琉球大学学術リポジトリ

ペラグラ患者病変皮膚におけるランゲルハンス細胞の消失

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Depletion of epidermal Langerhans cells in the skin lesions of pellagra patients

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**Key words** pellagra, niacin, Langerhans cell, photosensitivity

Running head: Depletion of Langerhans cells in pellagra
Introduction

Pellagra is a type of dietary deficiency disease, and is caused by an insufficiency in niacin or tryptophan. Diseases of malnutrition also include acrodermatitis enteropathica due to zinc deficiency, and necrolytic migratory erythema due to glucagonoma and biotin deficiency. Patients with these dietary deficiencies often present with some degree of skin dermatosis. The skin lesions usually form on body sites that are susceptible to minor trauma such as the feet, the hands, and the perioral regions.

Symptoms of pellagra include diarrhea, dermatitis, and dementia. It is usually diagnosed based on a patient’s dietary background and clinical symptoms. Indeed, reductions in serum niacin, tryptophan, NAD, or NADP are usually, but not always, helpful for diagnosis.\(^1\) Photosensitivity is a unique symptom in pellagra, as it is not seen in zinc or biotin deficiencies. In pellagra patients, niacin deficiency was reported to induce ROS formation in keratinocytes, and the subsequent PGE\(_2\) production mediated photosensitivity.\(^2\) Skin lesions due to these nutritional deficiencies also showed common histological alterations, including vacuolization and necrosis of keratinocytes in the upper epidermis. Recent studies have demonstrated decreases in the number of
Langerhans cells in skin lesions from patients with necrolytic migratory erythema\textsuperscript{3} and acrodermatitis enteropathica.\textsuperscript{4} In zinc-deficient mice, used as a model for human acrodermatitis enteropathica, a substantial decrease in epidermal Langerhans cells preceded and was responsible for the development and persistence of irritant contact dermatitis. In the current study, we analyzed the changes in dendritic cells in the skin of pellagra patients. We also examined the non-lesional skin adjacent to severe dermatitis in one pellagra case.

**Methods**

**Patients**

The study was approved by the ethics committee at the University of the Ryukyu, Okinawa, Japan. Patients with pellagra were identified from the medical records of the dermatology branches at the University of the Ryukyu, Tone-Chuo Hospital, Gunma University, Wakayama Medical University, and Saitama Medical Center in Japan. Biopsies were obtained for histological diagnosis.

Seven pellagra patients (mean age 61 years; age range 33–77 years, 4 males and 3 females) (Table 1) were involved in this study. In cases 2 and 6, the serum levels of
niacin were normal, but those of vitamin B6 and tryptophan were significantly decreased, respectively. In case 7, although the niacin level was not determined, the patient developed dementia and typical photodermatitis. Histologically normal skin areas adjacent to excised benign skin tumors of 10 otherwise healthy persons (mean age 60.8 years; age range 35-76 years, 6 males and 4 females) were used as controls.

**Immunohistochemistry**

Formalin-fixed, paraffin-embedded tissue blocks were cut into 4-μm sections, heated at 50 °C for five hours, and subsequently deparaffinized in xylene and hydrated using a series of ethanol solutions. For antigen retrieval, slides were heated in a pressure cooker in Tris-EDTA buffer (pH 9.0) or sodium citrate (pH 6.0) for 3 or 5 minutes, depending on the antibody used (Table S1). Samples were then treated with 3% hydrogen peroxide for 30 minutes to quench endogenous peroxidase activity. After washing with phosphate buffered saline (PBS), slides were incubated with a blocking solution (Serum-free Protein Block, Dako, Glostrup, Denmark) for 10 minutes, followed by incubation with a primary antibody for more than 60 minutes at room temperature. Anti-Langerin, CD1c CD14, CD11c, CD141, CD4, and CD8 antibodies were purchased from Abcam
(Cambridge, MA, USA), anti-CD1a, HLA-DR, Melan-A, cytokeratin 20, and tryptase antibodies from Dako and CD25 from Nichirei (Tokyo, Japan). After washing, sections were incubated with biotinylated goat anti-rabbit or anti-mouse IgG for 30 minutes, followed by streptavidin peroxidase (LSAB2 kits, Dako). Samples were then visualized using AEC and DAB Substrate Chromogen (Dako), and counterstained with Mayer’s hematoxylin.

