Dermal dendritic cell number correlates with serum autoantibody titers in Brazilian pemphigus foliaceus patients

Abstract

Pemphigus foliaceus (PF) is an autoimmune bullous disease endemic in Brazil. Since serum IL-12 is increased in patients with PF and Langerhans cells (LC) produce IL-12, we titrated serum autoantibodies by indirect immunofluorescence, and quantified epidermal dendritic cells, known as LC, and dermal dendritic cells (DC). Biopsies of blistering lesions were obtained from 22 patients, 13 of whom were submitted to biopsy of both injured and of apparently healthy skin. The control groups consisted of skin from 8 cadavers and from 12 women submitted to breast plastic surgery. LC and DC were identified with anti-CD1a antibody and quantified by morphometric analysis. LC number in the lesion and in apparently healthy skin from PF patients was similar to that of both control groups. DC number in the injured skin (median = 0.94 DC/mm basement membrane) was higher than that of the cadaver group (median = 0.13 DC/mm basement membrane). In the 13 patients with biopsies of both injured and apparently healthy skin, LC and DC were present in larger numbers in the lesion. There was a direct correlation between DC number in the lesion of the PF group and serum autoantibody titers. This correlation was not observed for LC number. The increased number of DC in the lesion, as well as its direct correlation with serum autoantibody titers suggest the participation of DC in the pathogenesis of PF. The relationship between increased DC number and IL-12 in PF needs to be clarified.

Key words

- Anti-CD1a
- Dendritic cells
- Fogo selvagem
- Langerhans cells
- Pemphigus foliaceus

Correspondence

A.M. Roselino
Divisão de Dermatologia
Departamento de Clínica Médica
FMRP, USP
Av. Bandeirantes, 3900
14049-900 Ribeirão Preto, SP
Brasil
Fax: +55-16-633-6695
E-mail: amfrosel@fmrp.usp.br

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special interest. LC are epidermal dendritic cells with antigen-presenting and T lymphocyte-activating abilities (5,6). The LC population is reduced in the epidermis of PF patients and the number of dermal DC is higher in the lesional skin than in the perilesional skin (7).

DC produce cytokines which can induce T cell differentiation, and among them IL-12 has a primordial role by inducing the Th1 phenotype (8,9). In the serum of PF patients, IL-4, IL-5 and INF-γ are decreased, and IL-10 and IL-12 are increased compared to controls (10). The production of IL-4, IL-5 and IL-6 but not of IFN-γ by T lymphocytes that specifically proliferate in response to Dg1 has been detected in patients with PF, suggesting that a Th2 pattern participates in its pathogenesis (11).

Since serum IL-12 is increased in PF (10) and DC, which produce IL-12, have an antigen-presenting function, the purpose of the present study was to quantify LC and DC in the skin of patients with PF and to attempt to correlate their numbers with circulating serum autoantibody titers.

Twenty-two patients with a diagnosis of PF, with active disease (positive Nikolsky signal), attended at the University Hospital of the Faculty of Medicine of Ribeirão Preto, University of São Paulo, were included in the study after giving informed written consent to participate. The protocol was approved by the Hospital Ethics Committee. Patients taking anticoagulant drugs and pregnant women were excluded. Thirteen presented the localized clinical form and 9 the generalized form of PF. Eleven patients were under treatment with prednisone (4 of whom were taking it in combination with chloroquine). Biopsies of both blistering lesion and of healthy skin in a sun-protected area were collected from 13 patients on the same day. The reason for using skin not adjacent to a lesion was to avoid detecting alterations of the disease itself, and the reason to use skin from a sun-protected area was due to the reduction of DC in sunlight-exposed areas (12). Eight skin fragments obtained from the anterior thoracic region of cadavers (3 h after death, on average) and 12 skin fragments obtained from 12 women submitted to breast plastic surgery were used as controls. There was no statistical difference concerning race or age between the PF and control groups.

For IIF, a fragment of human skin obtained from an abdominoplasty was used as substrate. Five-micrometer sections were incubated with patient serum at increasing dilutions for 30 min at room temperature. After rinsing with PBS, the sections were incubated with fluorescein-conjugated anti-human IgG (anti-human IgG-FITC, Behringwerke AG, Marburg, Germany) at 1:20 dilution for 30 min. After rinsing with PBS, the slides were mounted with buffered glycerin. Sections incubated with normal human serum and anti-human IgG were used as a negative control, and slides incubated only with anti-human IgG were used for the control of nonspecific fluorescence.

For LC and DC identification by immunohistochemistry, the OKT6 monoclonal antibody obtained in mice (anti-CD1a, Immunotech, Westbrook, ME, USA) was applied to 5-µm sections previously deparaffinized in xylene and hydrated in alcohol. As a secondary antibody, biotinylated anti-mouse rabbit IgG (Dako A/S, Glostrup, Denmark) was used for 35 min, followed by the streptavidin-biotin-peroxidase complex (StreptAB Complex/HRP, Dako A/S). The sections were then developed with diaminobenzidine. As a negative control, the primary antibody was excluded.

LC and DC quantification was performed by morphometric analysis, and the number of LC/mm² epidermis, LC/mm stratum corneum (SC), and LC/mm basement membrane (BM) were obtained. LC and DC were counted using light microscopy, with a 40X magnification objective.

