

**Blind title page:**

**Category of paper:** Case report

**Article title:** Langerhans cell hyperplasia in the tumor stage of mycosis fungoides: a mimic of Langerhans cell histiocytosis.

**Running title not exceeding 50 characters:** Langerhans cells in mycosis fungoides

**Abstract:**

Mycosis fungoides is a form of cutaneous T-cell lymphoma (CTCL). Malignant CD4<sup>+</sup> T cells have been found to adopt the T-regulatory (Treg) cell phenotype and function. We present the case of a 66-year-old man diagnosed with mycosis fungoides that was progressing from the plaque to the tumor stage. The histopathological examinations showed that the Langerhans cell (LC) infiltration in the skin lesion of the tumor stage was greater than that in the patch/plaque stage; the tumor stage lesions resembled Langerhans cell histiocytosis pathologically. The associations between LCs, apoptotic tumor cells, Treg CTCL cells, and relevant cytokines are complex. Treg CTCL cells produce the immunosuppressive cytokines IL-10 and TGF- $\beta$ , which facilitate continuous recruitment of LCs and maintenance of long-term dendritic cell immaturity, thereby explaining the remarkable LC infiltration in the tumor stage samples from our patient. This phenomenon indicates that LCs accompanied by Treg CTCL cells may play an important synergistic role in the tumor progression. The development of immunotherapy direct against Treg CTCL cells and LCs overproduction, and other immunosuppressive cytokines may be a potent useful adjuvant and worthy of further investigation.

**Key words:**

Cutaneous T-cell lymphoma

Langerhans cell histiocytosis

Mycosis fungoides

T-regulatory cell

Tumor stage

## **Introduction**

Mycosis fungoides (MF) is a form of cutaneous T-cell lymphoma (CTCL) that is probably elicited by various factors of exogenous or endogenous origin. CTCL has been hypothesized to be a disease caused by chronic antigen stimulation. The unlimited lymphoid cell growth in CTCL is stimulated by immature dendritic cells (DCs).<sup>1</sup> A recent report suggests that CTCL cells, which are a malignant form of CD4<sup>+</sup> T cells, adopt T-regulatory (Treg) cell phenotypes (CD25<sup>+</sup>, CTLA-4<sup>+</sup>, and FoxP3<sup>+</sup>) and functions after undergoing stimulation by DCs loaded with apoptotic T cells.<sup>2</sup>

## Case report

A 66-year-old man presented with multiple dusky-red to violaceous, scaly patches/plaques and nodules of varying sizes on the trunk, scalp, and limbs. These lesions initially manifested 2 years back as annular erythematous or violet-red patches that were treated as dermatitis. However, the skin lesions persisted, disseminated, and enlarged with red-brown to purplish coloration and irregular plaques and tumor formation. The tumor present on the back also showed focal ulceration and necrosis. Laboratory studies did not reveal leukocytosis with abnormal lymphocytes. Serum lactate dehydrogenase and calcium were not elevated. No osteolytic lesion was found in the image study. Both peripheral blood smear and bone marrow examination were negative for atypical lymphocyte infiltration. He had no hepatosplenomegaly from the physical examination and image study. Serological examination for human T-cell leukemia virus type-1 antibody was negative. There were no further visceral involvements. MF progressing from the plaque to the tumor stage was clinically suspected. The patient underwent skin biopsies for 2 separate skin lesion samples from the patch/plaque stage (Figure 1A) and tumor stage (Figure 1B).

Histopathologically, the patch/plaque stage lesion showed patchy and focally diffuse infiltrates of small to medium-sized, irregular and angulated atypical lymphoid cells involving the perivascular and periadnexal spaces and the interstitium of the dermis (Figure 2A). We also observed foci of epidermal permeation of atypical lymphoid cells and Pautrier's microabscess (Figure 2B). In the immunohistochemical analysis, the atypical lymphoid cells were positive for CD4 (Figure 2C) but negative for CD8 and CD20 (not shown). The presence of numerous number of CD1a-positive histiocytoid cells with reniform nuclei was identified mainly in the intraepidermal and little in the dermal perivascular regions (Figure 3C). A constellation of

clinical manifestations, and histopathological and immunohistochemical features suggests diagnostic of patch/plaque stage MF with variant intraepidermal and dermal perivascular Langerhans cells (LCs) infiltrates.

