mediated apoptosis of keratinocytes, as reported in toxic epidermal necrolysis and Stevens-Johnson syndrome lesions.[26] Otherwise, experimental conditions suggested that NO protects keratinocytes from ultraviolet B radiation-induced apoptosis.[34]

PL is a disease characterized by interface dermatitis with lymphocytes as the predominant infiltrative cell. Although in PLEVA lesions there is a predominant cytotoxic CD8+ T-cell infiltrate,[4] and a dermal CD4+ T-cell infiltrate in PLC,[3] the present study did not disclose difference in iNOS expression when compared PLEVA with PLC lesions.

The NO generated from iNOS activity may also be involved in the regulation of T helper type 1 (TH1) cell population expansion and TH1-mediated immune responses.[20] PL is currently considered a cutaneous T-cell lymphoid dyscrasia, with persistent T-cell clones.[14] T-cell clonality is a common finding reported in both variants of PL,[3] with few reported cases evolving to cutaneous T-cell lymphoma.[13,36]

The effects of NO on controlling T-cell proliferation are conflicting. NO is considered a potent inhibitor of lymphocyte proliferation in response to a variety of stimulus.[37] On the other hand, intracellular NO activity may be a general mechanism by which transformed cell of hematopoietic lineage inhibits Fas-induced apoptosis.[38]

The iNOS expression found in T-cell lines has been related to NO inhibition of apoptotic death of leukemic cells in human T-cell leukemia virus type-1.[39] Based on literature data, our results from PL lesions suggest a correlation between the strong iNOS expression by cellular elements and the persistence of T-cell infiltrate, which is probably because of the anti-apoptotic effect of NO.

In view of our results, we conclude that iNOS may participate in the PL pathogenesis. An abnormal and persistent response to an unknown antigen, probably a pathogen, associated to the immunoregulatory NO functions could contribute to the relapsing course observed in both forms of the disease. On the other hand, the NO anti-apoptotic effect in lymphoid cells may play a role in the maintenance of reactive T cells, leading to a persisting and unbalanced state that may evolve to T-cell oligoclonal or clonal process. The presence of other host controlling mechanisms, could justify the only occasional PL progression to the T-cell epidermotropic lymphoma.

To our knowledge, this is the first demonstration of the iNOS expression in PL lesions. Further studies are needed to clarify the role of NO and its cellular interactions in the pathogenesis of both variants of PL.

## References


1. Romani J, Puigi L, Fernández-Figueras MT, de Moragas J. Pityriasis lichenoides in children: clinicopathologic review of 22 patients. Pediatr Dermatol 1998; 15: 1.
2. Bowers S, Warshaw EM. Pityriasis lichenoides and its subtypes. J Am Acad Dermatol 2006; 55: 557.
3. Shieh S, Mikkola DL, Wood GS. Differentiation and clonality of lesional lymphocytes in pityriasis lichenoides chronica. Arch Dermatol 2001; 137: 305.
4. Muhlbauer JE, Bhan AK, Harrist TJ, et al. Immunopathology of pityriasis lichenoides acuta. J Am Acad Dermatol 1984; 10 (Pt 1): 783.
5. Dupont C. Pityriasis lichenoides in a family. Br J Dermatol 1995; 133: 338.
6. English JC III, Collins M, Bryant-Bruce C. Pityriasis lichenoides et varioliformis acuta and group-A beta hemolytic streptococcal infection. Int J Dermatol 1995; 34: 338.
7. Ostlere LS, Langtry JA, Branfoot AC, Staughton RC. HIV seropositivity in association with pityriasis. Clin Exp Dermatol 1992; 17: 36.
8. Tsai KS, Hsieh HJ, Chow KC, Lin TY, Chiang SF, Huang HH. Detection of cytomegalovirus infection in a patient with febrile ulceronecrotic Mucha-Habermann's disease. Int J Dermatol 2001; 40: 694.
9. Klein PA, Jones EC, Nelson JL, Nelson JL, Clark RAF. Infectious causes of pityriasis lichenoides: a case of fulminant infectious mononucleosis. J Am Acad Dermatol 2003; 49 (Pt 3): 151.
10. Boralevi F, Cotto E, Baysse L, Jouveincel AC, Léauté-Labrèze C, Taïebis A. Varicella-zoster virus involved in the etiopathogeny of pityriasis lichenoides? J Invest Dermatol 2003; 121 (letter).
11. Tomasini D, Tomasini CF, Cerri A, et al. Pityriasis lichenoides: a cytotoxic T-cell mediated skin disorder. Evidence of human parvovirus B19 DNA in nine cases. J Cutan Pathol 2004; 31: 531.
12. Zechini B, Teggi A, Antonelli M, et al. A case report of pityriasis lichenoides in a patient with chronic hepatitis C. J Infect 2005; 51: E23.
13. Fortson JS, Schroeter AL, Esterly NB. Cutaneous T-cell lymphoma (parapsoriasis en plaque). An association with pityriasis lichenoides et varioliformis acuta in young children. Arch Dermatol 1990; 126: 1449.
14. Guitart J, Magro C. Cutaneous T-cell lymphoid dyscrasia: a unifying term for idiopathic chronic dermatoses with persistent T-cell clones. Arch Dermatol 2007; 143: 1449.

