Immunopathologic characterization of the tissue response in endemic pemphigus foliaceus (fogo selvagem)

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Background: The research on endemic pemphigus foliaceus (fogo selvagem) has mainly focused on the humoral immune response, but little attention has been given to the function of cell-mediated immune response and the nature of the cellular elements of the tissue reaction in the lesions of fogo selvagem.

Objective: The purpose of this study was the immunophenotype characterization of the inflammatory cells as well as the expression of adhesion molecules and HLA-DR in the perilesional and lesional skin of fogo selvagem.

Methods: Twenty biopsy specimens of lesional and perilesional skin were analyzed by immunohistochemical techniques. The panel of monoclonal antibodies consisted of CD8, CD4, CD1a, HLA-DR, IL-2R, LFA-1, ICAM-1, and PAN-B.

Results: The semiquantitative analysis of the cell population revealed a predominance of CD4 T lymphocytes in the tissue response of perilesional and lesional skin. The population of epidermal Langerhans cells was decreased in lesional skin when compared with the perilesional skin, whereas $CD1a^+$ dermal dendritic cells predominated in lesional skin. Keratinocyte expression of ICAM-1 and HLA-DR was negative in both lesional and perilesional skin.

Conclusion: The overall results suggest the participation of the cell-mediated immunity in endemic pemphigus foliaceus (fogo selvagem). The lack of keratinocyte ICAM-1 expression may be related to the pattern of cytokines secreted by the $CD4^+$ T cells of the tissue reaction in fogo selvagem. (J Am Acad Dermatol 2001;44:446-50.)

Fogo selvagem (FS) is an organospecific autoimmune disorder characterized by the onset of subcorneal acantholytic vesicles and by the presence of IgG autoantibodies that react with components of the desmosomal core, that is, desmoglein 1. FS is endemic in certain regions of Brazil, where there are more than 15,000 registered cases. The disease occurs in endemic fashion within the states of Goiás, Mato Grosso do Sul, Paraná, São Paulo, and Minas Gerais. It appears that the disease is spreading toward the northwest and west of the country. The epidemiologic findings show that the disease appears in wild areas being colonized and disappears as the area becomes urbanized. The majority of the patients are young peasants or children who live in close proximity to rivers with lots of insects, in particular the black fly *Simulium nigrimanum*. The involvement of environmental factors and infectious agents has been the subject of intensive studies. FS differs from classical pemphigus foliaceus (Cazenave) by the age of those affected, by geographic distribution, and by the presence of familial cases in genetically related persons. However, the clinical manifestations, the histologic findings, and the immunopathologic features are similar in these forms of pemphigus foliaceus.[1]

The autoantibodies occurring in FS are specific and pathogenic. The predominant disease autoantibodies, present in all patients, are of the IgG4 subclass.[2] These autoantibodies are directed against the desmosomal cadherin desmoglein 1, a 160-kd glycoprotein.[3]

Few studies are available in the literature about the involvement of cell-mediated immunity in bullous disease and especially in FS. In pemphigus vulgaris as well as in bullous pemphigoid, lymphocytes are the predominant mononuclear cells in the

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inflammatory infiltrate of dermis. The ratio of $CD4^+/CD8^+$ T lymphocytes is about 2:1 in both diseases. B cells and natural killer cells are absent. The dendritic cells are increased in lesional skin compared with perilesional skin.[4,5] The keratinocytes of bullous pemphigoid express ICAM-1 and HLA-DR. However, ICAM-1 and HLA-DR were not expressed on epidermal keratinocytes of pemphigus vulgaris.[6] High levels of interferon gamma (IFN-γ) were demonstrated in the blister fluids of bullous pemphigoid in comparison with those obtained from pemphigus vulgaris, second-grade dermal burn, and friction bulla.[7]

Only one study was undertaken to examine the quantification of $S\text{-}100^+$ dendritic cells in FS. These cells were decreased in lesional skin.[8]

The purpose of the present study was to investigate the immunophenotype characterization of the cellular elements of the inflammatory response as well as expression of the adhesion molecule ICAM-1 and the antigen HLA-DR in an attempt to elucidate the possible involvement of cellular immunity in the pathogenesis of FS.