The epidermal dimensions were obtained
by morphometric analysis using a videocamera coupled to a light microscope fitted with a 10X magnification objective. The image obtained on the video monitor was connected to an automatic image analyzer (KS300 Kontron Imaging System, Kontron Elektronik, Eching bei München, Germany) and epidermal area, BM and SC extension were measured.

Data concerning IIF and LC and DC quantification were analyzed statistically by the Kruskal-Wallis, Mann-Whitney and Wilcoxon tests, and the correlation between IIF titers and cell quantification was calculated by the Spearman test.

In patients with the localized form of the disease, serum antibody titration by IIF showed lower serum antibody titers (1:512 to 1:2048, median 1:1024) compared to patients with the generalized form (1:512 to 1:16384, median 1:4096; P = 0.0083).

There was a statistically significant difference (P = 0.0492) in LC quantification (Figure 1) between the two control groups (cadavers and women submitted to plastic breast surgery) both in number of LC/mm BM and number of LC/mm SC, a fact that did not permit their pooling into a single population for data analysis.

In the 13 patients with PF from whom skin biopsies were obtained from both apparently healthy and injured skin, the LC number was significantly higher in the lesional skin when LC/mm2 (P = 0.0134) and LC/mm SC (P = 0.0215) were quantified, but not significantly higher when LC/mm BM was measured.

DC numbers differed between the control groups (P = 0.0122) in dermal DC quantification (Figure 2). In the PF lesion, DC were present in higher numbers (0.94/mm BM) than in cadaver skin (0.13/mm BM; P = 0.0329). In the same patient of the PF group, the DC number in the injured skin was higher compared to apparently healthy skin (P = 0.0049).

There was a correlation between dermal DC number/mm BM in the PF lesion and serum antibody titers by IIF (r = 0.4779, P < 0.05), whereas this correlation was not sig-

![Figure 1. Langerhans cells (LC) in skin biopsies from patients submitted to breast plastic surgery (N = 12), from cadavers (N = 8), and from healthy (N = 13) and injured (N = 22) skin of pemphigus foliaceus (PF) patients. LC number/mm basement membrane (BM) and LC number/mm stratum corneum (SC) are indicated on the right Y axis and LC/mm2 of epidermis is on the left Y axis. Horizontal lines are the medians. * vs *: P = 0.0134, and # vs #: P = 0.0215 (Wilcoxon test).](image1)

![Figure 2. Dermal dendritic cells (DC) in basement membrane (BM). Comparison between skin biopsies from patients submitted to breast plastic surgery (N = 12), from cadavers (N = 8), and from healthy (N = 13) and injured (N = 22) skin of pemphigus foliaceus (PF) patients. The horizontal lines are the medians. A vs B: P = 0.0122 and B vs D: P = 0.0329 (Mann-Whitney test); * vs *: P = 0.0134, and # vs #: P = 0.0215 (Wilcoxon test).](image2)
nificant in the apparently healthy skin. This correlation was also not significant for LC number. There are no reports in the literature to permit us to make a comparison of our results concerning correlation between DC number in bullous lesion and serum autoantibody titers in PF.

In the present study we used the anti-CD1a monoclonal antibody for LC and DC labeling but we should point out that the values obtained may have been underestimated since DC subpopulations that do not express the CD1a surface antigen were not counted (13), and furthermore the method does not determine whether the cells are functionally active or not (14).

The choice of cadaver skin and skin from women submitted to breast plastic surgery as controls was based on the premise that these skin samples could represent a unique control group, since the DC number detected in these two kinds of skin has been reported to be similar in the literature (15,16). However, we detected higher LC and DC numbers in the skin from patients submitted to plastic surgery, which may have been due to the age difference between the two control groups, since the biopsies were obtained from the same area (anterior thorax). Thus, the cadaver group presented a smaller number of LC, probably because it consisted of older individuals. Another hypothesis could be that in the cadaver group the LC may lose the expression of surface proteins post mortem, although the literature does not show differences between living persons and cadavers (15,16).

The LC number in the PF lesion was similar to that of the control groups. When the blistering lesion was compared to apparently healthy skin in the PF group, the value was higher in injured skin. Only one report by Santi and Sotto (7) is available in the literature, which demonstrates that the LC population was decreased in the lesioned epidermis compared to the perilesional epidermis in PF.

We also point out that in the injured area, due to the bulla, the LC number in some sections may have been underestimated due to inclusion of the bulla in the total area calculated. Thus, when we found a larger number of LC in lesioned skin than in apparently healthy skin of PF patients, this should be taken into consideration.

The DC number in the blistering lesion of the PF group was increased compared to the cadaver group and was also increased compared to apparently healthy skin. This finding agrees with the results reported by Santi and Sotto (7) in a study comparing lesioned skin with perilesional skin.

When we divided the patients into groups with and without treatment, a higher DC number persisted in the dermis of both of them compared to control groups (data not shown).

Considering the patient group as a whole and overlooking its heterogeneity, we may state that LC number is unchanged in PF lesions, whereas the number of dermal DC is significantly increased compared to control groups. In the PF group, both the LC and DC were present in higher numbers in the lesion compared to apparently healthy skin. The significant correlation between serum antibody titers and the number of dermal DC in the PF lesion suggests that DC may play a role in the pathogenesis of PF. The relationship between increased DC number and IL-12 in PF needs to be clarified.

References


