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Kimura's disease presenting with a giant suspensory tumor and associated with membranoproliferative glomerulonephritis

Kimura's disease is a rare chronic inflammatory disease, which typically occurs in middle-aged Asian men. This benign lymphoproliferative disorder with tissue eosinophilia is clinically characterized by painless subcutaneous swelling or induration, affecting the head and neck region. Here we present a 42-year-old Japanese woman with a giant tumor on the right inguinal region. The tumor was diagnosed as Kimura's disease, because of massive infiltration of lymphoid follicle-forming lymphocytes and eosinophils with elevated serum immunoglobulin E and blood eosinophilia. She also had nephrotic syndrome histopathologically diagnosed as membranoproliferative glomerulonephritis (MPGN). Renal involvement is known as one of the associated conditions with Kimura's disease. The skin and renal diseases were successfully treated with corticosteroids and surgical removal of the mass.

Key words: Kimura's disease, membranoproliferative glomerulonephritis, Th2 cell

Kimura's disease is a chronic, lymphoproliferative and inflammatory disease that develops in the dermis, soft tissues and lymph nodes. This intriguing condition was first described from China in 1937 [1], and named Kimura's disease after similar cases were reported by Kimura *et al.* in 1948 [2]. It is characterized clinically by subcutaneous masses or indurations occurring predominantly on the head and neck region [2] and histologically by the formation of lymphoid follicles and marked infiltration of eosinophils. The other predilection sites are the orbit, lacrimal gland, arm, and lymph nodes of the axilla and inguinal regions [3, 4]. The associated laboratory findings include blood eosinophilia and elevated serum immunoglobulin E (IgE) [3-5]. It has been shown that mRNA expression of interleukin (IL)-4, IL-5, and IL-13 is elevated in peripheral blood mononuclear cells in patients with Kimura's disease, suggesting that these Th2 cytokines play an essential role in the dysregulation of eosinophilia and serum IgE level [6].

Kimura's disease occasionally involves the kidney, usually manifesting as proteinuria and nephrotic syndrome [7-9]. Membranous glomerulonephritis and minimal change nephropathy, known as T helper 2 (Th2) type disease [10], may be the histological types associated with Kimura's disease. Here we report a Japanese woman with Kimura's disease, who presented with a large tumor on the inguinal region, and nephrotic syndrome showing the histological changes of membranoproliferative glomerulonephritis (MPGN).

Case report

A 42-year-old Japanese woman was referred to us because of an inguinal tumor that persisted for 10 years and enlarged recently. The patient had no history of atopic dermatitis or asthma, and her family history was unremarkable. Clinical examination revealed a large, pedunculated, nontender tumor, which measured 10.5 × 9.5 × 11.9 cm and was located on the right inguinal region *(figure 1A)*. The overlying skin color was brownish with mild erythema. Bilateral inguinal lymphadenopathy was noted.

We initially suspected inguinal herniation or ectopic endometriosis, which was excluded by gynecological and radiological examinations. A magnetic resonance imaging exhibited an iso-high intensitive mass by T1 intensity *(figure 2B, left)*, and high intensive irregular nodules existing in the mass by T2 intensity *(figure 2B, right)*. The tumor and enlarged lymph nodes were reinforced uniformly in contrast media. A computed tomography scan showed a protruded tumor in the right genitocrural region *(figure 2C, left)*. Multiple swollen lymph nodes were seen in both groin areas, and the lymphadenopathy was more conspicuous in the right area *(figure 2C, right)*.

A skin biopsy specimen from the mass revealed multiple lymphoid follicles with distinct germinal centers *(figiure 2A)* and a dense diffuse infiltrate of eosinophils and lymphocytes *(figure 2B)*. Vascular proliferation consisting of blood vessels lined by enlarged endothelial cells was also seen. There was at least no substantial number of mast

