Figure legends

Fig.1. Numerical alterations of cutaneous DCs after UVB irradiation of the skin.

Single-cell suspensions were stained with APC-conjugated anti-MHC class II and APC-Cy7-conjugated CD11c antibodies and subjected to flow cytometric analysis. A, Non-irradiated skin. B, UVB-irradiated skin. C, With the use of anti-EpCAM and anti-Langerin antibodies, DCs from non-irradiated skin were clearly sorted out into the three categories: LCs (Langerin+ EpCAM+), Langerin+ dDCs (Langerin+ EpCAM-), and Langerin- dDCs (Langerin- EpCAM-). D, In UVB-irradiated skin, Langerin+ dDCs were diminished. E, Total cell number of each DC subsets. F, Apoptotic cell number of LCs and Langerin+ dDCs as assessed by flow cytometric analysis (7-AAD- and Annexin+).

Fig. 2. Numbers of LCs, Langerin+ dDCs, and Langerin- dDCs in DLNs after FITC application to UVB-irradiated or non-irradiated skin.

Mice were irradiated with UVB (300 mJ/cm^2) on day -2 or non-irradiated, and sensitized with FITC on day 0. On day 1 to 4, DLNs were collected and stained for CD11c, Langerin, and EpCAM. We gated on the FITC+CD11c+ population and counted the EpCAM+ Langerin+ (LCs), EpCAM- Langerin+ (Langerin+ dDCs), EpCAM- Langerin- (Langerin- dDCs) cells. A, The number of LCs was gradually increased after

FITC application in the UVB irradiated and not-irradiated skin. B, The number of Langerin+ dDCs was increased sharply at day 3 in non-irradiated mice but not increased in UVB-irradiated mice. C, The number of Langerin- dDCs peaked at day 1 in the UVB-irradiated mice.

Fig. 3. Expression of surface CD86, intracellular IL-10, and surface OX40L in LCs and Langerin- dDCs from the skin and the darining lymph nodes.

A, Epidermal cell suspensions were obtained from UVB-irradiated skin 24 hours after UVB exposure, or non-irradiated skin. Solid line, UVB-irradiated skin; dotted line, non-irradiated skin; and closed shadow, isotype-matched control.

B, Cell suspensions were obtained from the draining lymph nodes of mice receiving UVB irradiation (day -2) and FITC paining (day 0) or mice receiving FITC painting without UVB irradiation. Lymph nodes were taken on day 1 and migrating LCs were identified as FITC+CD11c+EpCAM+Langerin+ cells, and migraining Langerin- dDCs were as FITC+CD11c+EpCAM-Langerin- cells. Solid line, UVB-irradiated mice; dotted line, non-irradiated mice; and closed shadow, isotype-matched control.

Fig. 4. RANKL expression in apoptotic keratinocytes from UVB-irradiated skin and RANKL promotion of LC IL-10 production.

A, Epidermal cell suspensions were obtained from UVB (300 mJ/cm^2)-irradiated skin

24 hours after irradiation. Keratinocyte were identified as the MHC class II- and γδTCR- fraction by flow cytometry. a, live keratinocyte; b, apoptotic keratinocyte, c; dead keratinocytes. B, Apoptotic keratinocytes expressed RANKL at a higher degree than live and dead keratinocytes. Solid line, UVB-irradiated skin; dotted line, non-irradiated skin; and closed shadow, isotype-matched control. C, Epidermal cell suspensions were cultured with or without recombinant RANKL for 24 hour. Intracellular IL-10 was measured by FACS. IL-10 production in Langerhans cell was increased by the addition of recombinant RANKL, and the increased IL-10 production was reduced by the further addition of anti-RANK antibody.

Fig. 5. Effects of administration of IL-10 neutralizing and OX40L blocking antibodies. Mice were irradiated with UVB (300 mJ/cm^2) on day -2, sensitized with DNFB on day 0, and challenged with DNFB on day 5. IL-10 neutralizing antibody (25 μg per mouse), OX40 blocking antibody (10 μg per mouse), or PBS (for control) was injected intraperitoneally on days 0 to 3. Positive control mice were sensitized and challenged, and negative control mice were challenged without sensitization. *$P < 0.05$.

Fig. 6. Effect of dissection of UVB-irradiated and/or hapten-applied skin on CHS.

A, The number of LCs migrating to the lymph nodes on day 5 in mice receiving skin dissection on day 1. The LC counts were significantly decreased in mice receiving skin

dissection (white bar) compared to non-disected mice (black bar). B, Mice were irradiated with UVB (300 mJ/cm^2) on day -2, sensitized with DNFB on day 0, and challenged with DNFB on day 5. On day 1, a group of mice were skin-dissected. Positive control mice were sensitized and challenged, and negative control mice were challenged without sensitization. *$P < 0.05$.

Fig. 7. UVB-induced immunosuppression in LC-depleted mice.

LCs (EpCAM+ Langerin+ cells) and Langerin+ dDCs (EpCAM- Langerin+ cells) were depleted by DT (100 ng per mouse) in Langerin-DTR-knocked-in mice, Langerin+ dDCs repopulated 10 days later. A, Non-irradiated skin. B, 24 hours after DT injection. C, 10 days after DT injection. D, LCs were depleted in Langerin-DTR-knocked-in mice by DT (day -3) before UVB irradiation (day -2). They were sensitized (day 0) and challenged (day 5) with DNFB. E, LCs were depleted in Langerin-DTR-knocked-in mice by DT 10 days (day -12) before UVB irradiation (day -2). They were sensitized (day 0) and challenged (day 5) with DNFB. *$P < 0.05$.

Figure 1

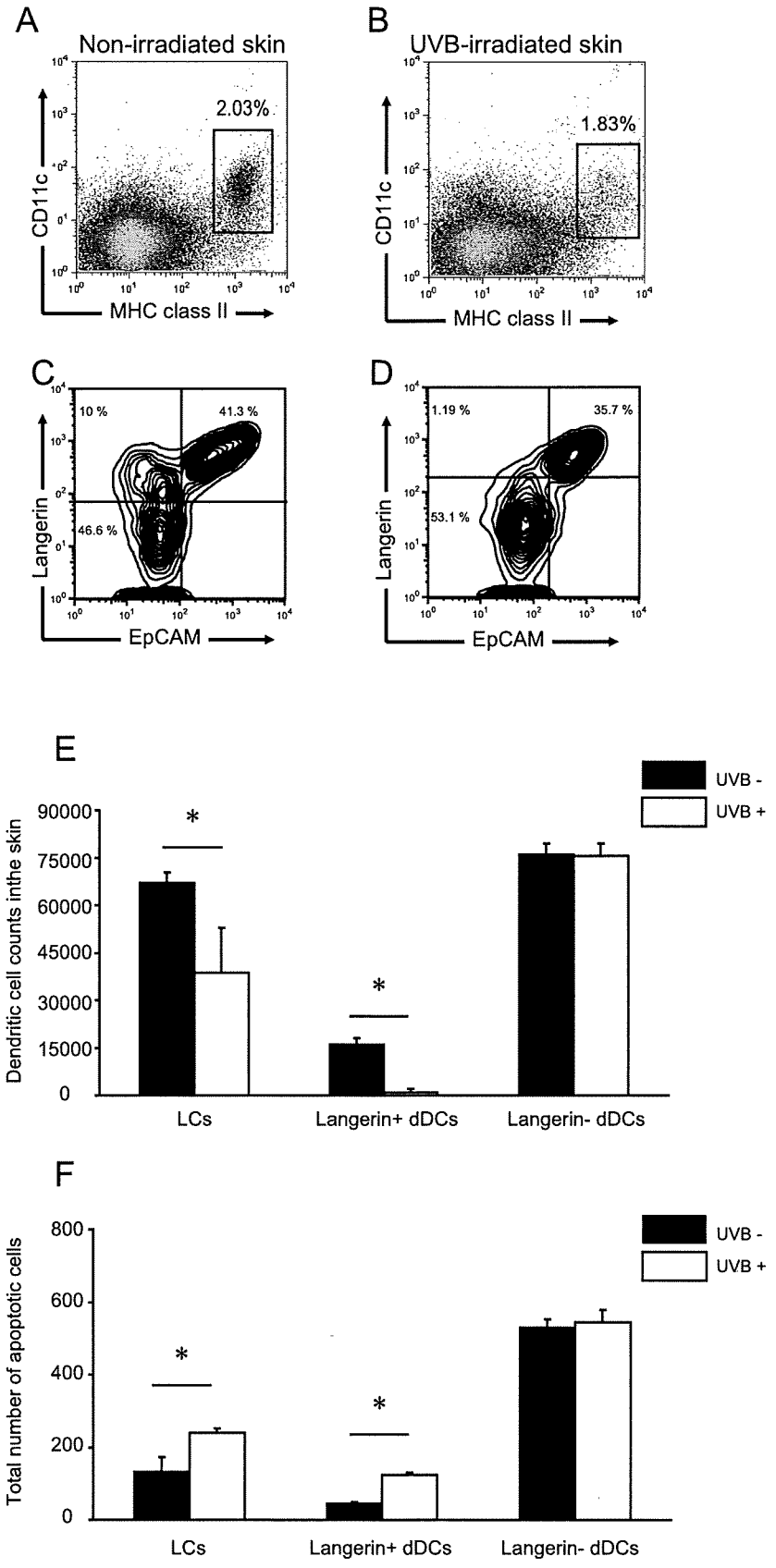

A
Non-irradiated skin
2.03%
CD11c
MHC class II
B
UVB-irradiated skin
1.83%
CD11c
MHC class II
C
10 %
41.3 %
46.6 %
Langerin
EpCAM
D
1.19 %
35.7 %
53.1 %
Langerin
EpCAM
E
UVB -
UVB +
Dendritic cell counts inthe skin
90000
75000
60000
45000
30000
15000
0
*
*
LCs
Langerin+ dDCs
Langerin- dDCs
F
UVB -
UVB +
Total number of apoptotic cells
800
600
400
200
0
*
*
LCs
Langerin+ dDCs
Langerin- dDCs