MATERIAL AND METHODS

Skin biopsies

Biopsy specimens of lesional (full developed blister with duration of about 24 hours) and perilesional skin (located 1.5 cm from the lesion) were obtained from 11 patients with the generalized form of the disease and 9 patients with the localized form of FS. The clinical classification used in this study was that proposed by the Cooperative Research Group for Study of Fogo Selvagem.[1] All patients showed high epidermal acantholysis and positive direct and indirect immunofluorescence for antiepithelial antibody, except for 2 patients for whom the indirect immunofluorescence was negative. Both showed the localized clinical form of FS. No patients were under treatment when biopsies were performed.

Immunohistochemical staining

The specimens were snap frozen and submitted to cryomicrotomy. Cryostat-cut sections (5 μm) were fixed in acetone, incubated with primary mouse monoclonal antibody and treated by avidin-biotin method modified as previously described.[9] The mouse monoclonal antibodies used in this study were anti-CD8 for a subpopulation of cytotoxic-suppressor lymphocytes, anti-CD4 for a subpopulation of helper lymphocytes, anti-CD25 for interleukin 2 receptor cells (IL-2R), anti-CD1a for Langerhans cells, anti-CD54 intercellular adhesion molecule-1 (ICAM-1), anti-CD11 lymphocyte function-associated antigen-1 (LFA-1), anti-CD19 for the subpopulation of B lymphocytes, and major histocompatibility complex (MHC) molecules class II (HLA-DR). All monoclonal antibodies were obtained from Dakopatts, except for the last one, which was a donation from the Transplant Immunology Laboratory of the Heart Institute of the University of São Paulo. The reaction was developed with the chromogen diaminobenzidine plus nickel chloride (Sigma) and counterstained with methyl green. A specimen of lichen planus was used as positive control for the reaction. The negative control was obtained by omitting the primary antibodies.

The population of cells immunostained in the dermis was semiquantitatively analyzed as previously described and was scored as follows by two independent investigators[10]: 0, negative immunolabeling; 1, up to one third of positive stained mononuclear cells in the dermal cell infiltrate; 2, more than one third up to two thirds of positive stained mononuclear cells in the dermal cell infiltrate; 3, more than two thirds of positive stained mononuclear cells in the dermal infiltrate.

A different scale was used to quantify immunolabeling of the epidermis[11]: 0, negative immunolabeling; 1, small number of positive stained dendritic cells with their cellular body well demarcated and with rare dendritic processes; 2, regular number of positive stained dendritic cells with defined and confluent dendritic processes; 3, large number of positive stained dendritic cells with confluence of the dendritic processes giving a uniform staining aspect to the epidermis.

In case of discordance between the two independent investigators an independent third investigator provided consensus about the final score.

The results of the lesional and perilesional skin as well as of the generalized and localized clinical forms of the disease were compared by the nonparametric statistical method of Mann-Whitney. The subpopulations of $CD4^+$ and $CD8^+$ T lymphocytes were also compared in all biopsy specimens, in lesional and perilesional skin, and in both clinical forms of FS, by Spearman's rank correlation.[12] The level of significance was set at $P = .05$.

RESULTS

$CD4^+$ (Fig 1) and $CD8^+$ T lymphocytes were found in the dermal mononuclear infiltrate of all FS biopsy specimens. Comparison of the subpopulations of $CD4^+$ and $CD8^+$ T cells for all specimens as a whole showed a predominance of $CD4^+$ T cells ($P = .001$). The $CD4^+$ T-cell population also predominated when the specimens were analyzed separately, both in the lesional skin ($P = .001$) and in the perilesional skin ($P = .012$). Both clinical forms of the