15. Dereure O, Levi E, Kadin ME. T-cell clonality in pityriasis lichenoides et varioliformis acuta. A heteroduplex analysis of 20 cases. Arch Dermatol 2000; 136: 1483.
16. Magro C, Crowson N, Lin T, Sood N. Pityriasis lichenoides chronica: stratification by molecular and phenotypic profile. Hum Pathol 2007; 38: 479.
17. Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V. Nitric oxide in human skin: current status and future prospects. J Invest Dermatol 1998; 110: 1.
18. Weller R, Pattullo S, Smith L, Golden M, Ormerod A, Benjamin N. Nitric oxide is generated on the skin surface by reduction of sweat nitrate. J Invest Dermatol 1996; 107: 327.
19. Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V. Nitric oxide and its implications in skin homeostasis and disease. Arch Dermatol Res 1998; 290: 643.
20. Rowe A, Farrell AM, Bunker CB. Constitutive and inducible nitric oxide synthase in inflammatory dermatoses. Br J Dermatol 1997; 136: 18.
21. Tsukazaki N, Watanabe M, Shimizu K, Hamasaki Y, Katayama I. Photoprovocation test and immunohistochemical analysis of inducible nitric oxide synthases expression in patients with Sjogren's syndrome associated with photosensitivity. Br J Dermatol 2002; 147: 1102.
22. Bruch-Gerharz D, Schnorr O, Suschek C. Arginase 1 overexpression in psoriasis: limitation of inducible nitric oxide synthase activity as a molecular mechanism for keratinocyte hyperproliferation. Am J Pathol 2003; 162: 203.
23. Ferrini M, Magee TR, Vernet D, Rajfer J, González-Cadavid NF. Aging-related expression of inducible nitric oxide synthase and markers of tissue damage in the rat penis. Biol Reprod 2001; 64: 972.
24. Giustizieri ML, Albanesi C, Scarponi C, De Pita O, Girolomoni G. Nitric oxide donors suppress chemokine production by keratinocytes in vitro and in vivo. Am J Pathol 2002; 161: 1749.
25. Kuhn A, Fehsel K, Lehmann P, Krutmann J, Ruzicka T, Kolb-Bachofen V. Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus. J Invest Dermatol 1998; 111: 147.
26. Lerner LH, Qureshi AA, Reddy BV, Lerner EA. Nitric oxide synthase in toxic epidermal necrolysis and Stevens-Johnson syndrome. J Invest Dermatol 2000; 114: 196.
27. Schön T, Hernandez, Pando RH, et al. Expression of inducible nitric oxide synthase and nitrotyrosine in borderline leprosy lesions. Br J Dermatol 2001; 145: 809.
28. Qadoumi M, Becker I, Donhauser N, Röllinghoff M, Bogdan C. Expression of inducible nitric oxide synthase in skin lesions of patients with American cutaneous leishmaniasis. Infect Immun 2002; 70: 4571.
29. Lim Y-J, Chang S-E, Choi J-H, et al. Expression of inducible nitric oxide synthase in skin lesions of acute herpes zoster. J Dermatol Sci 2002; 29: 89.
30. Weller R, Price RJ, Ormerod AD, Benjamin N, Leifert C. Antimicrobial effect of acidified nitrite on dermatophyte fungi, Candida and bacterial skin pathogens. J Appl Microbiol 2001; 90: 648.
31. Heck DE, Laskin DL, Gardner CR, Laskin JD. Epidermal growth factor suppresses nitric oxide and hydrogen peroxide production. J Biol Chem 1992; 267: 21277.
32. Black MM, Marks R. The inflammatory reaction in pityriasis lichenoides. Br J Dermatol 1972; 87: 533.
33. Kim YM, Bombeck CA, Billiar TR. Nitric oxide as a bifunctional regulator of apoptosis. Circ Res 1999; 84: 253.
34. Weller R, Schwentker A, Billiar TR, Vodovotz Y. Autologous nitric oxide protects mouse and human keratinocytes from ultraviolet B radiation-induced apoptosis. Am J Physiol Cell Physiol 2003; c1140.
35. Albina JE, Cui S, Mateo RB, et al. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J Immunol 1993; 150: 5080.
36. Thomson KF, Whittaker SJ, Russell-Jones R, Charles-Holmes R. Childhood cutaneous T-cell lymphoma in association with pityriasis lichenoides chronica. Br J Dermatol 1999; 141: 1136.
37. Mahidhara RS, Hoffman RA, Huang S, et al. Nitric oxide-mediated inhibition of caspase-dependent T lymphocyte proliferation. J Leukoc Biol 2003; 74: 403.
38. Mannick JB, Miao XQ, Stamler JS. Nitric oxide inhibits Fas induced apoptosis. J Biol Chem 1997; 272: 24125.
39. Mori N, Nunokawa Y, Yamada Y, Ikeda S, Tomonaga M, Yamamoto N. Expression of human inducible nitric oxide synthase gene in T-cell lines infected with human T-cell leukemia virus type-I and primary adult T-cell leukemia cells. Blood 1999; 74: 2655.