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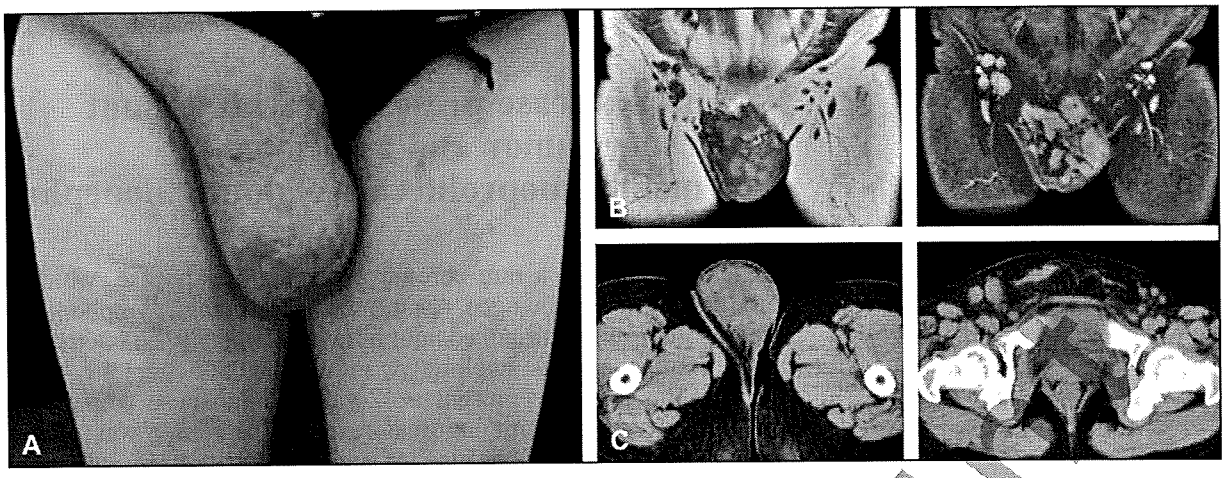

Figure 1. Clinical appearance, magnetic resonance image and computed tomography. **A)** A large suspensory tumor on the right inguinal region. **B)** There is an iso-high intensitive mass on the groin region (left, T1 intensity), and some irregular nodules are seen in the mass (right, T2 intensity). **C)** There is an inarticulate tumor in the right groin to genitocrural region (left), and swollen lymph nodes are seen in the both groin areas (right).

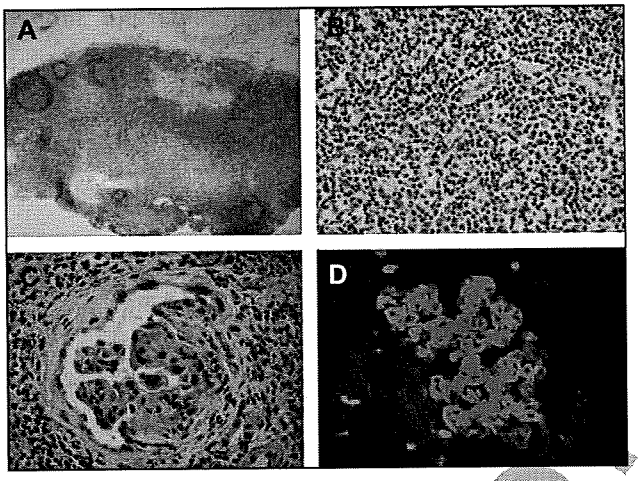

Figure 2. Histopathology of the skin lesion and the kidney. **A, B)** A massive infiltrate of lymphocytes and eosinophils with multiple lymphoid follicles (hematoxylin-eosin, original magnification × 40; A, × 200; B). **C)** Increased mesangial cells and matrix with thickened glomerular basement membrane (hematoxylin-eosin, original magnification × 100). **D)** Immunofluorescence study showing deposition of IgE (**D**) (original magnification × 100).

cells by toluidine blue staining. Immunohistochemically, centrocytes in the germinal center were positive for CD20 + B cells, and lymphocytes surrounding the follicles were CD3+ T cells, consisting of both CD4+ and CD8+ populations. The tumor was histologically diagnosed as Kimura's disease.