Figure 2

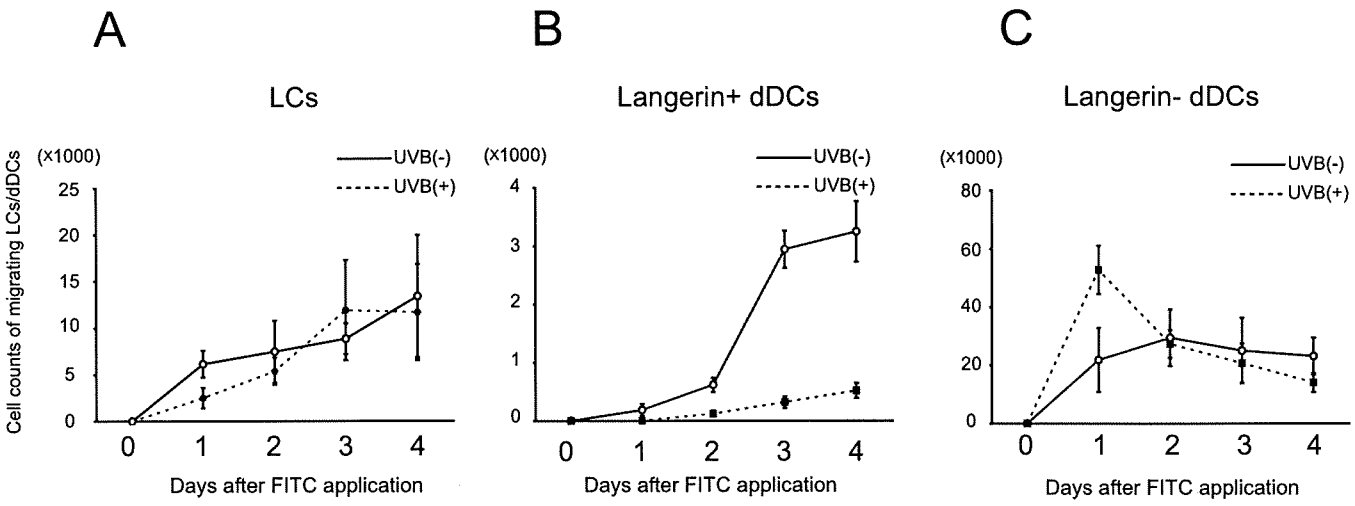

A
LCs
B
Langerin+ dDCs
C
Langerin- dDCs
Cell counts of migrating LCs/dDCs
(x1000)
UVB(-)
UVB(+)
Days after FITC application

Figure 3

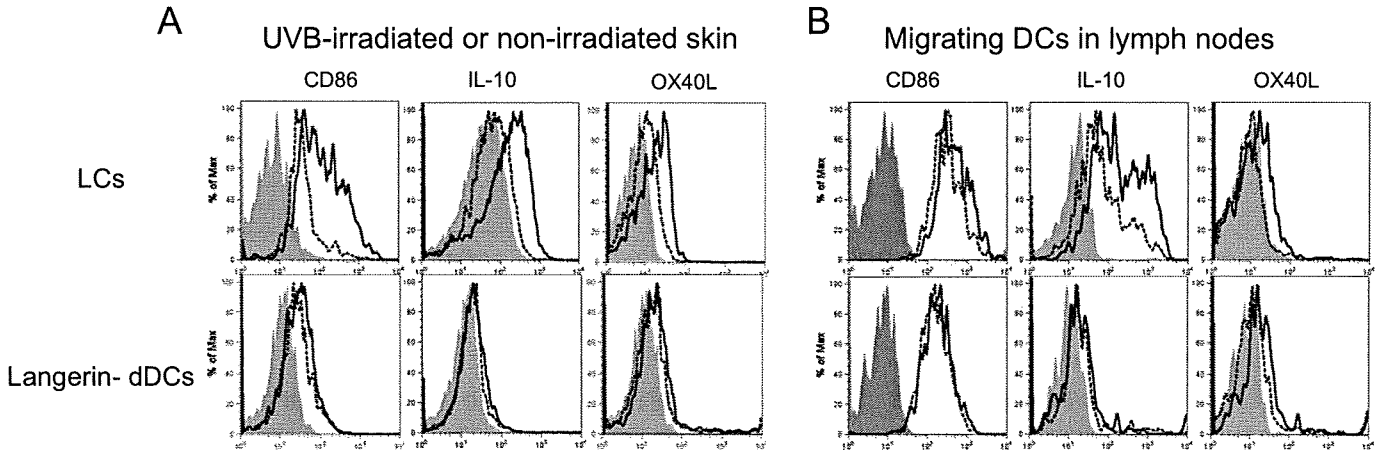

A
UVB-irradiated or non-irradiated skin
CD86
IL-10
OX40L
LCs
Langerin- dDCs
% of Max
B
Migrating DCs in lymph nodes
CD86
IL-10
OX40L
% of Max

Figure 4

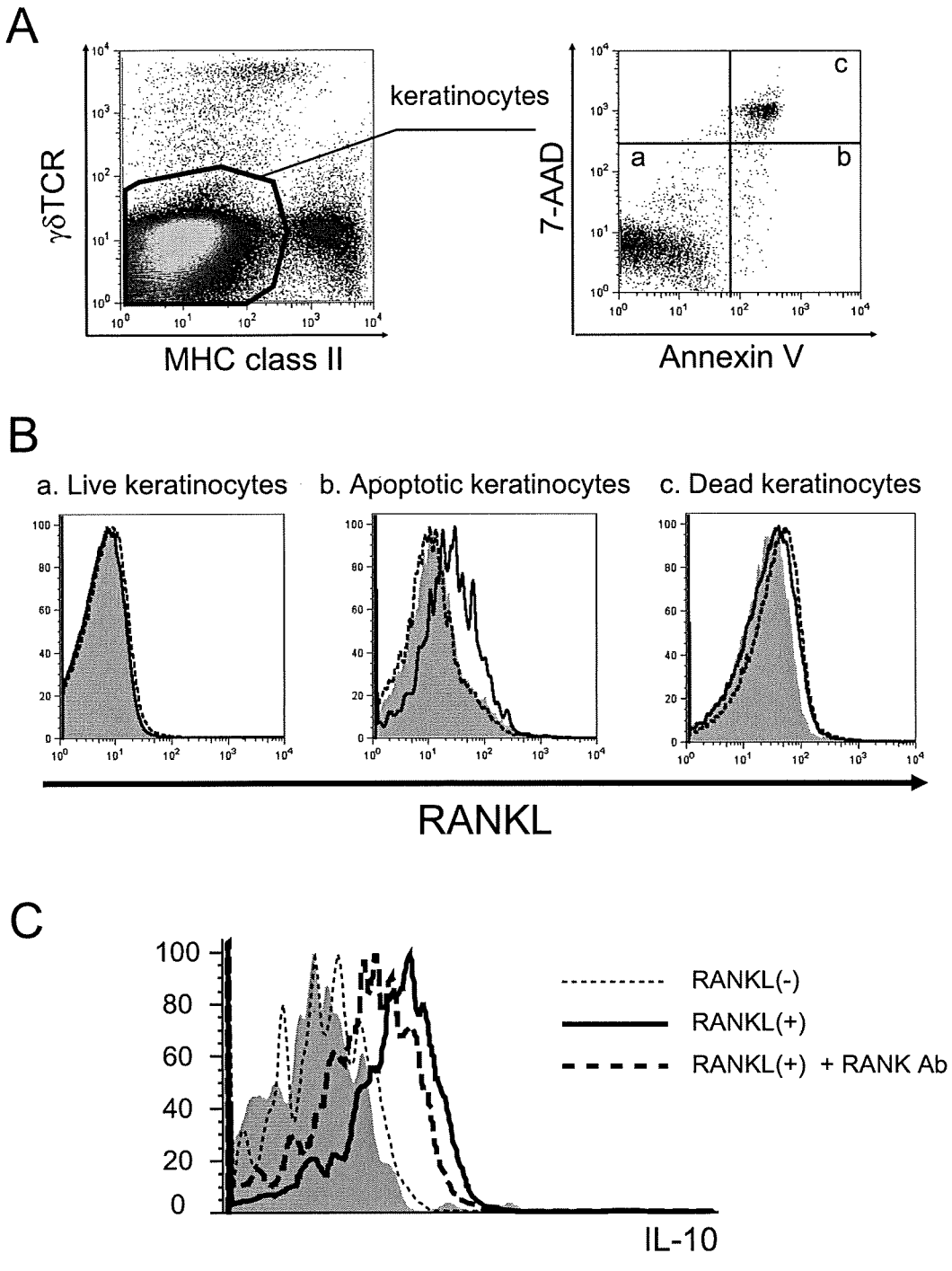

A
keratinocytes
γδTCR
MHC class II
7-AAD
Annexin V
a
b
c
B
a. Live keratinocytes
b. Apoptotic keratinocytes
c. Dead keratinocytes
RANKL
C
RANKL(-)
RANKL(+)
RANKL(+) + RANK Ab
IL-10