However, the tumor nodule showed dense infiltration of dermal aggregates of atypical lymphoid cells, numerous large histiocytoid cells with grooved, reniform nuclei closely interspersed among tumor cells, and scattered eosinophils (Figures 3A and 3B). The histiocytoid cells showing membranous staining for CD1a (Figure 3D) and cytoplasmic staining for S-100 protein densely distributed in the epidermis and entire dermis (not shown). This confirmed their identity as LCs. In the present case, a large number of CD68-positive cell infiltrates mainly in the dermis was also observed (not shown). Based on the overall features, it was diagnostic of tumor stage MF with numerous LC proliferations. He received regular medication treatment with topical and systemic steroids, methotrexate, and phototherapy. There were no further visceral involvements. Unfortunately, the patient died of pneumonia and sepsis seven months later.

## Discussion

Cutaneous DCs, including dermal and epidermal DCs, play an important role in the induction of immune responses against tumor cells. When the immune system encounters emerging tumor cells, regional tissue immature DCs ingest tumor cells or their tumor antigens. Then, the tumor-specific antigens are processed and displayed by the class I and class II major histocompatibility complex (MHC) molecules on the DCs. These fragmented peptides may be presented to cytotoxic T-cells. The process of induction of an anti-tumor T-cell response is called cross-presentation. Eventually, tumor-specific CD8<sup>+</sup> cytotoxic T lymphocytes gain the ability to recognize and kill tumor cells by inducing them to undergo apoptosis. At present, the activation of naïve CD8<sup>+</sup> T cells to differentiate into active cytotoxic T-lymphocytes is known to require recognition of antigens, costimulatory signals, and perhaps help from class II MHC-restricted CD4<sup>+</sup> T cells. In a previous conducted in vitro study, apoptotic MF cells were recognized as tumor-specific antigens by the immune system in the latter phase of tumor growth.<sup>3</sup>

It is of particular interest that tumors are frequently infiltrated with DCs and the presence of an increased number of DCs within the tumor has been postulated to indicate a better prognosis in certain cancers.<sup>4</sup> However, LCs, which constitute a subset of DCs and function as antigen presenting cells (APCs), are important regulators of T-cell immunity and most prevalent in the epidermis. LCs are characterized by positive staining for CD1a, S-100 protein, and, more specifically, langerin, which is a LC-specific C-type lectin and imparts a specific functional role to LCs in facilitating antigen presentation by CD1a.<sup>5</sup> In the other study, of meaningful finding is that the proportion of LCs intermingled with malignant lymphoid cells in the tumor stage infiltrate is significantly higher than that in the patch/plaque stage infiltrate.<sup>4</sup> In our case, the findings of immunohistochemical studies are consistent with those of previous reports; these

studies also showed that the number of LCs in the tumor stage MF lesions is significantly higher than the corresponding number in the patch/plaque stage samples. The presence of pronounced infiltrates of CD1a<sup>+</sup>/S-100<sup>+</sup> LCs among atypical lymphoid cells in the tumor stage MF lesions may lead to misinterpretation as LCH histologically. It brings us a big puzzle as well.

To the best of our knowledge, LCH is a different disease entity caused by the infiltration of LCs in one or more organs, among which cutaneous involvement is traditionally considered to portend a multisystemic involvement and has a poor prognosis.<sup>6</sup> Previous studies demonstrated that LCH is characterized by clonal proliferation. The LCs of LCH form a monoclonal population, indicating that LCH is neoplastic.<sup>7</sup> However, other studies state that the excess of LC in lymphoproliferative disorders or lymphomas is polyclonal.<sup>8,9</sup> This may support that the accumulation of LC in this circumstance is in favor of a reactive nature. Elsewhere, the absence of thrombocytopenia, hepatosplenomegaly, or pulmonary infiltrates clinically is definitely against the diagnosis of LCH.