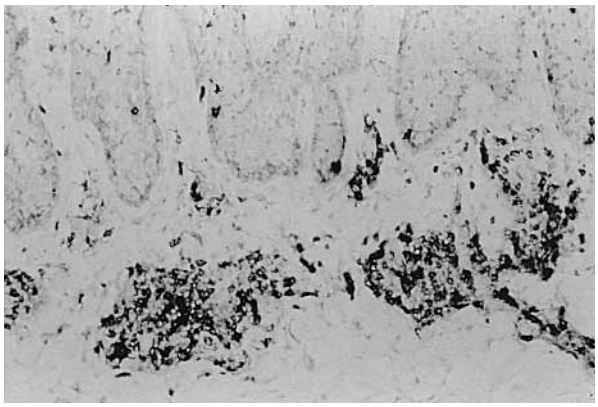

Fig 1. FS. $CD4^+$ lymphocytes (score 3) around vessels of superficial dermis of lesional skin. (Immunoperoxidase ABC technique; original magnification ×200.)

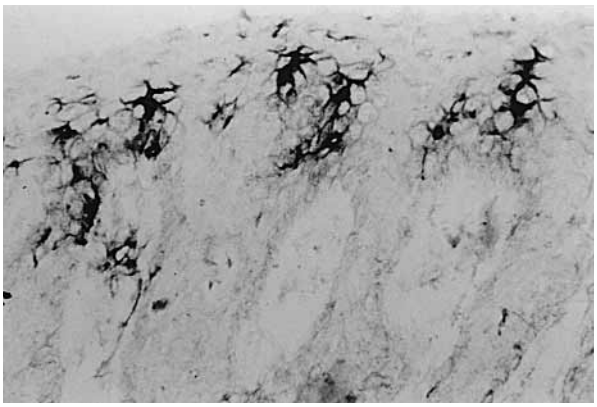

Fig 3. FS. Langerhans cells grouped in clusters with short dendrites within epidermis of lesional skin. (Immunoperoxidase ABC technique [CD1a monoclonal antibody]; original magnification ×400.)

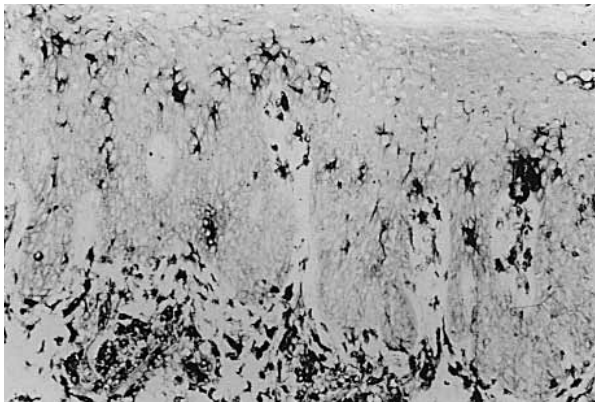

Fig 2. FS. HLA-DR expression of dendritic cells within epidermis (score 2) and mononuclear cells of papillary and superficial reticular dermis (score 3) of lesional skin. (Immunoperoxidase ABC technique; original magnification ×200.)

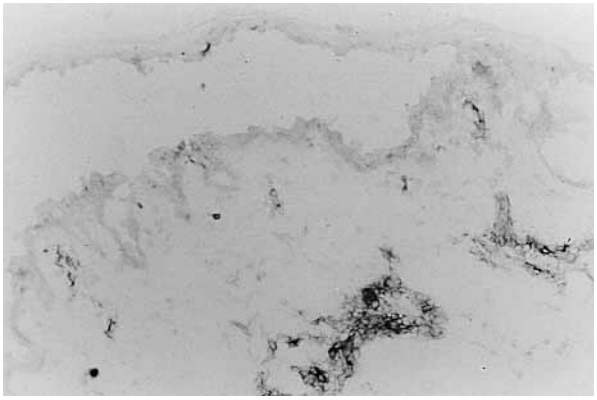

Fig 4. FS. ICAM-1 expression of endothelial and few mononuclear cells of dermis. Note absence of ICAM-1 expression by epidermal keratinocytes of acantholytic blister in lesional skin. (Immunoperoxidase ABC technique; original magnification ×100.)

disease showed the same results, with the subpopulations of $CD4^+$ T lymphocytes predominating in both the localized form ($P = .002$) and the generalized form ($P = .003$).