Figure 5

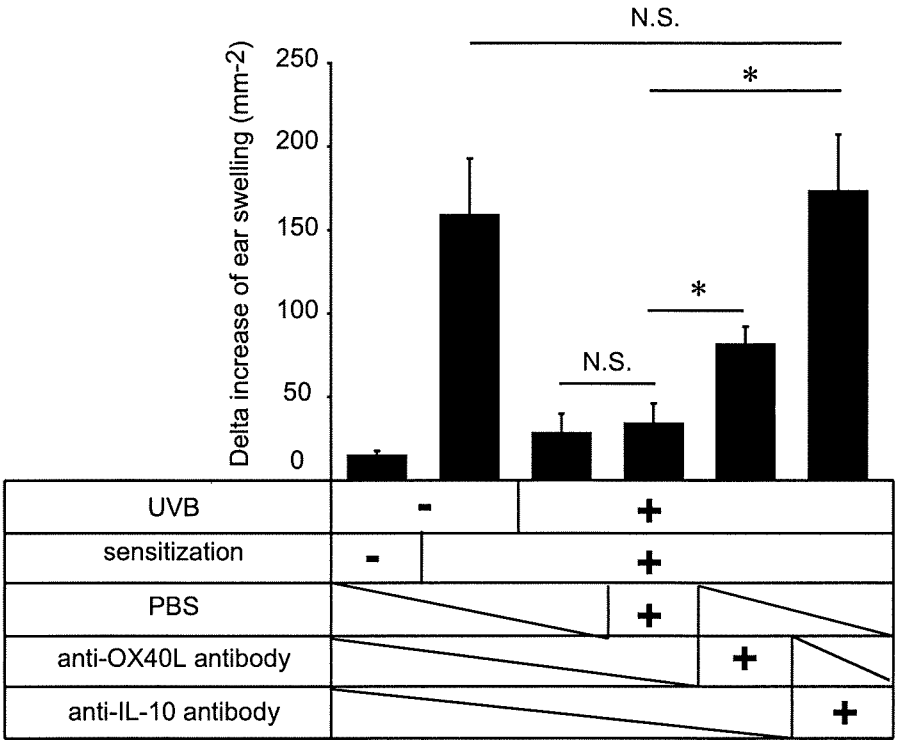
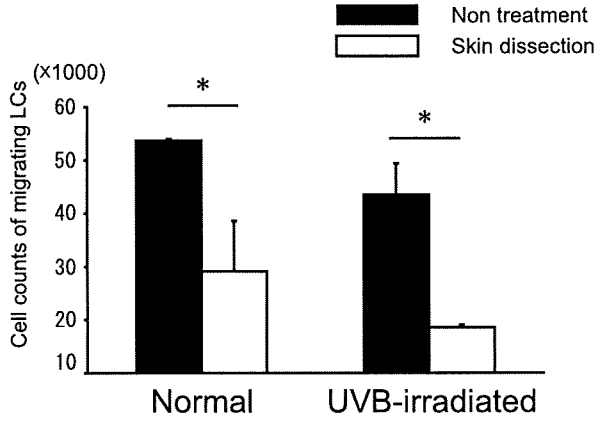

N.S.
*
*
N.S.
Delta increase of ear swelling (mm-2)
250
200
150
100
50
0
UVB
sensitization
PBS
anti-OX40L antibody
anti-IL-10 antibody
-
+
-
+
+
+
+

Figure 6

A


Non treatment
Skin dissection
(X1000)
Cell counts of migrating LCs
60
50
40
30
20
10
*
*
Normal
UVB-irradiated

B

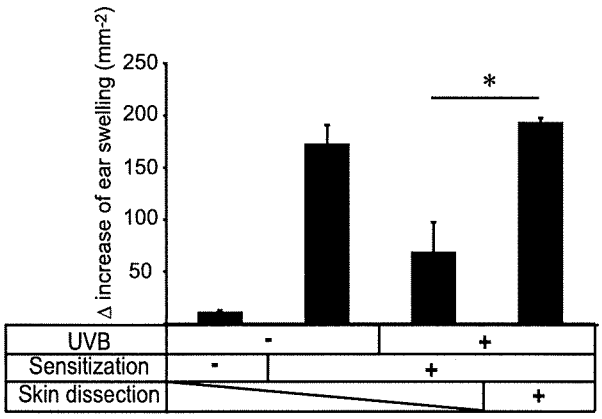
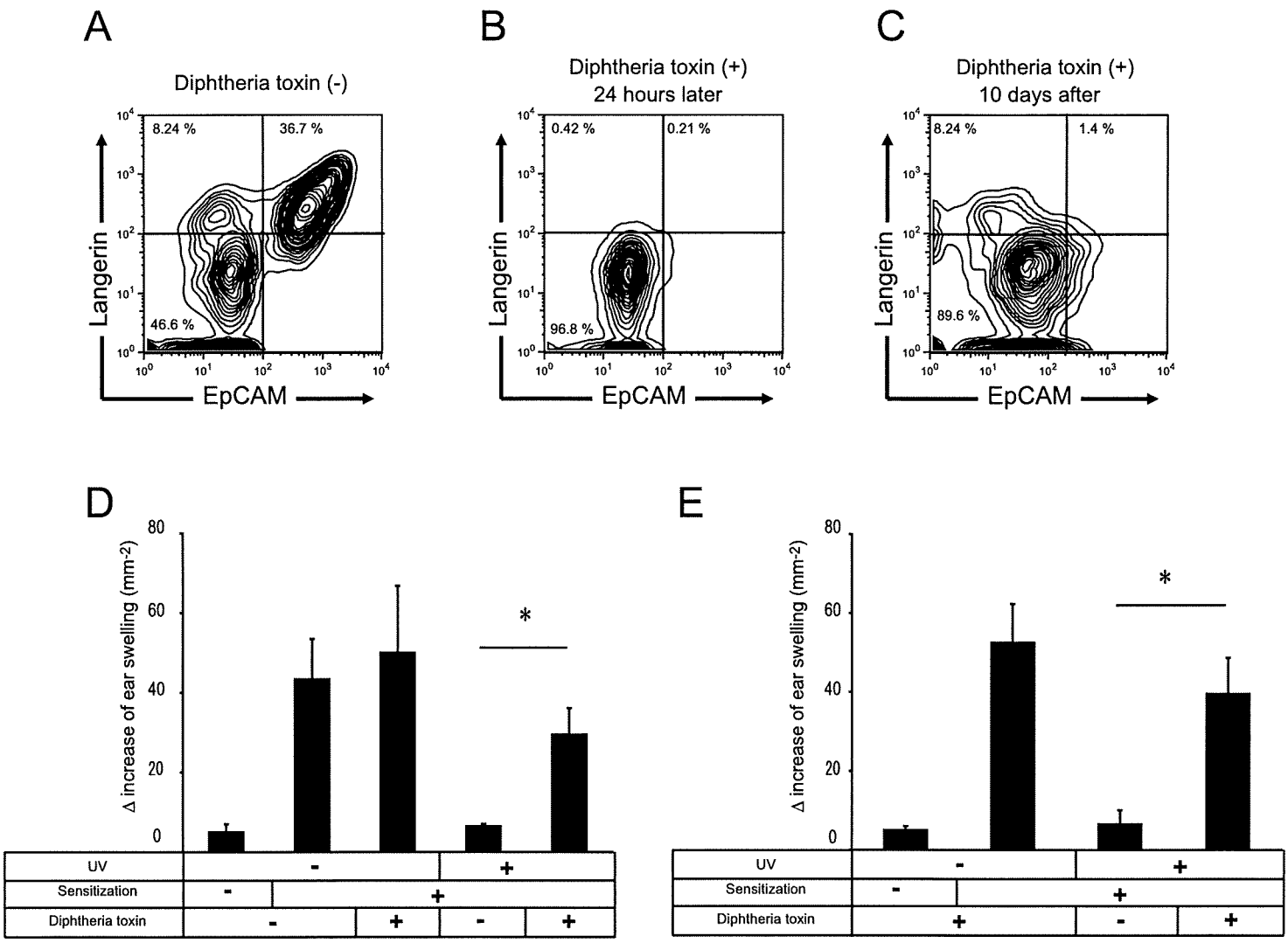

Δ increase of ear swelling (mm-2)
250
200
150
100
50
*
UVB
Sensitization
Skin dissection
-
+
-
+
+

Figure 7


A
Diphtheria toxin (-)
8.24 %
36.7 %
46.6 %
Langerin
EpCAM
B
Diphtheria toxin (+)
24 hours later
0.42 %
0.21 %
96.8 %
Langerin
EpCAM
C
Diphtheria toxin (+)
10 days after
8.24 %
1.4 %
89.6 %
Langerin
EpCAM
D
Δ increase of ear swelling (mm-2)
80
60
40
20
0
*
UV
Sensitization
Diphtheria toxin
E
Δ increase of ear swelling (mm-2)
80
60
40
20
0
*
UV
Sensitization
Diphtheria toxin

Acta Derm Venereol 2010; 90: 68–72

CLINICAL REPORT

Leukaemic Cutaneous T-cell Lymphoma Manifesting Papuloerythroderma with CD3$^-$ CD4$^+$ Phenotype

Takatoshi SHIMAUCHI, Kazunari SUGITA, Motonobu NAKAMURA and Yoshiki TOKURA
Department of Dermatology, University of Occupational and Environmental Health, Kitakyusyu, Japan

The leukaemic form of cutaneous T-cell lymphoma, as represented by Sézary syndrome, exhibits erythroderma. We describe here an indolent leukaemic patient with cutaneous T-cell lymphoma, who initially had a nodulotumourous eruption with a crop of solid papules, but finally presented with papuloerythroderma. Histologically, the skin lesions showed non-epidermotropic dermal infiltration of atypical lymphocytes with lymphoid follicles and a granulomatous change. The circulating malignant CD4$^+$CCR4$^+$ T cells lacked the expression of T-cell receptor and did not respond to concanavalin A. The unresponsiveness of T cells to the T-cell mitogen may be associated with the non-epidermotropic behaviour of the tumour cells and the initially non-erythrodermic eruption. ***Key words: cutaneous T-cell lymphoma; papuloerythroderma; phenotype.***

(Accepted September 24, 2009.)