Hematology revealed an elevated count of leukocytes (12,500/μL) with 49.0% (6,125/μL) eosinophils. Serum IgE was markedly elevated (110,000 U/mL; normal, 0-170 U/mL), with increased levels of particular cytokines: IL-4, 9.6 pg/mL (normal, < 6.0); IL-5, 19.4 pg/mL (normal, < 10); and IL-6, 1.6 pg/mL (normal, < 4.0). She had hypoalbuminemia (1.9 g/dL; normal, 4.0-5.0 g/dL) and hypercholesterolemia (365 mg/dL; normal, 128-256 mg/dL). Her serum creatinine was 1.6 mg/dL (normal, 0.4-0.7 mg/dL), and her urine contained erythrocytes 20-29/HPF, and her mean systolic and diastolic blood pressure were 146/86 mmHg, respectively. Furthermore, the amount of protein in the 24 hour urine sample was 10.12 g/day. Therefore, she was diagnosed as having nephrotic syndrome and referred for a kidney biopsy. A biopsy specimen demonstrated remarkable increments of mesangial cells and matrix with thickened glomerular basement membrane and an infiltrate of eosinophils *(figure 2C)*. An immunofluorescence study showed deposition of IgE *(figure 2D)*. Thus, the renal condition was diagnosed as MPGN.

The patient was treated with half-pulse methylprednisolone (500 mg daily for 3 days), followed by oral prednisolone (30 mg daily). The tumor shrank and was reduced in size. Laboratory data were improved as follows: eosinophils, 0.8% (90/μL); IgE, 24,000 U/mL; and urinary protein, 7.16 g/day in the 24 hour sample. After the mass size reduction by the steroids, total surgical removal of the tumor was performed. Histologically, the tumor was encapsulated with scar tissue, and a reminiscence of lymphocytic infiltrates was present. She was additionally given 150 mg daily of mizoribine, and the combination of prednisolone and mizoribine continued for 7 months. Currently, 11 months after cessation of the therapy, she is free of relapse with normalized laboratory data (eosinophils, 0.2% or 264/μL; creatinine, 1.2 mg/dL; hematuria, 1-4/HPF; blood pressure, 135/72 mmHg), except for moderately elevated urinary protein (1.22 g/day) and serum IgE level (1,787 U/mL).

Discussion

Although the inguinal region is one of the predilection sites of Kimura's disease, our patient is characterized by a giant tumor occurring at the site. The etiology and pathogenesis of Kimura's disease remains unclear. It has been speculated that the disorder represents an allergic or autoimmune disease triggered by bacterial, viral, parasitic or fungal infection, or their toxins [11-13]. Marked eosino-

philia, elevated serum IgE, and atopic history have implicated that Th2 cells are involved in the pathogenesis of this disorder [13]. Katagiri *et al.* [10] demonstrated the overexpression of Th2 cytokines, IL-4, IL-5 and IL-13, in the peripheral blood mononuclear cells from a patient with Kimura's disease. In addition, IL-5 is known to be produced by lymphocytes from the patient's skin lesions and lymph nodes after stimulation with *Candida* antigen [14, 15]. Elevated IL-4 and IL-5 levels in our case are in accordance with these observations.

Proteinuria was reported in 12-16% of patients with Kimura's disease, and 59-78% of those cases had nephrotic syndrome [16-18]. Renal diseases may precede, follow, or coincide with Kimura's disease. In accordance with the finding that Th2 cytokines promote the permeability of the glomerular basement membrane [6, 19], Th2 type renal diseases, such as membranous glomerulonephritis and minimal change nephropathy, are occasionally associated with Kimura's disease [10, 20, 21]. Although some authors have suggested that MPGN is a Th1 immune response according to the histological patterns [20, 21], few studies are available to allow assessment of the polarization of Th responses in MPGN. In glomeruli, IgG3 (Th1 type) is a predominant subclass, and IgG4 and IgE (Th2 type) are recessive ones. Given that the activation of C1q is restricted to IgG3, our observation of IgE presence, and C1q absence in the kidney specimen suggests that the patient's MPGN represents Th2 type renal disease.

A number of treatment modalities have been reported for Kimura's disease [6-10, 15, 18], including intralesional corticosteroids, cyclosporine, electrodessication, curettage, cryotherapy, radiotherapy, interferon-α, and chemotherapy [10, 22-26]. Systemic treatment with corticosteroids is effective in reducing the size of lesions, but they can recur when steroids are ceased [27]. Surgical resection of the lesions is recommended by some authors [28]. Our patient was successfully treated with systemic steroids with a following excision of the mass.