The roles of diverse cutaneous DC populations in the induction of immunity and tolerance are also currently popular and under research. There is some indication that dermal DCs and LCs might have different roles in the immune responses.<sup>10</sup> The role of cutaneous LCs in inducing T-cell-mediated immune responses appears to be important for the induction of T-cell responses, whereas dermal DCs may elicit humoral responses.<sup>10</sup> Immature DCs should be restricted to inflamed epithelium at the site of antigen entry. When encountering with foreign antigens, DCs use their surface receptors to capture and endocytose foreign antigens. The activated DCs are on the way of migration through lymphatic circulation to regional lymph nodes. During the route of migration, DCs mature from cells capable of capturing antigens into APCs. After reaching the hilum of lymph node, APCs are quickly directed to T cells zones by chemoattracting cytokines



produced in the microenvironment of T-cell zones. While APCs encounter with naïve T cells, the former are efficient at presenting MHC class II-restricted foreign peptides to the latter, resulting in activation of a subset of effector T cells.<sup>11</sup> The existence of a novel Langerin<sup>+</sup> DC subset in the dermis has been identified that is independent of epidermal LCs by several study groups recently. Instead of LCs, Langerin<sup>+</sup> dermal DCs are suggested to be critical for the initiation of efficient T cell response in contact hypersensitivity study using animal model.<sup>12,13</sup> The finding seems contradictory to original recognition of LCs function. In brief summary, DCs will go through a maturation process, so their surface molecules may change into distinct profiles. Importantly, to recognize the cellular behavior of DCs may give us better insight into how this process can occur.

In recent years, a population of T cells with the CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> phenotype, i.e., Treg cells, has been shown to play a key role in the suppression of immune response.<sup>14</sup> Berger CL et al.<sup>2</sup> concluded that the malignant cells of CTCL adopt Treg cell phenotype and functions. Treg CTCL cells are an important population that plays a crucial role in the maintenance of tumor self-tolerance. From in vitro studies, loading of immature DCs with apoptotic CTCL cells and exposing them to CD4<sup>+</sup> CTCL cells would result in clonal expansion of Treg CTCL cells with upregulation of cytoplasmic cytotoxic T lymphocyte antigen-4 (CTLA-4), FoxP3, and membrane CD25. The inductions of all these molecules lead to the differentiation of Treg cells along with inhibition of normal T-cell poiesis.<sup>15</sup>

These Treg CTCL cells may engage T-cell inhibitory pathways mediated by secretion of IL-10 and TGF- $\beta$  and expression of CTLA-4.<sup>4,16</sup> IL-10 produced by Treg CTCL cells maintains long-term DC immaturity, thereby ensuring continued phagocytosis of apoptotic cells and further CTCL cell replication.<sup>11</sup> In other words, “immature DCs” are able to support CTCL cell replication. After maturation, DCs would eventually result in degranulation and death of DCs,

which is rapidly followed by death of CTCL cells.<sup>1</sup> Secretion of TGF- $\beta$  by Treg CTCL cells can also incite the differentiation of monocytes and CD34 precursors into immature DCs, thereby facilitating continual recruitment of LCs.<sup>17</sup> CTLA-4, a membrane molecule of Treg cells, also plays an important role in the downregulation of immunosurveillance.<sup>16</sup> The mechanism of immunopathology in CTCL is regulated by an intricate pathway that is closely associated with the interactions among LCs, apoptotic tumor cells, Treg CTCL cells, and relevant cytokine expression. This finding may imply that remarkable LC infiltration within tumor nodules, like that observed in our patient, may contribute to the growth of Treg CTCL cells and result in the progression of patch/plaque stage MF to tumor stage MF.