Acta Derm Venereol 2010; 90: 68–72.

Yoshiki Tokura, Department of Dermatology, University of Occupational and Environmental Health, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyusyu, 807-8555, Japan. E-mail: tokura@med.uoeh-u.ac.jp

Cutaneous T-cell lymphoma (CTCL) is a peripheral T-cell malignancy, which firstly involves the skin, then gradually the lymph nodes or other organs as the disease progresses (1). Sézary syndrome is the typical leukaemic variant of CTCL, presenting as erythroderma and lymphoadenopathy (1). However, atypical cases of leukaemic CTCL exhibiting skin eruptions other than erythroderma have been reported in relation to the aberrant surface phenotype (2). The tumour cells of CTCL usually have a CD3$^+$CD4$^+$ mature helper T-cell phenotype and express functional T-cell receptor (TCR). In addition, CD4$^+$ Sézary cells highly express cutaneous leukocytes antigen (CLA) and chemokine receptors CCR4, CCR7, and CCR10 (3). TCR and CD3 complex interacts with antigen/major histocompatibility complex on antigen-presenting cells, inducing T-cell proliferation and cytokine production via activated T-cell signalling pathways. Therefore, an abnormality in the expression of CD3/TCR complex may lead to unresponsiveness of tumour cells to external antigenic stimuli and unique skin eruptions. We report here an unusual case of CTCL with chronic leukaemia of T cells lacking the expression of CD3/TCR complex and exhibiting a papuloerythrodermatous eruption.

CASE REPORT

In July 2004, a 74-year-old Japanese man was referred to us for evaluation of a one-year history of topical corticosteroid-resistant, pruritic eruption on his trunk. On examination, there were coalesced nodules or tumours on his lower chest (Fig. 1a) and scattered red papules on the trunk and extremities (Fig. 1b). Lympho-

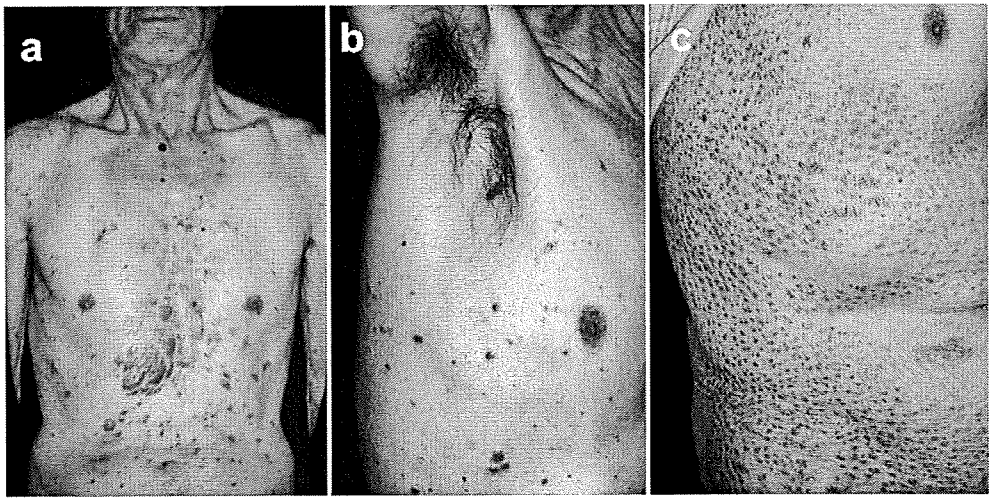


Fig. 1. Clinical appearance. (a and b) Initial well-defined, elevated nodules or tumours on the patient's trunk and extremities. (c) Evolved disseminated papular lesions forming deck-chair sign.

adenopathy was absent in both the axillae and groins. Peripheral blood examination showed normal counts of leukocytes (8.1×10^9/l) with 57.0% neutrophils, 1.0% eosinophils and 24.0% lymphocytes, but 13.0% of lymphocytes exhibited a medium-sized and irregular-shaped nucleus. Notably, serum immunoglobulin E level was high (49,000 U/ml; normal 170 < U/ml). The other blood chemistry values were normal, and circulating antibody against human T-cell lymphotropic virus type I was negative. Visceral involvement was absent as evaluated by roentgenographic examinations.

A skin biopsy specimen taken from a nodule on the chest revealed a dense infiltrate of atypical lymphocytes in the dermis (Fig. 2a). These tumour cells did not show epidermotropism. In some areas, there were lymphoid follicles (Fig. 2b) and Langhans-type giant cells surrounded by the tumour cells (Fig. 2c). The atypical lymphocytes had medium-sized convoluted nuclei (Fig. 2d). Immunohistochemical study showed that the tumour cells were positive for CD4, CD45RO, but negative for CD3. Large lymphocytes in the lymphoid follicles were $CD20^+$ B cells. While histiocytes infiltrating in the upper dermis were positive for CD68, Langhans type giant cells were negative for this marker.

A flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) showed 69.6% $CD4^+$ cells and 13.9% $CD8^+$ cells. The gated circulating $CD4^+$ T cells were positive for CD45RO (84.3%) and CCR4 (94.9%), but negative for CD3, TCRαβ, TCRγδ, CD7, CD45RA, CD56, CXCR3, and cutaneous lymphocyte-associated antigen (CLA) (Fig. 3). A standard Southern blot analysis of DNA extracted from PBMCs exhibited monoclonal rearrangement bands of TCRCβ1. Thus, the tumour cells possessed the CD3/TCR-defective helper T-cell phenotype.

The patient's condition was thus diagnosed as leukaemic CTCL. He was treated with oral administration of prednisolone, 10 mg daily, and etoposide, 25 mg daily for 7 days at a 4-week interval. Since 2004, his general and leukaemic conditions had been well controlled by the therapy, with flattened nodulo-tumours, except for transient pneumonia that was successfully treated with antibiotics. During this indolent clinical course, however, the skin eruption was gradually changed to multiple solid papules on the trunk and proximal limbs. In 2009, the papular lesions were disseminated but spared axillae, inguinal regions, antecubital and popliteal fossae, and in particular, abdominal folds, forming a deck-chair sign (Fig. 1c). We diagnosed the eruption as papuloerythroderma. His leukaemic state remained unchanged and the papuloerythrodermatous eruption persisted.

To examine the function of tumour cells, we isolated both $CD3^+CD4^+$ normal T cells and $CD3^-CD4^+$ tumour cells from the patient's PBMCs using BDTM IMag Cell Separation System with the anti-human CD3 and CD4 Particles-DM (BD Biosciences PharMingen, San Diego, CA, USA) according to the manufacturer's directions. The $CD3^-CD4^+$ tumour cells were isolated by magnetic immunoselection by sorting $CD4^+$ cells followed by depleting $CD3^+$ cells. The $CD3^+CD4^+$ normal T cells were obtained by sorting $CD4^+$ cells from the $CD3^+$ cells. The purity of each fraction was more than 90% (Fig. 4a). To confirm the monoclonal expansion of $CD3^-CD4^+$ tumour cells, we performed a

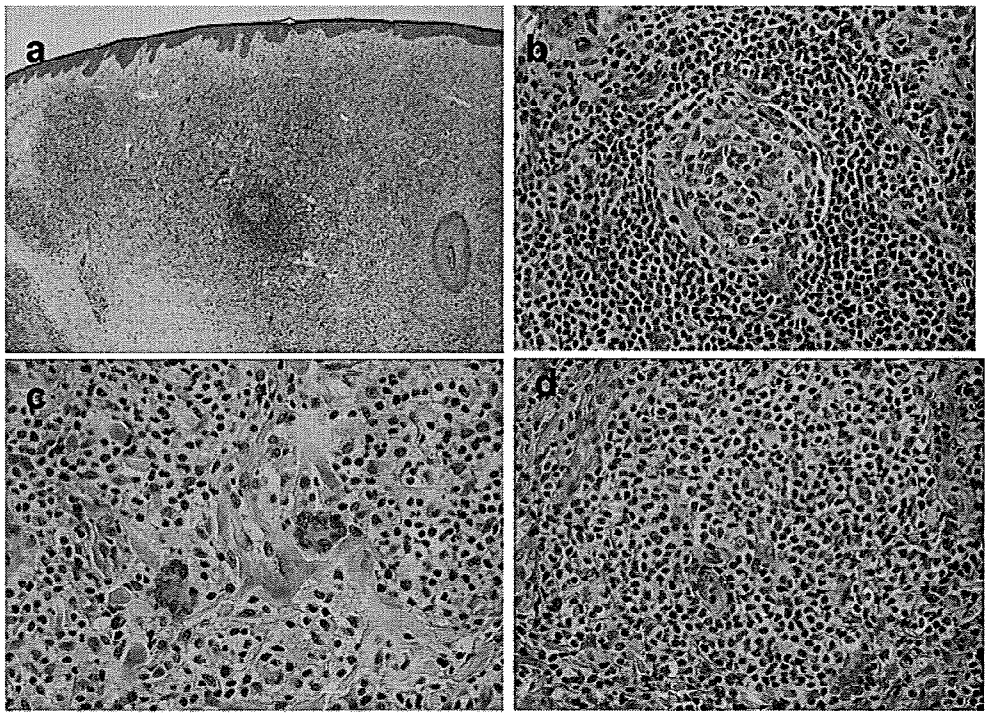


Fig. 2. Histological appearance. (a) Dense infiltrate of atypical lymphocytes in the dermis without epidermotropism. (b and c) Lymphoid follicles and Langhans-type giant cells surrounded by the tumour cells. (d) Atypical lymphocytes with medium-sized convoluted nuclei.