It seems that Kimura's disease and MPGN are both related to a Th2 dominant immune response. Our patient may represent an extreme case of Kimura's disease, as she had the large skin lesion and the coexistence of important renal disorders. ■

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ORIGINAL ARTICLE

Inducible Nitric Oxide Synthase Downmodulates Contact Hypersensitivity by Suppressing Dendritic Cell Migration and Survival

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Nitric oxide (NO) has several important roles in various physiological settings; one of the NO synthases, inducible NO synthase (iNOS), is induced by external stimulation of the skin. A prototypic example of external stimulation is hapten exposure, which induces the T-cell-mediated immune response known as contact hypersensitivity (CHS). We herein report on cutaneous dendritic cell (DC) function in the presence of an iNOS-specific inhibitor during the sensitization phase of CHS. First, we examined epidermal cell (EC) suspensions using flow cytometry with an iNOS antibody and confirmed that iNOS was expressed in the cytoplasm of Langerhans cells (LCs). We then studied the role of iNOS in CHS, and found that responses to DNFB were enhanced by the addition of an iNOS inhibitor during sensitization. Similarly, the iNOS inhibitor augmented FITC-induced migration of cutaneous DCs, including Langerin$^+$ LCs and Langerin$^-$ dermal DCs, to draining lymph nodes. Finally, we showed that iNOS inhibitor enhanced LC survival *in vitro*. We concluded that NO suppresses migration and survival of cutaneous DCs, resulting in a downmodulation of CHS.

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INTRODUCTION

Inducible nitric oxide (NO) synthase (iNOS) is one of the three isoenzymes that generate NO from its precursor L-arginine. In the skin, keratinocytes (Arany *et al.*, 1996), Langerhans cells (LCs) (Qureshi *et al.*, 1996), dermal fibroblasts (Wang *et al.*, 1996), and melanocytes (Rocha and Guillo, 2001) express iNOS upon stimulation with inflammatory cytokines and/or lipopolysaccharide (LPS). Although NO can be proinflammatory when produced in large amounts, it may also regulate adaptive immune responses (Kuchel *et al.*, 2003). The best characterized example is the induction of iNOS by LPS and IFN-γ in murine macrophages (Lu *et al.*, 1996), LCs (Qureshi *et al.*, 1996), and keratinocytes (Yamaoka *et al.*, 2000). Although some information has thus been accumulated regarding the *in vitro* effects of iNOS on skin immunocompetent cells, the *in vivo* actions of iNOS remain unknown.

Murine contact hypersensitivity (CHS) is an antigen-specific immune response consisting of the two phases, namely, sensitization and elicitation. The constituents involved in its pathogenesis are Th1/Tc1 cells serving as helper/effector cells (Akiba *et al.*, 2002); cutaneous dendritic cells (DCs), including epidermal LCs and dermal DCs (dDCs), as antigen-presenting cells (Kissenpfennig and Malissen, 2006); and keratinocytes as a source of IL-1α, tumor necrosis factor-α, and GM-CSF to the LCs (Sugita *et al.*, 2007). iNOS is induced in LCs and keratinocytes by contact allergens; this supports the view that iNOS has a role in CHS (Morita *et al.*, 1996). It has previously been reported that an iNOS inhibitor injected intradermally during the elicitation phase suppressed CHS responses (Ross *et al.*, 1998), but the specificity of this iNOS inhibitor is not clear; furthermore, the role of iNOS in the sensitization phase remains unknown.

In this study, we investigated the effects of an iNOS-specific inhibitor in order to determine whether iNOS functions as a positive or negative regulator in CHS. Our results show that iNOS suppresses the CHS response by downmodulating the migration and survival of DCs.

RESULTS

iNOS inhibitor enhances CHS response to DNFB

First, we tested the degree of CHS response in mice treated with L-N^6-iminoethyl-lysine (L-NIL), an iNOS inhibitor.