**Histological assessment**

Observations were conducted using direct microscopy, and images were recorded at 100× magnification. Numbers were counted on the printout data. In the epidermis, the numbers of positive cells were counted inside 1 mm in width. In the dermis, the numbers of positive cells were counted inside a square having a side of 1 mm from the basement membrane. Measurements were taken at several distinct areas within each section, and the mean number and standard error of the mean (SEM) were calculated. Because of the limited availability of unstained tissue sections, we analyzed CD4, 8, 25 lymphocytes in 4 pellagra cases, and CD141, CD11c dendritic cells in 6 pellagra cases.

**Statistical analysis**
The analysis of variance (ANOVA) was used to assess the difference between the mean values of each specimen. A post-hoc comparison between the groups was performed with Tukey's honestly significant difference (HSD) test. The significance level was set at $p < 0.05$.

**Results**

**Histological severity of pellagra lesions**

All cases of pellagra had relatively severe skin symptoms with an intense, vesicular erythema on the dorsum of the hands and feet, while the histological features ranged from mild lymphocytic inflammation ($n = 2$) and moderate vacuolization ($n = 3$) to severe cases with extensive necrosis of keratinocytes ($n = 2$) (Figs. 1, S1). We analyzed the presence of epidermal Langerhans cells, dermal dendritic cells, T lymphocytes, melanocytes, Merkel cells, and mast cells in the lesional skin.

**Substantial decrease of epidermal Langerhans cells**

A significant reduction in epidermal Langerhans cells was observed in the lesional skin of pellagra patients compared to normal skin (Figs. 1, S1, S2). The mean number of Langerin$^+$ cells in the normal human epidermis was $17.7 \pm 5.9$ cells/mm, whereas...
patients with mild, moderate, or severe pellagra had 4.2 (± 1.8) cells/mm, 2.9 (± 3.2)
cells/mm, and 1.6 (± 2.5) cells/mm in the lesional epidermis, respectively (Table S2).
Cells expressing CD1a in the normal human epidermis numbered 20.1 (± 4.6) cells/mm,
whereas patients with mild, moderate or severe pellagra had 8.8 (± 4.5) cells/mm, 4.9 (±
3.9) cells/mm, and 2.0 (± 1.8) cells/mm in the lesional epidermis, respectively (Table
S2). The extent of the decrease in epidermal Langerhans cells was ascertained
statistically in accordance with the severity of the histological alteration in pellagra
lesions ($p < 0.01$) (Fig. 2a, Table S2). The epidermal HLA-DR+ cells in the skin lesion
of severe pellagra cases were significantly reduced or disappeared in a same manner
observed for Langerin and CD1a positive cells ($p < 0.01$), while those of moderate
pellagra cases were slightly increased (Fig. 2a, Table S2).

**Preservation of other cutaneous cells**

The numbers of dermal dendritic cells identified as CD141+, CD1c+ and CD11c+ cells
did not differ from those of the normal skin, while that of CD14+ cells was significantly
higher in pellagra skin lesion (Figs. 2b, S3, S4, Table S2). The extent of infiltration of
CD4+ and CD8+ cells did not show any significant difference, as these cells were
distributed irrespective of the severity of pellagra lesions. However, the numbers of
CD25+ cells were reduced in the severe pellagra skin lesions compared to mild cases
(data not shown).

The number of Melan-A+ melanocytes was higher in pellagra skin lesions compared
to normal skin; however, the degree of the increase of these melanocytes was not
correlated with the histological severity of pellagra lesions. (Figs. S3, S4, Table S2).
The numbers of tryptase+ mast cells (Figs. S3, S4, Table S2) and keratin 20+ Merkel
cells (data not shown) were not affected.