From a therapeutic point of view, it already shows that, in the in vivo study, depletion of these Treg cells by administration of anti-CD25 monoclonal antibody has indeed suppressed growth of a variety of different tumors.<sup>18,19</sup> The elimination of CD25<sup>+</sup> Treg cells facilitates long-term CD4<sup>+</sup> T cell-mediated tumor immunity and is a potent useful adjuvant in immunotherapy.<sup>18</sup> The development of certain receptor-specific antagonists may inhibit the overproduction of LCs and excessive immunosuppression mediated by IL-10, TGF- $\beta$  and other cytokines produced by Treg CTCL cells. Perhaps an immunotherapy strategy designed for interruption of the pathway of LCs and Treg CTCL cell overproduction possibly resulting in refractory MF, is worthy of further investigation and may contribute to the development of new therapeutic approaches.

In conclusion, this case indicates that LCs accompanied by Treg CTCL cells may play an important synergistic role in the tumor progression to a more advanced stage of MF. The histological finding in nodular lesion of tumor stage MF shows prominent LC proliferation that may cause a difficult distinction between reactive LC proliferation and LCH. The excess of LC in this microenvironment is presumed to be polyclonal and reactive in nature. As a general rule,

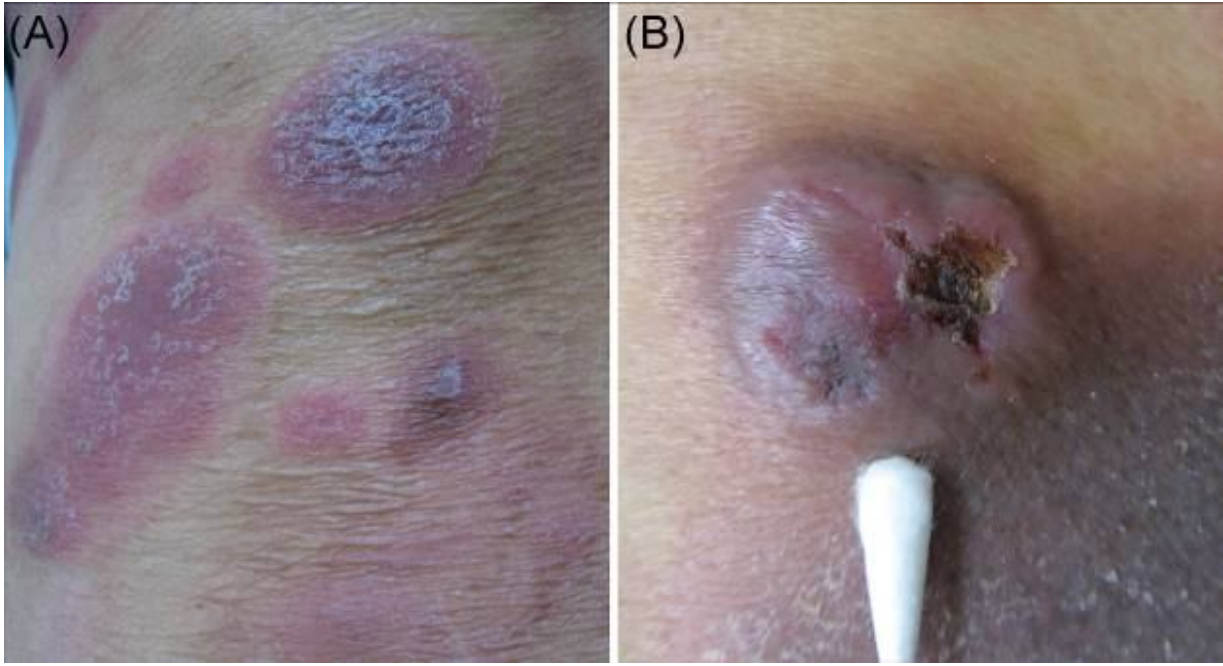
mature DCs may express a defensive immune response against tumor cells. On the contrary, the antitumor immunity may deviate to the tumor-progression state under the influence of LCs, apoptotic tumor cells, and Treg CTCL-cell differentiation.  $CD4^+CD25^+ Foxp3^+$  Treg CTCL cells may inhibit the generation of immune responses and hamper the development of effective tumor immunity. The excessive immunosuppression mediated by intricate interaction between LCs, apoptotic cells and Treg CTCL cells, and accumulation of genetic alteration of tumor cells presumably contributes to the tumor progression of CTCL.