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Abbreviations: Ab, antibody; B6, C57BL/6; CCL21, CC chemokine ligand 21; CCR7, CC chemokine receptor 7; CHS, contact hypersensitivity; DC, dendritic cell; dDC, dermal DC; EC, epidermal cell; iNOS, inducible nitric oxide synthase; LC, Langerhans cell; L-NIL, L-N^6-iminoethyl-lysine; LPS, lipopolysaccharide; NO, nitric oxide; PBS, phosphate-buffered saline

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The mice were sensitized and challenged with DNFB, and their ear swelling responses were measured 24 hours after the challenge. A significantly higher degree of ear swelling response was observed in C57BL/6 (B6) mice treated intraperitoneally with L-NIL throughout the sensitization phase than in non-treated control mice (Figure 1a). Similar results were obtained 48 hours after the challenge (data not shown). In addition, histological analysis of the L-NIL-treated mice showed a remarkable infiltration of lymphocytes into the edematous dermis, which was not seen in untreated mice (Figure 1b). To confirm that L-NIL was biologically active in the skin when administered systemically, we measured the NOx ($NO_2^- + NO_3^-$) concentration of DNFB-sensitized skin. NOx production induced by DNFB was inhibited by an intraperitoneal injection of L-NIL (Supplementary Figure S1), suggesting that L-NIL is biologically active in lesional skin even when it is administered systemically.

iNOS expression in keratinocytes and LCs

Freshly isolated murine epidermal cells (ECs) were incubated for 24 hours in a culture medium, and the LCs and keratinocytes among them were analyzed for iNOS expression with flow cytometry. Both the keratinocytes and the LCs bore iNOS in the cytoplasm (Figure 2a). iNOS expression was greater in the mature LCs (major histocompatibility complex (MHC) class II high expression) than in the immature LCs (MHC class II intermediate expression). We carried out the same analysis on ECs that had been cultured for 24 hours in the presence of LPS, and found that LPS increased the number of LCs that highly expressed iNOS (Figure 2b).

iNOS inhibitor increases cutaneous DC accumulation in regional lymph nodes

To investigate the *in vivo* significance of iNOS for cutaneous DCs, we performed an FITC-induced cutaneous