**Langerhans cells remained in the follicular epithelia**

In cases of histologically severe pellagra (case 6), Langerhans cells were completely
absent in the interfollicular epidermis(Figs. 3a), although a few remained in the
follicular epithelia (Figs. 3b).

**Langerhans cells persisted in the undamaged epidermis**

In case 5, a biopsy was taken from the intense erythematous skin lesion together with
the adjacent non-erythematous skin. In the inflamed lesion, the number of epidermal
Langerhans cells was significantly low (1.0 cells/mm), similar to other moderate
pellagra cases. However, in the histologically unaffected skin neighboring the intense
skin lesion, the numbers of Langerin\textsuperscript{−} and CD1a\textsuperscript{−} Langerhans cells were 8 cells/mm and
17 cells/mm, respectively, and were maintained at a level similar to the healthy controls
(Fig. 4).

Discussion

Our data suggest that solely epidermal Langerhans cells were affected in all pellagra
cases, while the numbers of other cutaneous cells such as mast cells and Merkel cells as
well as dermal dendritic cells were not affected. The increase of epidermal HLA-DR\textsuperscript{+}
cells in the moderate pellagra (Fig. 2a) might reflect the induction of HLA-DR
expression in the inflammatory skin lesion, which is kept in relatively low level at
non-inflamed skin. The numbers of HLA-DR\textsuperscript{+} cells did not always follow a linear
relationship with disease severity, as we observed a decrease of cell counts in mild cases,
mixed results in moderate cases, and a complete disappearance of these cells in severe
cases. In one moderate pellagra (case 5), the number of CD14\textsuperscript{+}, HLA-DR\textsuperscript{+}, and CD1a
negative cells increased in the epidermis (data not shown). Thus, HLA-DR\textsuperscript{+} cells
increased in the average values of moderate pellagra cases. The dermal CD14\textsuperscript{+} cells,
those increased in pellagra lesion, were considered to be monocytes derived macrophages massively induced by inflammation.\textsuperscript{5}

Although numbers of melanocytes were higher in pellagra skin lesions, the pathophysiological implications were unclear, as the changes might be the result of a distinct melanocyte distribution depending on the affected patients, and the duration of the prolonged inflammation.

Epidermal Langerhans cells are capable of modulating or even halting the inflammatory reaction in addition to their role in initiating a specific immune response by processing and presenting antigens.\textsuperscript{6} Thus, the depletion of Langerhans cells observed in the reported zinc-deficient mice model was considered to contribute to the epidermal necrosis secondary to prolonged inflammation.\textsuperscript{4} Since the dermatoses in pellagra often develop in exposed and vulnerable body sites, including hands, feet, and perioral regions, we think that the depletion of Langerhans cells can also involve increased and sustained photosensitivity dermatitis.

Murine Langerhans cells migrate into the epidermis through the infundibulum and isthmus of hair follicular epithelia.\textsuperscript{7} Langerhans cells were completely absent in the
interfollicular epidermis, although a few remained in the follicular epithelia. The presence of residual Langerhans cells in lesional hair follicles, even in severe pellagra cases, can provide an insight into the migratory process and microenvironment of human Langerhans cells.

Langerhans cells were completely absent in the lesional pellagra skin, while Langerhans cells survived and their numbers were maintained in the closely adjacent histologically undamaged epidermis. When dermatitis is provoked by physiological irritation including UV exposure, activated Langerhans cells migrate to the lymph nodes through dermal lymph vessels from the epidermis. Consequently, in normal skin, Langerhans cells are immediately replenished from dermal CD1c+ dendritic cells and monocytes, and the number of Langerhans cells is maintained. However, in pellagra, Langerhans cells cannot be replenished and are lost in severe lesions. The resultant decrease in Langerhans cells, which act to suppress inflammatory reactions, is considered to further prolong the dermatitis. In conclusion, the results of this study suggest that the depletion of epidermal Langerhans cells did not precede skin damage as observed in case 5. In pellagra patients, Langerhans cells might not be regenerated due
to the shortage of some trophic factors, consequently, the exhaustion of Langerhans cells may cause the characteristic prolonged dermatitis.