## References

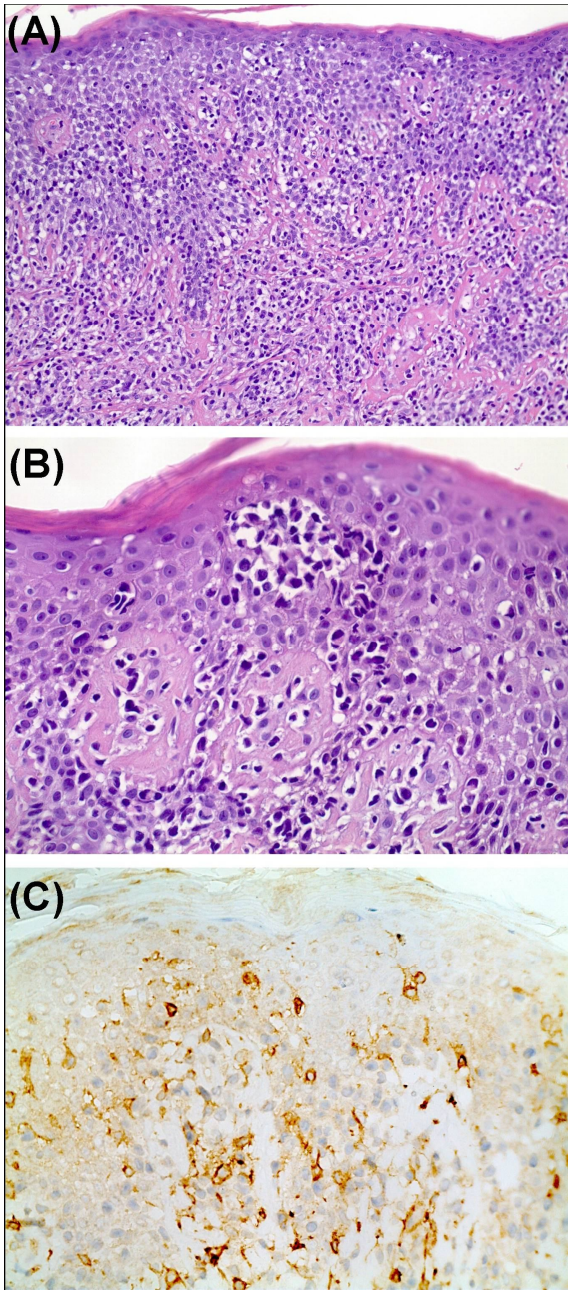
1. Berger CL, Hanlon D, Kanada D. The growth of cutaneous T-cell lymphoma is stimulated by immature dendritic cells. *Blood*. 2002; 99: 2929-39.
2. Berger CL, Tigelaar R, Cohen J, et al. Cutaneous T-cell lymphoma: malignant proliferation of T-regulatory cells. *Blood*. 2005; 105:1640-7.
3. Goteri G, Filosa A, Mannello B, et al. Density of neoplastic lymphoid infiltrate, CD8<sup>+</sup> T cells, and CD1a<sup>+</sup> dendritic cells in mycosis fungoides. *J Clin Pathol*. 2003; 56: 453-8.
4. Lüftl M, Feng A, Licha E, Schuler G. Dendritic cells and apoptosis in mycosis fungoides. *Br J Dermatol*. 2002; 147: 1171-9.
5. Hunger RE, Sieling PA, Ochoa MT, et al. Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells. *J Clin Invest*. 2004; 113: 701-8.
6. Yi-Chin Shih, Wan-Lung Lin, I-Hsin Shih. Langerhans Cell Histiocytosis in Taiwan: A Retrospective Case Series in a Medical Center. *Dermatol Sinica* 2009; 27: 93-102.
7. Gong L, Zhang WD, Li YH, et al. Clonal status and clinicopathological features of langerhans cell histiocytosis. *J Int Med Res*. 2010; 38: 1099-105.
8. Benharroch D, Guterman G, Levy I, Shaco-Levy R. High content of Langerhans cells in malignant lymphoma--incidence and significance. *Virchows Arch*. 2010; 457: 63-7.
9. Christie LJ, Evans AT, Bray SE, et al. Lesions resembling Langerhans cell histiocytosis in association with other lymphoproliferative disorders: a reactive or neoplastic phenomenon? *Hum Pathol*. 2006; 37: 32-9.
10. Stoitzner P. The Langerhans cell controversy: are they immunostimulatory or immunoregulatory cells of the skin immune system? *Immunol Cell Biol*. 2010; 88: 348-50.
11. Sen D, Forrest L, Kepler TB, et al. Selective and site-specific mobilization of dermal