B lymphocytes were rarely found in FS. Only 3 biopsy specimens of lesional skin and 2 of perilesional skin from 4 different patients showed a few B lymphocytes (score 1). No significant difference was observed when the labeled cell elements present in the tissue inflammatory reaction were compared in the localized and generalized forms of the disease.

There were few $IL\text{-}2R^+$ cells among the dermal inflammatory elements. No significant difference in CD25 expression was found between the lesional and perilesional skin ($P = .619$).

Expression of HLA-DR occurred only in epidermal cells with dendritic morphology (ie, Langerhans cells) (Fig 2). The lesional epidermis showed more $HLA\text{-}DR^+$ dendritic cells than the perilesional epidermis ($P = .039$). The keratinocytes and acantholytic cells were all negative for this marker. In the dermis, besides the lymphocytes, the endothelial cells, other mononuclear cells, and dendritic cells also expressed HLA-DR. This expression predominated in the lesional skin ($P = .012$) compared with the perilesional skin.

The $CD1a^+$ Langerhans cells appeared as isolated cells or in clusters in the epidermis, with their cellular bodies well demarcated in black and with less evident dendrites (Fig 3). In a few cases the labeled Langerhans cells gave a network appearance to the epidermis with confluence of the dendrites between the keratinocytes. A few $CD1a^+$ dermal dendritic cells were present around the dermal adnexa and

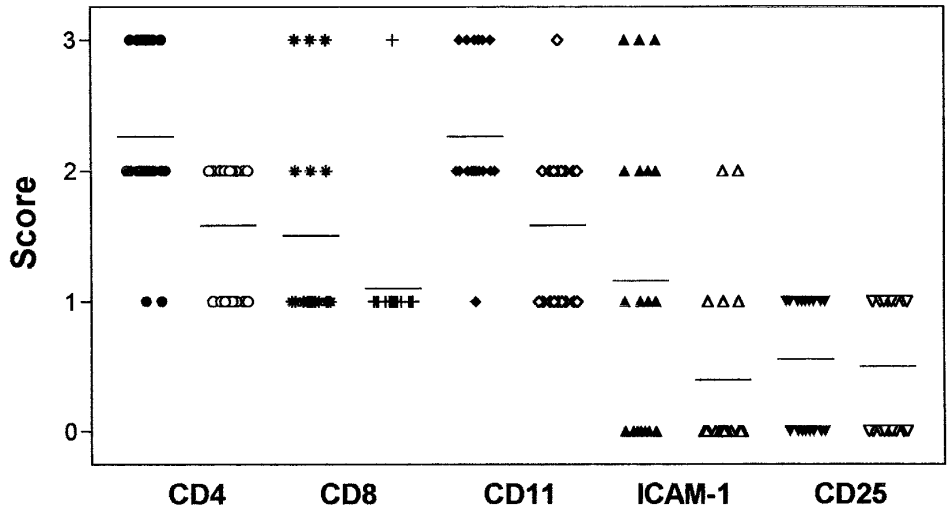

Fig 5. Distribution of semiquantitative scores of labeled cells in dermis from patients with FS. *Closed symbols* and *asterisk* represent lesional skin; *open symbols* and *plus sign* represent perilesional skin. *Bars* represent mean of values.