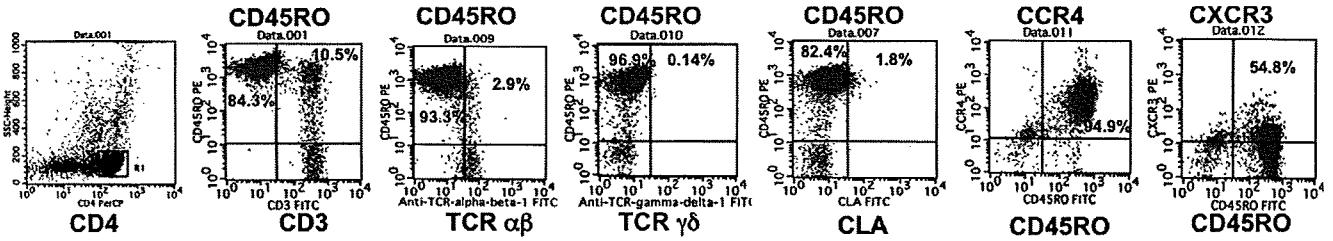


Fig. 3. Flow cytometric analysis of peripheral blood mononuclear cells. Circulating $CD4^+$ T cells were positive for CD45RO (84.3%) and CCR4 (94.9%), but negative for CD3, TCRαβ, TCRγδ, CD7, CD45RA, CD56, CXCR3, and CLA.

polymerase chain reaction (PCR) analysis of the TCR β gene. A monoclonal band was detected in $CD3^-CD4^+$ cells, but not in $CD3^+CD4^+$ cells (Fig. 4b). Next, we analysed the cytokine production pattern of these two fractions. Each fraction (1×10^6/ml in 24-well plates) was cultured along with the unfractionated samples under stimulation with concanavalin A (Con A, 2 μg/ml) for 72 h. The culture supernatants were measured for IFN-γ and IL-4 by using enzyme-linked immunosorbent assay kits (Genzyme/Techne, Minneapolis, MN, USA) according to the manufacturer's directions. As control, healthy subject's PBMCs were used. Upon stimulation with Con A, the patient's PBMCs and healthy donor's PBMCs produced high amounts of IFN-γ. It was noted that the patient's $CD3^-CD4^+$ tumour cells secreted a low level of IFN-γ compared with $CD3^+CD4^+$ normal T cells even after Con A stimulation (Fig. 4c). On the other hand, none of them secreted detectable levels of IL-4 (data not shown). These findings suggested that the tumour cells were unresponsive to CD3/TCR-mediated stimuli presumably because of the lack of expression of CD3/TCR complex.

DISCUSSION

The case of leukaemic CTCL described here is characterized by the unique skin manifestation and the aberrant tumour cell phenotype. The patient initially presented with nodules/tumours and a crop of solid papules on the trunk. The papular lesions gradually increased in number and spread over the trunk and other sites, but spared the abdominal creases, forming papuloerythroderma. It is known that papuloerythroderma represents a sign of internal malignancies (4, 5), or a manifestation of drug allergy (6) or CTCL (7–9). Our case documented that nodulo-tumourous lesions of CTCL may be changed into papuloerythroderma during the indolent long-term clinical course.

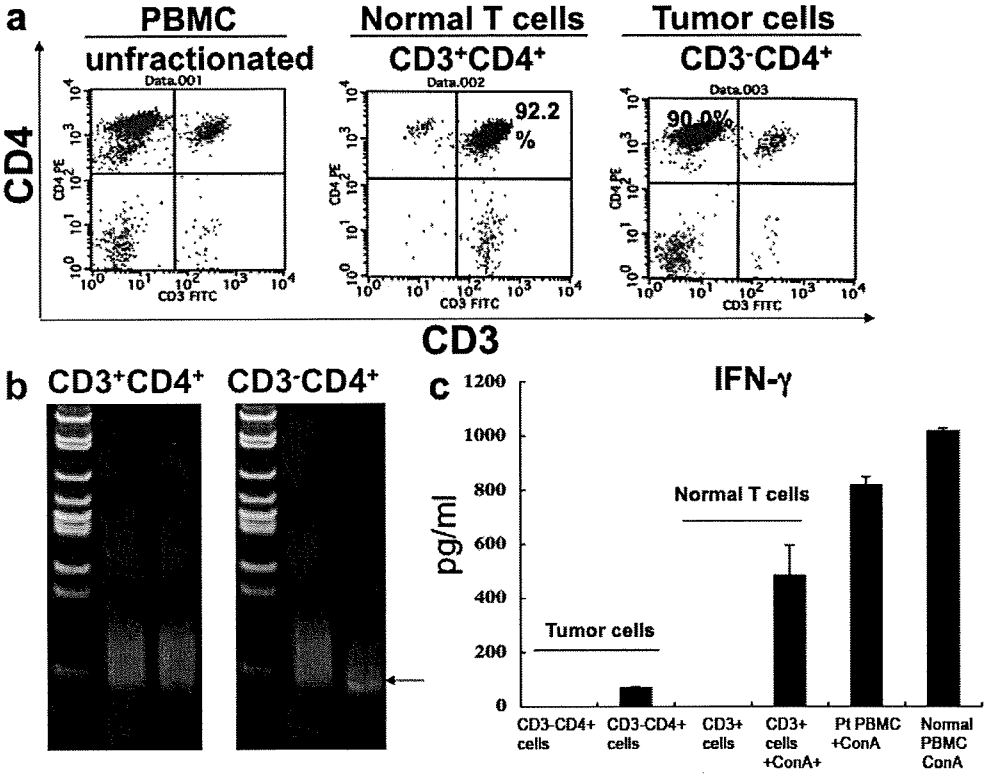


Fig. 4. Biological features of malignant T cells. (a) Flow cytometric analysis, showing successful separation of $CD3^+CD4^+$ normal T cells and $CD3^-CD4^+$ tumour cells from the patient's peripheral blood mononuclear cells (PBMCs). (b) PCR analysis of the TCR β gene. A monoclonal band was detected in $CD3^-CD4^+$ cells, but not in $CD3^+CD4^+$ cells. (c) Cytokine production pattern of tumour cells. $CD3^-CD4^+$ tumour cells, $CD3^+CD4^+$ normal T cells, patient's (Pt) PBMCs, and normal control's PBMCs (1×10^6 /well) were cultured with or without Con A (2 μg/ml) for 72 h. The patient's $CD3^-CD4^+$ tumour cells secreted a low level of IFN-γ compared with $CD3^+CD4^+$ normal T cells even after Con A stimulation.

Papuloerythroderma histologically shows skin infiltration of eosinophils, as historically reported by Ofuji et al. (10). In drug-induced papuloerythroderma, patients have high percentages of circulating Th2 cells reacting with the causative drug as well as tissue and blood eosinophilia (6, 11). Thus it has been suggested that Th2 cells are involved in the formation of papuloerythrodermatous lesions. Since neoplastic T cells of leukaemic CTCL or Sézary syndrome usually have the cytokine expression pattern of Th2 cells (12, 13), the development of papuloerythroderma in CTCL patients may be reasonable. In the present case, the neoplastic cells expressed Th2 chemokine receptor CCR4 (14, 15) and secreted a very low level of IFN-γ even upon stimulation with Con A, compared with normal T cells. Although we could not detect IL-4 secreted from the tumour cells, their inability to produce IFN-γ may result in a Th2-preponderant condition and allows papuloerythroderma to develop. This is consistent with the finding that a significant decrease in the number of IFN-γ-producing T cells occurs with disease progression from mycosis fungoides to Sézary syndrome (16).

Cases of CTCL with CD3/TCR-lacking phenotype have rarely been reported, and the skin eruptions in those patients are varied from poikiloderma (17), nodulotumours (2), to papuloerythroderma as shown here. This aberrant expression might affect the behaviour of tumour cells. Surface expression of a fully assembled TCR/CD3 complex is required for the responses to normal mitogen and superantigen (18). Given that CTCL cells are chronically activated by some antigens in the skin milieu, the TCR/CD3-negative malignant cells could not be driven to proliferate in response to antigens indigenous to the epidermis, resulting in the non-epidermotropic, atypical erythrodermic clinical manifestation. A patient with Sézary syndrome, expressing superantigenic stimulus-transducible TCR, despite lack of CD3 expression, exhibits erythroderma (19). Taken together, the expression of functional TCR may be associated with the formation of erythroderma. In addition, the circulating tumour cells in the present case and our reported case (2) did not express CLA, whereas approximately 60% of Sézary cells bear this skin-homing molecule (20). This lack of CLA expression also may provide an explanation for development of atypical erythroderma in our patient.