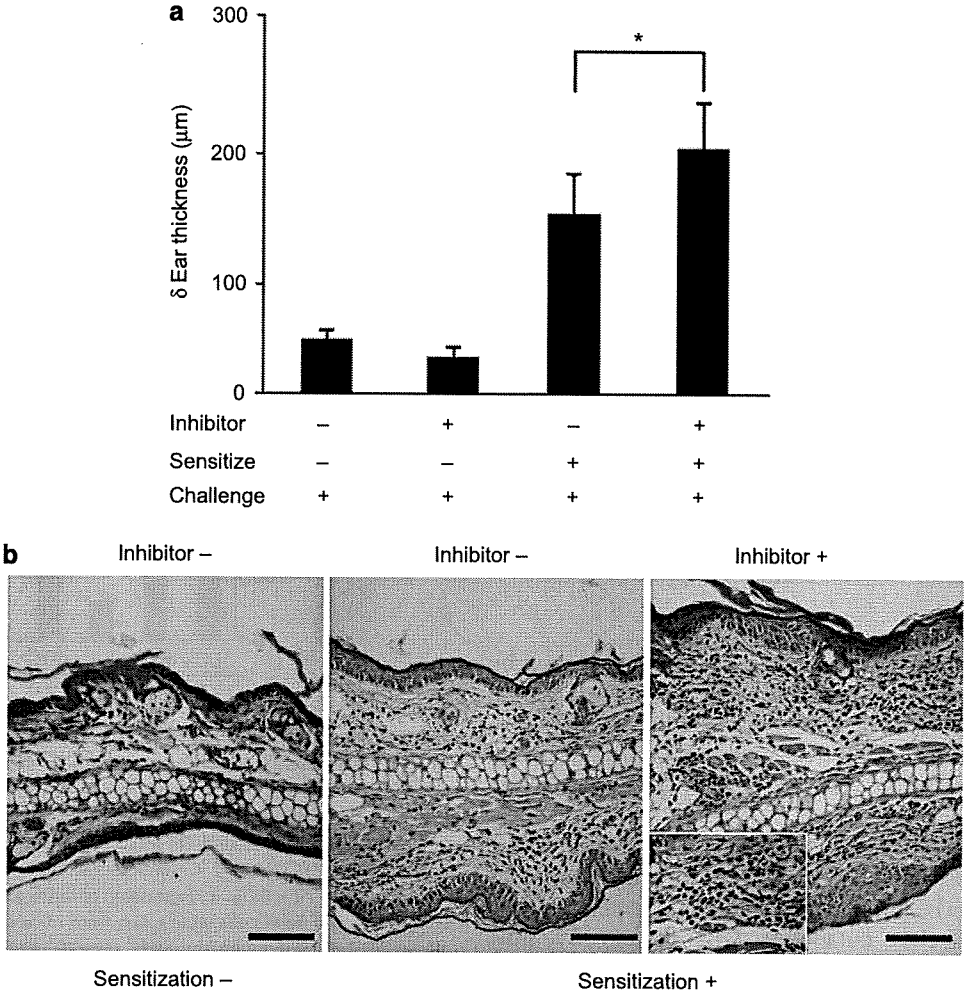

Figure 1. Increased CHS response to DNFB caused by blockade of iNOS. (**a**) For CHS model, B6 mice were immunized by the application of 0.5% DNFB to their shaved abdomens. They were challenged on both ears with 0.3% DNFB. iNOS inhibitor was applied through intraperitoneal injection (2.5 mg in 0.5 ml PBS twice daily). Ear thickness swelling was measured 24 hours later. Data are expressed as the mean ± SD of five mice. $*P<0.05$. (**b**) Non-sensitized ears, challenged ears, and challenged ears from non-treated mice (inhibitor –) were stained with hematoxylin and eosin. Inset: close-up view of hematoxylin and eosin staining of ears from mice treated with iNOS inhibitor, showing perivascular lymphocytic infiltration. Bar = 80 μm. Data are from three independent experiments.

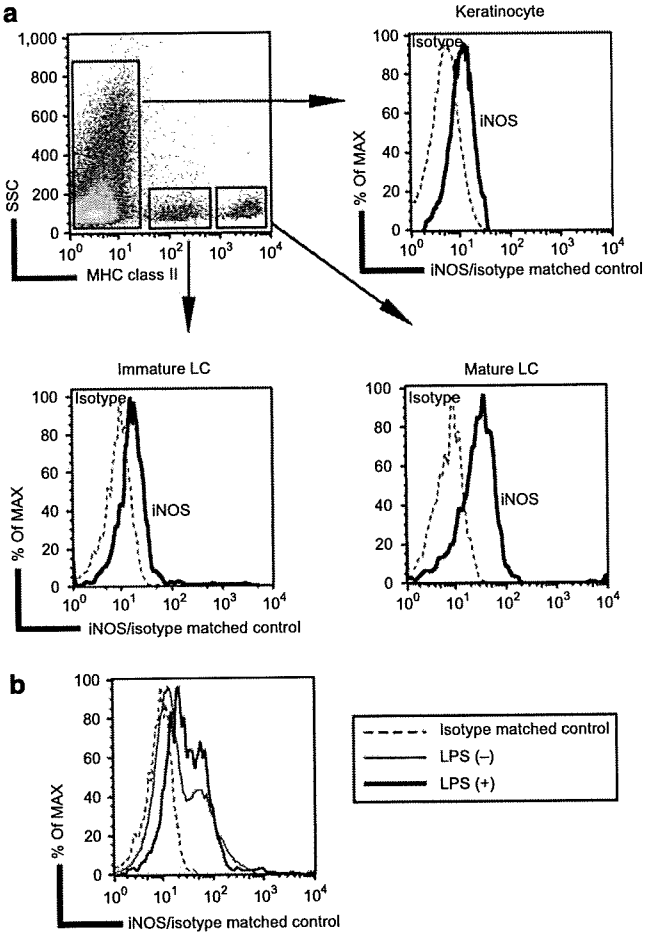

Figure 2. Expression of iNOS by both keratinocytes and LCs. (**a**) EC suspensions were analyzed for the expression of iNOS by means of flow cytometry. For intracellular detection of iNOS, cell fixation-permeabilization was performed before immunolabeling with anti-iNOS and anti-MHC class II mAbs. LCs or keratinocytes were gated by MHC class II positivity. (**b**) EC suspensions from naive mice were cultured with or without LPS (1 $\mu g\,ml^{-1}$) for 24 hours. The cultured cells were subjected to a flow cytometric analysis, which allowed us to measure the expression of iNOS. Data are from three independent experiments.