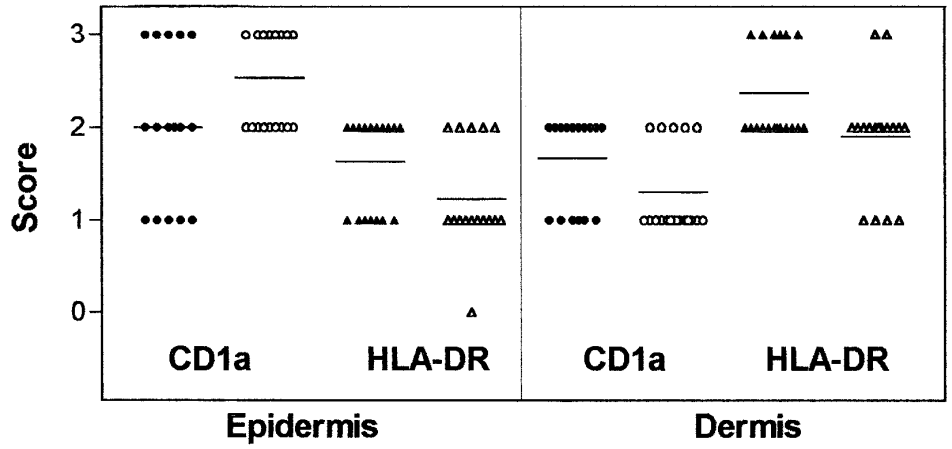

Fig 6. Distribution of semiquantitative scores of labeled cells in FS skin. *Closed symbols* represent lesional skin; *open symbols* represent perilesional skin. *Bars* represent mean of values.

vessels. The epidermal Langerhans cells were decreased in lesional skin when compared with perilesional skin ($P = .039$), whereas the opposite occurred in the dermis. CD1a$^+$ dendritic dermal cells predominated in the lesional dermis ($P = .039$).

ICAM-1 was expressed by the vascular endothelial cells and by the mononuclear cells surrounding the dermal vessels. The keratinocytes did not show any expression of ICAM-1 (Fig 4). The dermal inflammatory cells also expressed LFA-1 antigen. No epidermotropic LFA-1$^+$ T lymphocytes were observed in the perilesional or lesional epidermis. Cellular expression of ICAM-1 ($P = .013$) and LFA-1 ($P = .002$) was increased in the dermis of lesional skin, compared with the dermis of perilesional skin.

There was no statistically significant difference concerning all the cell elements population studied or ICAM-1 and HLA-DR expression when we compared the groups of patients with the generalized and localized form of FS.

Figs 5 and 6 show the scattergrams of the distribution of the semiquantitative score of each immunolabeled cell population in lesional and perilesional skin of FS.

DISCUSSION

CD4$^+$ and CD8$^+$ T lymphocytes were observed in the inflammatory reaction of FS. The CD4$^+$ T cells predominated in the lesional and perilesional skin as well as in both clinical forms of the disease (localized and generalized). Although the mononuclear cells in

the dermis expressed HLA-DR, only a few lymphocytes expressed CD25 (IL-2R), always with the lowest score. These cells may represent the resident lymphocytes in the skin.[13]

The epidermal keratinocytes did not express ICAM-1 or HLA-DR in lesional or perilesional skin of FS, and LFA-1$^+$ epidermotropic lymphocytes were also absent.

In vitro studies have demonstrated that IFN-γ-treated keratinocytes express ICAM-1 and HLA-DR.[14] The dermatoses that exhibit keratinocyte expression of ICAM-1 and HLA-DR have in common the production of IFN-γ by activated T lymphocytes.[15]

In bullous pemphigoid the keratinocytes express ICAM-1 and HLA-DR. In pemphigus vulgaris the keratinocytes are neither immunoreactive to ICAM-1 nor to HLA-DR.[6] The blister fluid of bullous pemphigoid has high levels of IFN-γ in comparison with that obtained from pemphigus vulgaris, second-grade dermal burn, and friction bulla.[7] Recently, however, Rico et al[16] (1999) demonstrated the predominance of helper type 2 T cells (T_H2) cytokines in bullous pemphigoid lesions.

In general there is a good correlation between the cytokine profile produced by T cells and the expression of adhesion molecules both in the endothelial cells and epidermal keratinocytes. Psoriatic lesions have a T_H1 cytokine profile (ie, IL-2, IFN-γ and tumor necrosis factor α) accompanied by expression of ICAM-1 on epidermal keratinocytes.[17]

The lack of keratinocyte ICAM-1 expression in FS may be related to the pattern of cytokines secreted by the CD4$^+$ T cells of the tissue reaction, as demonstrated in Sézary syndrome by Griffiths et al[15] in 1989. This observation and the evidence that in FS the pathogenic autoantibodies are of the IgG 4 subclass[2] would suggest that in FS, as was demonstrated with pemphigus vulgaris,[18] the T-cell population may be of the T_H2-like cytokine profile.