In association with the atypical eruption, the histological findings are unique in this case. In addition to the absence of epidermotropism, the presence of lymphoid follicles and granulomatous change are the characteristic features, which indicate the nodulo-tumourous eruption are reactive as well as neoplastic. In fact, the patient's clinical course was indolent and not aggressive, despite having tumours. The association of these histological features with the CD3/TCR-lacking phenotype is speculative, but it is possible that the inactive state of the malignant cells might induce the various chronic anti-tumour responses of inflammatory cells and result in pseudolymphomatous and granulomatous changes.

REFERENCES

1. Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, et al. Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the Internationa Society for Cutaneous lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood 2007; 110: 1713–1722.
2. Tokura Y, Yagi H, Seo N, Takagi T, Takigawa M. Nonerythrodermic leukemic variant of cutaneous T-cell lymphoma with indolent cinical course: Th2-type tumor cells lacking T-cell receptor/CD3 expression and coinfiltrating tumoricidal CD8+ T cells. J Am Acad Dermatol 2000; 43: 946–954.
3. Sokolowska-Wojdylo M, Wenzel J, Gaffal E, Lenz J, Speuser P, Erdmann S, et al. Circulating clonal CLA+ and CD4+ T cells in Sézary syndrome express the skin-homing chemokine receptors CCR4 and CCR10 as well as the lymph node-homing chemokine receptor CCR7. Br J Dermatol 2005; 152: 258–264.
4. Nazzari G, Sabatini C. Ofuji's papuloerythroderma: an association with early gastric cancer. Eur J Dermatol 1999; 9: 317–318.
5. Schepers C, Malvehy J, Azon-Masoliver A, Navarra E, Ferrando J, Mascaro JM. Papuloerythroderma of Ofuji: a report of 2 cases including the first European case associated with visceral carcinoma. Dermatology 1996; 193: 131–135.
6. Sugita K, Koga C, Yoshiki R, Izu K, Tokura Y. Papuloerythroderma caused by aspirin. Arch Dermatol 2006; 142: 792–793.
7. Shimauchi T, Ohshima A, Tokura Y. Folliculotropic mycosis fungoides presenting as papuloerythroderma. J Dermatol 2006; 33: 498–500.
8. Nashan D, Faulhaber D, Ständer S, Luger TA, Stadler R. Mycosis fungoides: a dermatological masquerader. Br J Dermatol 2007; 156: 1–10.
9. Martínez-Barranca ML, Muñoz-Pérez MA, García-Morales I, Fernández-Crehuet JL, Segura J, Camacho F. Ofuji papuloerythroderma evolving to cutaneous T-cell lymphoma. J Eur Acad Dermatol Venereol 2005; 19: 104–116.
10. Ofuji S, Furukawa F, Miyachi Y, Ohno S. Papuloerythroderma. Dermatologica 1984; 169: 125–130.
11. Sugita K, Kabashima K, Nakashima D, Tokura Y. Papuloerythroderma of Ofuji induced by furosemid. J Am Acad Dermatol 2008; 58: 54–55.
12. Vowels BR, Lessin SR, Cassin M, Jaworsky C, Benoit B, Wolfe JT, et al. Th2 cytokine mRNA expression in skin in cutaneous T-cell lymphoma. J Invest Dermatol 1994; 103: 669–673.
13. Seo N, Tokura Y, Matsumoto K, Furukawa F, Takigawa M. Tumor-specific cytotoxic T lymphocyte activity in Th2-type Sézary syndrome: its enhancement by interferon-g (IFN-γ) and IL-12 and fluctuations in association with disease activity. Clin Exp Immunol 1998; 112: 403–409.
14. Imai T, Baba M, Nishimura M, Kakizaki M, Takagi S, Yoshie O. The T cell-directed CC chemokine TARC is a highly specific biological ligand for CC chemokine receptor 4. J Biol Chem 1997; 272: 15036–15047.
15. Imai T, Chantry D, Raport CJ, Woods CL, Nishimura M, Godiska R, et al. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J Biol Chem 1998; 273: 1764–1768.

16. Lee BN, Duvic M, Tang CK, Bueso-Ramos C, Estrov Z, Reuben JM. Dysregulated synthesis of intracellular type 1 and type 2 cytokines by T cells of patients with cutaneous T-cell lymphoma. Clin Diagn Lab Immunol 1999; 6: 79–84.
17. Fischer MB, Hauber I, Fodinger M, Wolf HM, Thon V, Donath P, et al. Defective TCR surface expression associated with impaired TCR beta-chain assembly in a patient with cutaneous T-cell lymphoma. J Invest Dermatol 1995; 104: 537–550.
18. Tokura Y, Heald PW, Yan SL, Edelson RL. Stimulation of cutaneous T-cell lymphoma cells with superantigenic staphylococcal toxins. J Invest Dermatol 1992; 98: 33–37.
19. Sano S, Matsui Y, Itami S, Yoshikawa K. Immunological study on CD3 defective cutaneous T cell lymphoma cells from a patient with Sézary syndrome. Clin Exp Immunol 1998; 113: 190–197.
20. Heald PW, Yan SL, Edelson RL, Tigelaar R, Picker FJ. Skin-selective lymphocyte homing mechanisms in the pathogenesis of leukemic cutaneous T-cell lymphoma. J Invest Dermatol 1993; 101: 222–226.

Correspondence

CD30-positive primary cutaneous anaplastic large-cell lymphoma and definite squamous cell carcinoma

doi: 10.1111/j.1365-2230.2009.03232.x

CD30-positive primary cutaneous anaplastic large cell lymphoma (PCALCL) is one of several primary cutaneous CD30-positive lymphoproliferative disorders. The overlying epidermis often shows epidermal hyperplasia with ulceration, and pseudocarcinomatous hyperplasia has been reported in a small number of CD30-positive PCALCL cases.[1–3] However, separate and distinct squamous cell carcinoma (SCC) has rarely been seen in CD30-positive PCALCL, and only one SCC (keratoacanthoma type) was reported by Cespedes *et al.*[2] We report a rare case of CD30-positive PCALCL with SCC that infiltrated the deep dermis.

A 20-year-old woman presented with a 3-month history of an enlarging hyperkeratotic tumour (50 mm in diameter) on the left thigh.

Histological examination of a biopsy from the tumour showed infiltrative proliferation of atypical keratinocytes (Figs 1 and 2a) as well as a diffuse and dense background infiltrate of large atypical lymphoid cells mixed with many eosinophils in the dermis (Fig. 2c). The atypical keratinocytes were well differentiated (Fig. 2b). Large atypical lymphoid cells strongly expressed CD30 (Fig. 2d) and CD3, but were negative for ALK-1. There was no evidence of extracutaneous involvement of the tumour based on the findings from chest and abdominal computed tomography and 67-Ga scintigraphy.

The diagnosis of PCALCL with well-differentiated SCC was made. The lesion was completely excised and has not recurred in > 11 years.

Some reports have described epidermal hyperplasia together with CD30-positive PCALCL. The pathogenesis of prominent epidermal hyperplasia in association with CD30-positive PCALCL has been attributed to a variety of mediators including epidermal growth factor (EGF), transforming growth factor (TGF)-α and epidermal growth factor receptor (EGFR).[1] Courville *et al.* reported stronger expression of EGF and TGF-α in T cells and stronger epidermal expression of EGFR in cutaneous T-cell lymphoma (CTCL) with pseudocarcinomatous hyperplasia than in control cases of CTCL without pseudocarcinomatous hyperplasia.[1] EGF causes epidermal proliferation and TGF-α is an important factor in wound healing and carcinogenesis. EGFR is the receptor for both EGF and TGF-α, and amplification or overexpression of EGFR has been seen in SCC of the skin.[4] It is currently thought that EGFR is important in squamous cell carcinogenesis, and anti-EGFR antibody (cetuximab) has been used to treat cutaneous SCC.[5] From the common association of CD30-positive PCALCL with epidermal hyperplasia and less commonly, with SCC, we speculate that these mediators may play a role in epidermal proliferation and tumorigenesis.

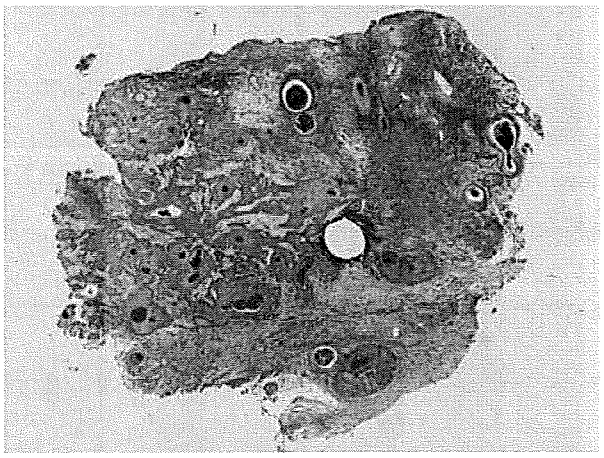


Figure 1 A skin biopsy taken from the hyperkeratotic plaque on the left thigh showed deep infiltrative proliferation of atypical keratinocytes in the reticular dermis near the subcutaneous tissue (haematoxylin and eosin, original magnification × 10).

Treatment for SCC or epidermal hyperplasia overlying CD30-positive PCALC should be carefully planned. Scarisbrick *et al.*[3] reported that epidermal hyperplasia in CD30-positive PCALCL may be mistakenly diagnosed as SCC, thereby leading to inappropriate overtreatment. We agree with this assessment and that epidermal hyperplasia with CD30-positive PCALCL need not to be treated with wide local excision, but definite SCC such as in our case does need wide local excision.

In conclusion, our case of CD30-positive PCALCL coexisting with SCC is very rare. It suggests that CD30-positive PCALCL may induce not only epidermal hyperplasia but also SCC in specific cases.