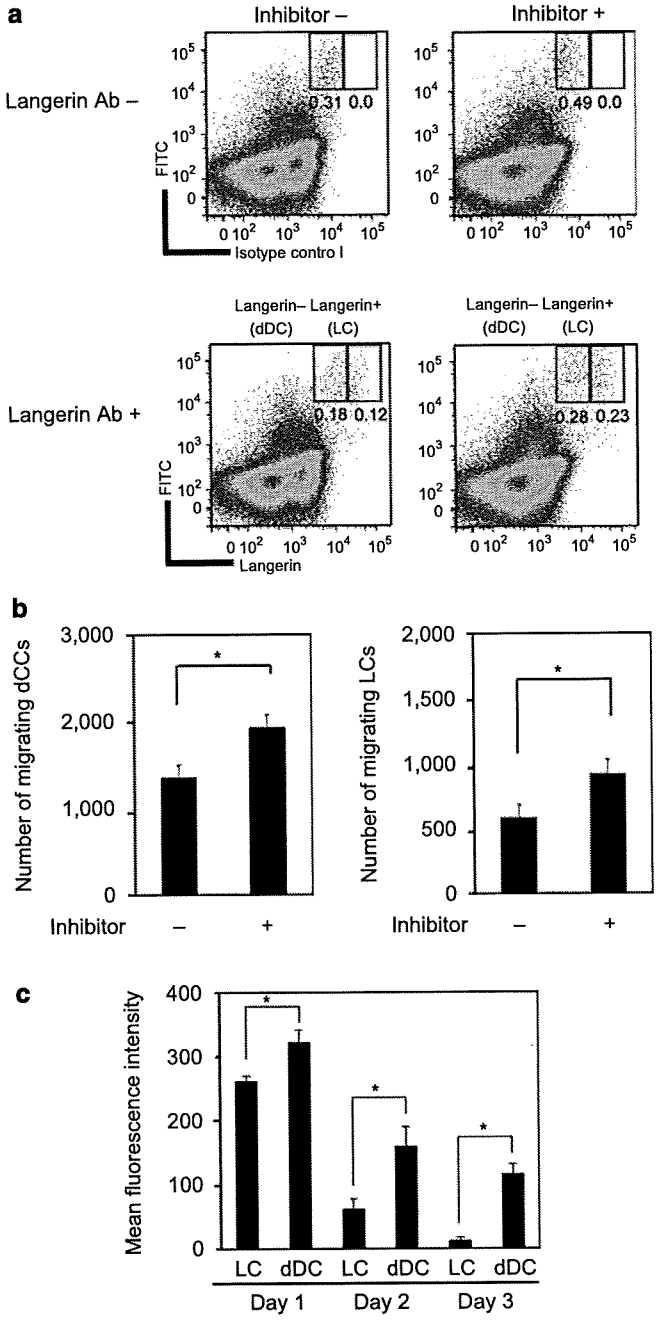

Figure 3. Augmented cutaneous DC accumulation in regional lymph nodes by iNOS blockade. (**a**) Langerin expression and FITC fluorescence in cells derived from regional lymph nodes were analyzed by means of flow cytometry 72 hours after the application of 200 µl of 2% FITC. The percentage of migrating LCs is indicated. (**b**) Migrating dDCs or LCs were counted 72 hours after FITC painting. Columns show the mean ± SD from at least four mice per group. *$P<0.05$. (**c**) Expression of iNOS in migrating LCs and dDCs. Draining lymph node cells were taken from mice painted with FITC on the abdomen and stained with anti-MHC class II, Langerin, and iNOS mAbs. Days 1, 2, and 3 indicate the number of days since FITC painting. Data are expressed as mean fluorescence intensity (MFI) for iNOS. MFI was the value of LCs or dDCs subtracted from that of the isotype-matched control. Columns show the mean ± SD. *$P<0.01$. Results are representative of three independent experiments.