Acknowledgements

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References


Legends

Figure 1

The clinical appearance, histological and immunohistochemical analyses of representative pellagra cases. Hematoxylin-eosin staining (×200), and the presence of Langerin (×400) and CD1a (×400) positive cells in normal skin, mild pellagra, and severe pellagra.

Figure 2

(a) Mean and SEM of Langerin-, CD1a-, and HLA-DR-positive cells in the lesional epidermis, and (b) HLA-DR-, CD14-, CD141-, CD1c, and CD11c -positive cells in the lesional dermis. Dermis from normal and mild pellagra patients are compared to severe pellagra patients.

Figure 3

(a) The absence of Langerhans cells absence in the interfollicular epidermis (×200), and (b) the presence of residual Langerhans cells at the lesional hair follicle (×400) of a severe pellagra (case 6), anti-Langerin staining.
Figure 4

Histological evaluation of pellagra (case 5) whose lesional and adjacent non-lesional skin were analyzed. Langerin, CD1a, HLA-DR positive cells were retained in the non-lesional skin, whereas these cells disappeared substantially in the lesional epidermis. The presence of dermal HLA-DR, CD1c, CD11c, CD14, and CD141 positive cells were similar between the inflamed lesion and non-lesional skin.
Supplemental data

Figure S1.

The clinical appearance, histological, and immunohistochemical examinations of Langerin, CD1a, and HLA-DR expression in the skin lesions of pellagra cases (original magnification 200× for HE, 400× for immunohistochemistry). Pellagra 1: mild, 67 y.o. male; Pellagra 2: mild, 77 y.o. male; Pellagra 3: moderate, 42 y.o. female; Pellagra 4: moderate, 66 y.o. male; Pellagra 5: moderate, 74 y.o. male; Pellagra 6: severe, 33 y.o. female; Pellagra 7: severe, 68 y.o. female.

Figure S2.

Histological (HE) and immunohistochemical results of Langerin, CD1a, and HLA-DR expression in representative normal skin from cases 1-5 (original magnification 200× for HE, 400× for immunohistochemistry). Normal skin 1: 74 y.o., male; Normal skin 2: 35 y.o., female; Normal skin 3: 53 y.o., female; Normal skin 4: 54 y.o., male; Normal skin 5: 76 y.o., male. Normal skin 6: 73 y.o., female; Normal skin 7: 42 y.o., female; Normal skin 8: 51 y.o., male; Normal skin 9: 74 y.o., male; Normal skin 10: 76 y.o.,
Figure S3.

Expression of CD1c, CD14, CD141, CD11c, Melan-A, and tryptase in the skin lesions of pellagra cases (original magnification 400× for CD1c, CD14 and Melan-A, 200× for CD141, CD11c and tryptase).

Figure S4.

Expression of CD1c, CD14, CD141, CD11c, Melan-A, and tryptase in normal skin 1-5 (original magnification 400× for CD1c, CD14 and Melan-A, 200× for CD141, CD11c and tryptase).

Table S1.

Antibodies and antigen retrieval methods used for immunohistochemistry.

Table S2.

Enumeration of the mean number with SEM of dendritic cells and other cutaneous cells in the skin lesions of mild, moderate, and severe pellagra groups, as well as normal controls.
Normal skin  |  Mild pellagra  |  Moderate pellagra  |  Severe pellagra

HE  |  Langerin  |  CD1a
Fig. 2
Fig. 3
Fig. 4
Supplemental data
Fig S2

Normal skin 1  Normal skin 2  Normal skin 3  Normal skin 4  Normal skin 5

HE

Langerin

CD1a

HLA-DR
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Fig S4

Normal skin 1  Normal skin 2  Normal skin 3  Normal skin 4  Normal skin 5

CD1c

CD14

CD141

CD11c

Melan-A

Tryptase