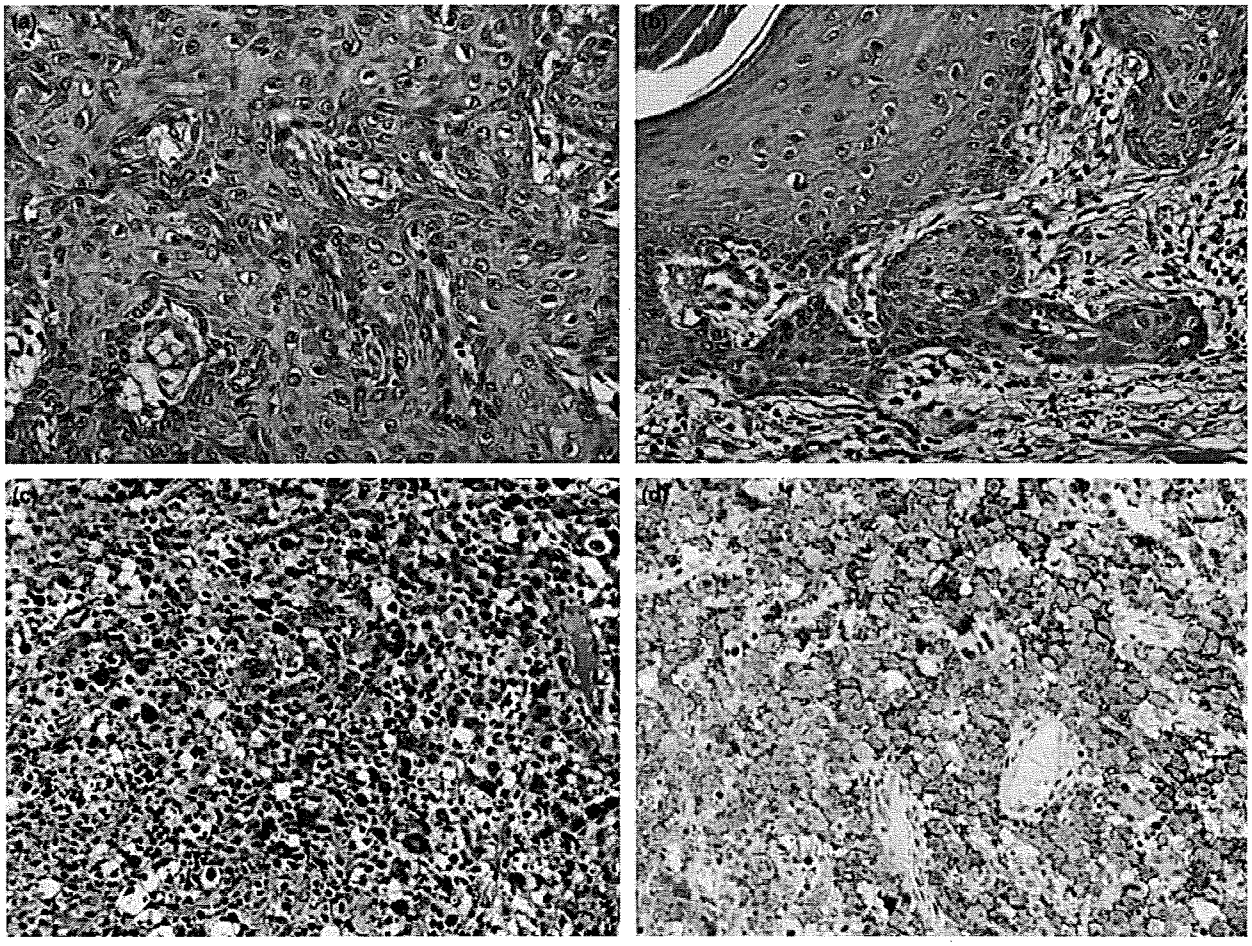


Figure 2 (a) Loss of the normal orderly stratified arrangement of the epidermis; (b) central keratinization and horn pearl formation, which suggested well-differentiated squamous cell carcinoma; (c) the typical keratinocyte infiltrates were associated with both diffuse and dense infiltration of large atypical lymphoid cells mixed with many dermal eosinophils. Haematoxylin and eosin, original magnification (a–c) × 100. (d) Large atypical lymphoid cells strongly expressed CD30 (original magnification × 100).

T. Yanagi, T. Shimizu,* K. Kodama, I. Nemoto-Hasebe, M. Kasai and H. Shimizu
*Department of Dermatology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo 060-8638, Japan, and *Department of Dermatology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani, Toyama, Japan*
E-mail: yanagi@med.hokudai.ac.jp
Accepted for publication 19 September 2008
Conflict of interest: none declared.

References

1 Courville P, Wechsler J, Thomine E *et al.* Pseudoepitheliomatous hyperplasia in cutaneous T-cell lymphoma. A clinical, histopathological and immunohistochemical study with particular interest in epithelial growth factor expression. The French Study Group on Cutaneous Lymphoma. *Br J Dermatol* 1999; **140**: 421–6.
2 Cespedes YP, Rockley PF, Flores F *et al.* Is there a special relationship between CD30-positive lymphoproliferative disorders and epidermal proliferation? *J Cutan Pathol* 2000; **27**: 271–5.
3 Scarisbrick JJ, Calonje E, Orchard G *et al.* Pseudocarcinomatous change in lymphomatoid papulosis and primary cutaneous CD30+ lymphoma: a clinicopathologic and immunohistochemical study of 6 patients. *J Am Acad Dermatol* 2001; **44**: 239–47.
4 Shimizu T, Izumi H, Oga A *et al.* Epidermal growth factor receptor overexpression and genetic aberrations in metastatic squamous-cell carcinoma of the skin. *Dermatology* 2001; **202**: 203–6.
5 Bauman JE, Eaton KD, Martins RG. Treatment of recurrent squamous cell carcinoma of the skin with cetuximab. *Arch Dermatol* 2007; **143**: 889–92.

6. Mohanty SK, Arora R, Kakkar N, Kumar B. Clear cell papulosis of the skin. *Ann Diagn Pathol.* 2002;6(6):385-388.
7. Kumarasinghe SP, Chin GY, Kumarasinghe MP. Clear cell papulosis of the skin: a case report from singapore. *Arch Pathol Lab Med.* 2004;128(11): e149-e152.
8. Chong WS, Ong BH, Kumarasinghe SP. Hypopigmented papules in an Asian boy. *Pediatr Dermatol.* 2005;22(3):268-269.
9. Benouni S, Kos L, Ruggeri SY, North PE, Drolet BA. Clear cell papulosis in Hispanic siblings. *Arch Dermatol.* 2007;143(3):358-360.
10. Toker C. Clear cells of the nipple epidermis. *Cancer.* 1970;25(3):601-610.
11. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation to relapse and survival with amplification of the HER2/neu oncogene. *Science.* 1987;235(4785):177-182.
12. Di Tommaso L, Franchi G, Destro A, et al. Toker cells of the breast: morphological and immunohistochemical characterization of 40 cases. *Hum Pathol.* 2008;39(9):1295-1300.

Bloody Nipple Discharge in an Infant

Bloody nipple discharge (BND) is occasionally observed in women with mammary disorders such as mastitis, intraductal papilloma, or breast carcinoma. However, this phenomenon is rarely seen in infants and children; BND in infants has seldom been reported in the dermatologic literature.

Report of a Case. A 4-month-old girl was referred to our clinic with a 1-week history of unilateral BND. Her mother reported a spontaneous and intermittent BND from the infant's left breast and denied breast manipulation or trauma. The infant was healthy except for BND and had no history of taking medication. The mother had no history of drug ingestion during either pregnancy or breastfeeding.

Physical examination of the chest and nipples showed no remarkable findings such as erythema, heat, tenderness, palpable mass, or enlargement of tissue. Pressure on the areolar area resulted in a bloody discharge from the left nipple (**Figure**, A). Ultrasonography of the left breast demonstrated dilatation of the retroareolar mammary ducts (Figure, B). The results of a blood cell count and coagulation tests were within the normal range. Culture of the bloody discharge revealed no bacterial growth. Cytologic examination of the secretion showed abundant erythrocytes but no other atypical cells. Based on these findings, bacterial infection and breast carcinoma were ruled out as a cause of the BND, and we decided to observe her without any treatment.