- dendritic cells and Langerhans cells by Th1- and Th2-polarizing adjuvants. *Proc Natl Acad Sci U S A.* 2010; 107: 8334-9
12. Igyarto BZ, Kaplan DH. The evolving function of Langerhans cells in adaptive skin immunity. *Immunol Cell Biol.* 2010;88:361-5.
  13. Bursch LS, Wang L, Igyarto B, et al. Identification of a novel population of Langerin<sup>+</sup> dendritic cells. *J Exp Med.* 2007;204:3147-56.
  14. Levings MK, Bacchetta R, Schulz U, Roncarolo MG. The role of IL-10 and TGF-beta in the differentiation and effector function of T-regulatory cells. *Int Arch Allergy Immunol.* 2002; 129: 263-76.
  15. Sakaguchi S, Sakaguchi N, Shimizu J, et al. Immunologic tolerance maintained by CD25<sup>+</sup> CD4<sup>+</sup> regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev.* 2001; 182: 18-32.
  16. Loser K, Beissert S. Dendritic cells and T cells in the regulation of cutaneous immunity. *Adv Dermatol.* 2007; 23: 307-33.
  17. Jaksits S, Kriehuber E, Charbonnier AS, et al. CD34<sup>+</sup> cell-derived CD14<sup>+</sup> precursor cells develop into Langerhans cells in a TGF-beta 1-dependent manner. *J Immunol.* 1999; 163: 4869-77.
  18. Nagai H, Horikawa T, Hara I, et al. In vivo elimination of CD25<sup>+</sup> regulatory T cells leads to tumor rejection of B16F10 melanoma, when combined with interleukin-12 gene transfer. *Exp Dermatol.* 2004; 13: 613-20.
  19. Jones E, Dahm-Vicker M, Simon AK, et al. Depletion of CD25<sup>+</sup> regulatory cells results in suppression of melanoma growth and induction of autoreactivity in mice. *Cancer Immun.* 2002; 2: 1.

**Figure 1** Skin specimens from 2 separate lesions: patch/plaque stage (A) and tumor stage (B) of mycosis fungoides.



**Figure 2** Histopathological and immunohistochemical features. The typical features of the patch/plaque stage lesion are composed of diffuse infiltrates of atypical lymphoid cells in the dermis, foci of epidermatropism (A), and Pautrier’s microabscess (B). Immunohistochemical staining revealed CD4-positive cells (C). Original magnification: (A) hematoxylin and eosin (H&E), ×200; (B) H&E, ×400; (C) ×400.



**Figure 3** Histopathological and immunohistochemical features. The tumor stage lesion shows dense infiltration of histiocytoid cells with grooved, reniform nuclei interspersed among atypical lymphoid cells and scattered eosinophils (A,B), which mimics the features of Langerhans cell histiocytosis. (C) Numerous CD1a-positive cells in the patch/plaque stage lesion. (D) The histiocytoid cells are positive membranous staining for CD1a in the tumor stage lesion. Original magnification: (A) H&E,  $\times 200$ ; (B) H&E,  $\times 400$ ; (C)  $\times 100$ ; (D)  $\times 400$ .