The function of keratinocytes in the cell-mediated response of FS seems to be unknown. In pemphigus vulgaris it was demonstrated that the antigenic response to desmoglein 3–specific T cells was restricted to HLA-DR.[18] Probably in FS the cell-mediated immunity is also triggered by the Langerhans cells.

According to the results obtained, the distinct clinical forms of FS (localized and generalized) could not be related to differences in nature or intensity of the tissue response of the skin lesions.

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REFERENCES

1. Diaz LA, Sampaio SAP, Rivitti EA, Martins CR, Cunha PR, Lombardi C, et al. Endemic pemphigus foliaceus (fogo selvagem). I: clinical features and immunopathology. J Am Acad Dermatol 1989;20:657-69.
2. Rock B, Martins CR, Theofilopoulos AN, Balderas RS, Anhalt GJ, Labib RS, et al. The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). N Engl J Med 1989;320:1463-9.
3. Stanley JR, Klaus Kovtum V, Sampaio SAP. Antigenic specificity of fogo selvagem autoantibodies is similar to North American pemphigus foliaceus and distinct from pemphigus vulgaris autoantibodies. J Invest Dermatol 1986;87:197-201.
4. Zillikens D, Ambach A, Schüssler M, Dummer R, Hartmann AA, Burg G. The interleukin-2 receptor in lesions and serum of bullous pemphigoid. Arch Dermatol Res 1992;284:141-5.
5. Zillikens D, Ambach A, Zentner A, Dummer R, Schüssler M, Burg G, et al. Evidence for cell-mediated immune mechanisms in the pathology of pemphigus. Br J Dermatol 1993;128:636-43.
6. Karashima T, Hachisuka H, Okubo K, Sasai Y. Epidermal keratinocytes of bullous pemphigoid express intercellular adhesion molecule-1 (ICAM-1). J Dermatol 1992;19:82-6.
7. Takiguchi Y, Kamiyama O, Saito E, Nagao E, Kaneko F, Minagawa T. Cell-mediated immune reaction in the mechanism of blister formation in bullous pemphigoid. Dermatologica 1989; 179(Suppl 1):137.
8. Maceira JP, Marques AS. Análise imuno-histoquímica das células S-100 positivas em lesões de pênfigo. An Bras Dermatol 1989;64:5-9.
9. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577-80.
10. Tebbe B, Mazur L, Stadler R, Orfanos CE. Immunohistochemical analysis of chronic discoid and subacute cutaneous lupus erythematosus—relation to immunopathological mechanisms. Br J Dermatol 1995;132:25-31.
11. Bennion SD, Middleton MH, David Bajar KM, Brice S, Norris DA. In three types of interface dermatitis, different patterns of expression of intercellular adhesion molecule-1 (ICAM-1) indicate different triggers of disease. J Invest Dermatol 1995;105:71S-9.
12. Feinstein AR. Clinical epidemiology: the architecture of clinical research. Philadelphia: WB Saunders; 1985.
13. Bos JD, Zonneveld I, Das PK, Krieg SR, Van Der Loos CM, Kapsenberg ML. The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin. J Invest Dermatol 1987;88:569-73.
14. Barker JNWN, Allen MH, Macdonald DM. The effect of in vivo interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. J Invest Dermatol 1989;93:439-42.
15. Griffiths CEM, Voorhees JJ, Nickoloff BJ, Michigan AA. Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: modulation by recombinant gamma interferon and tumor necrosis factor. J Am Acad Dermatol 1989;20:617-29.
16. Rico MJ, Benning C, Weingart S, Streilein RD, Hall RP III. Characterization of skin cytokines in bullous pemphigoid and pemphigus vulgaris. Br J Dermatol 1999;140:1079-86.
17. Uyemura K, Yamamura M, Fivenson DF, Modlin RL, Nickoloff BJ. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. J Invest Dermatol 1993;101:701-5.
18. Lin MS, Swartz SJ, Lopez A, Ding X, Fernandez-Vina MA, Stastny P, et al. Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. J Clin Invest 1997;99:31-40.