Comment. Bloody nipple discharge in infants, first described by Berkowitz and Inkelis,[1] occurs unilaterally or bilaterally in both sexes. Most patients older than 1 year show a palpable mass or breast enlargement, whereas infantile patients sometimes present with a normal appearance.[2] In laboratory examinations, coagulation test results and serum hormone levels are usually found to be normal, and culture of the discharge is usually negative.[2]

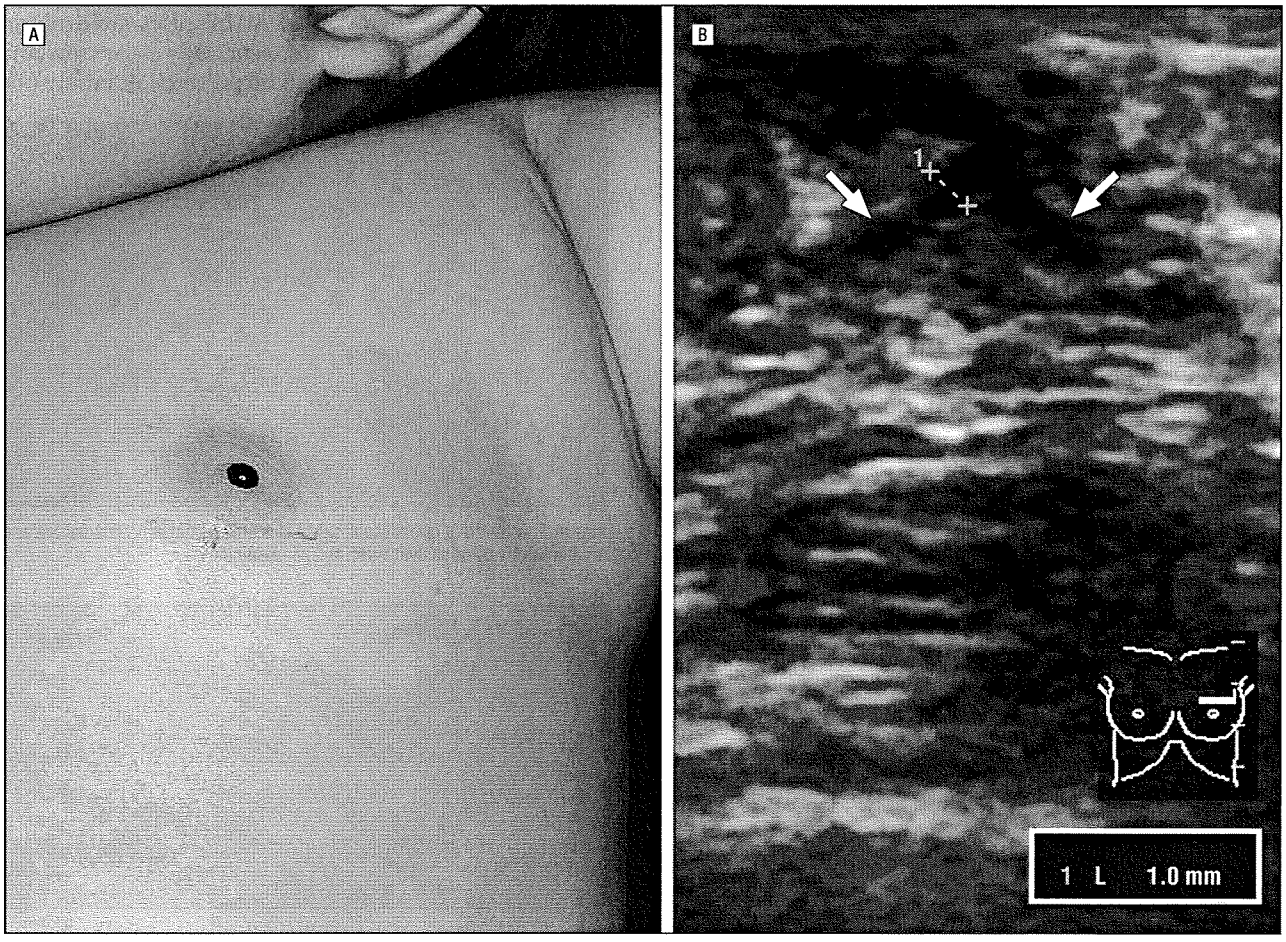


Figure. Bloody nipple discharge. A, Clinical appearance of bloody nipple discharge from the left breast. B, Ultrasonographic image of the left breast showing dilatation of the subareolar mammary ducts (arrows).

Histopathologically, mammary duct ectasia, a benign lesion characterized by dilated ducts surrounded by fibrosis and inflammation, has been proven in more than half of childhood BND cases with palpable masses treated surgically.[2] Mammary duct ectasia has also been detected by ultrasonography even in infantile cases with nonpalpable masses.[3,4] Underlying breast carcinoma should definitely be ruled out when we see patients with BND, but to our knowledge, it has never been reported in infants.[4,5] From these data, mammary duct ectasia is the most likely cause of BND in infants and children, although the specific cause of duct ectasia remains unclear.

To our knowledge, all but 1 of the reported BND cases in infants has achieved spontaneous resolution within 9 months.[3-5] The 1 case that did not resolve spontaneously was treated surgically. These facts suggest that BND in infants is a benign and self-limiting condition. Therefore, invasive intervention, including biopsy, should be avoided, especially in girls, because even minimal operative injury to the breast bud may produce severe tissue damage resulting in functional disability and persistent disfigurement.[4,5] Noninvasive investigations such as culture of the discharge and ultrasonographic evaluation are recommended as well as a careful physical examination and close clinical follow-up. Only if ultrasonography reveals a mass or abnormality other than mammary duct ectasia, or if the discharge persists for more than 9 months, should further investigations, including invasive interventions, be considered.[5]

Hideyuki Ujiie, MD
Masashi Akiyama, MD, PhD
Rinko Osawa, MD
Satoru Shida, MD, PhD
Satoru Aoyagi, MD, PhD
Hiroshi Shimizu, MD, PhD

Correspondence: Dr Ujiie, Department of Dermatology, Hokkaido University Graduate School of Medicine, North 15 W 7, Kita-ku, Sapporo 060-8638, Japan (h-ujiie@med.hokudai.ac.jp).
Financial Disclosure: None reported.

1. Berkowitz CD, Inkelis SH. Bloody nipple discharge in infancy. *J Pediatr*. 1983; 103(5):755-756.
2. Imamoglu M, Cay A, Reis A, Ozdemir O, Sapan L, Sarihan H. Bloody nipple discharge in children: possible etiologies and selection of appropriate therapy. *Pediatr Surg Int*. 2006;22(2):158-163.
3. Weimann E. Clinical management of nipple discharge in neonates and children. *J Paediatr Child Health*. 2003;39(2):155-156.
4. De Praeter C, De Coen K, Vanneste K, Vanhaesebrouck P. Unilateral bloody nipple discharge in a two-month-old male. *Eur J Pediatr*. 2008;167(4):457-459.
5. Kelly VM, Arif K, Ralston S, Greger N, Scott S. Bloody nipple discharge in an infant and a proposed diagnostic approach. *Pediatrics*. 2006;117(4):e814-e816.

Acute Generalized Exanthematous Pustulosis Caused by Rifabutin

Acute generalized exanthematous pustulosis (AGEP), first named by Beylot et al[1] in 1980, is a clinical reaction pattern that is principally drug induced.[2,3] Its incidence is probably underestimated because many cases are either unrecognized or confused with pustular psoriasis.[3] We report herein a case of AGEP caused by rifabutin, an antituberculous agent.

Report of a Case. A 58-year-old man with hypertension, coronary artery disease, and schizophrenia was admitted to our hospital for cervical nontuberculous mycobacterial lymphadenitis. He had a history of drug allergy to trimethoprim-sulfamethoxazole presenting as a generalized nonpustular exanthematous eruption. After 10 days of treatment with rifabutin, he developed a fever with temperatures up to 38°C accompanied by numerous nonfollicular sterile pustules on widespread edematous erythema over the trunk and all extremities without mucous membrane involvement (**Figure 1**).

Laboratory examinations revealed leukocytosis with left shift and mild eosinophilia. (The white blood cell count was 11200/μL; neutrophil-bands, 12%; eosinophil-bands, 7%; to convert white blood cells to 10^9/L, multiply by 0.001.) Histopathologic evaluation showed spongiform subcorneal pustules with a predominance of neutrophils and eosinophils and papillary dermal edema with perivascular inflammatory cell infiltrate (**Figure 2**).

Rifabutin treatment was discontinued, and intravenous hydrocortisone, 100 mg, was administered every 6 hours. The pustules resolved rapidly with generalized desquamation and have not recurred. Acute generalized exanthematous pustulosis was confirmed by validation score of the EuroSCAR study group.[2]

Comment. More than 90% of AGEP cases are drug induced, mainly by antibiotics, especially ß-lactams and macrolides.[2,3] Our patient had no personal or family his-

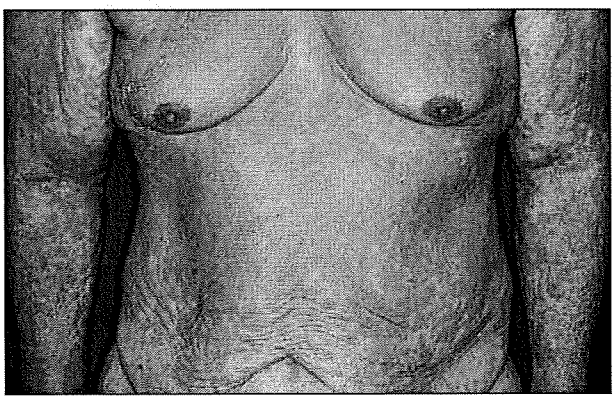


Figure 1. Numerous nonfollicular pinhead sterile pustules on edematous and erythematous plaques over trunk and all extremities.

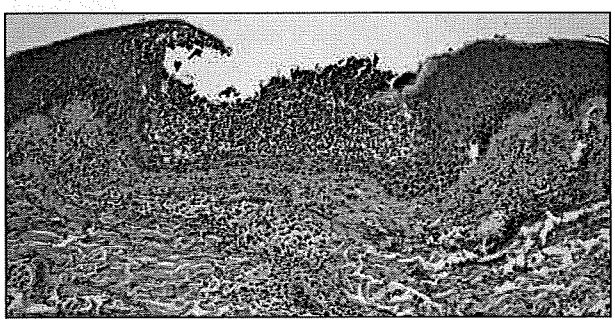


Figure 2. Spongiform subcorneal pustule with mixed neutrophils and eosinophils and a focal necrosis of keratinocytes in the epidermis (hematoxylin-eosin, original magnification ×100).