DC migration assay. FITC applied to the skin is taken up by cutaneous DCs, which subsequently migrate to the draining lymph nodes as $FITC^+$ MHC class II^+ cells. We intraperitoneally injected L-NIL, an iNOS inhibitor (2.5 mg in 0.5 ml phosphate-buffered saline (PBS) twice daily for 4 consecutive days) or the equivalent amount of PBS into mice; 24 hours after the last injection, we applied FITC to the abdomen. We then isolated axillary and inguinal draining lymph node cells 72 hours after FITC application and characterized the $FITC^+$MHC class II^+ cutaneous DCs therein by flow cytometry. Staining for Langerin showed that two subsets of the $FITC^+$ MHC class II^+ cutaneous DCs, the dDCs and LCs, were present in significantly greater numbers because of treatment with iNOS inhibitor (Figure 3a and b). Therefore, the blockade of iNOS promoted lymph node accumulation of cutaneous DCs in response to skin exposure to an antigen.

iNOS expression in migrating LCs and dDCs

We examined iNOS expression in freshly isolated LCs and dDCs, both of which are capable of migrating into the lymph nodes on sensitization. The expression of iNOS in these cells was examined with FITC and anti-Langerin mAb. FITC was applied to the abdomen, and draining lymph node cells were sampled 24, 48, and 72 hours later. These cells were then labeled with anti-MHC class II mAb, anti-Langerin Ab, and anti-iNOS Ab. Although LCs are positive for Langerin, most dermal DCs are negative for Langerin (Nagao *et al.*, 2009), iNOS was present in both LCs and dDCs. The mean fluorescence intensity for iNOS was as follows: LC, 11.9 ± 4.1; dDC, 36.6 ± 20.5 (mean ± SD of three mice). These data suggested that iNOS was weakly expressed only in freshly isolated LCs and dDCs, in amounts too small to be statistically significant. Nevertheless, we were able to observe that the dDCs showed a higher mean fluorescence intensity of iNOS expression than the LCs did (Figure 3c).

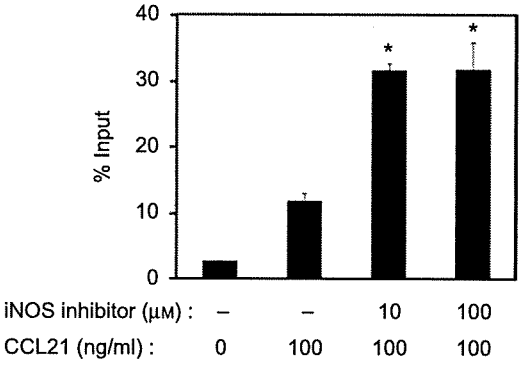

Figure 4. Chemotactic activity of epidermal LCs to CCL21. EC suspensions were incubated with or without iNOS inhibitor plus LPS in culture medium for 9 hours and applied to a transwell. CCL21 at 100 ng ml^{-1} was administered to the lower chamber. Migrating epidermal LCs in the lower chamber were identified as belonging to the MHC class II$^+$ subset. The number of migrating LCs was calculated. Columns represent the mean ± SD of triplicated transwells, and data are from three independent experiments. *$P<0.01$.

Chemotactic activity of LCs to CCL21

EC suspensions were incubated with LPS in a culture medium for 9 hours and applied to transwells in the presence or absence of L-NIL, an iNOS inhibitor. The migrating LCs in the lower chamber were identified as MHC class II$^+$ cells. CCL21 (CC chemokine ligand 21), a cytokine expressed in secondary lymphoid organs that mediates the chemotaxis of lymphocytes and DCs through its receptor, CCR7 (CC chemokine receptor 7; Saeki *et al.*, 1999), was then added to the lower chamber. All LCs exhibited a strong chemotactic response to this chemokine, but this response was significantly increased by the iNOS inhibitor (Figure 4).

iNOS inhibitor caused no alteration of the expression of co-stimulatory molecules or CCR7

The chemotaxis-promoting activity of the iNOS inhibitor, described above, raised the possibility that the iNOS inhibitor upregulates the expression of co-stimulatory molecules and CCR7. To determine whether this is the case, freshly isolated ECs were cultured for 24 hours in the presence or absence of the iNOS inhibitor, and the expression levels of these molecules were monitored by gating for MHC class II$^+$ LCs. After 24 hours of culture, a single population of LCs usually divides into two populations, with different expression levels of co-stimulatory molecules and CCR7 (Sugita *et al.*, 2007) (Figure 5a). The addition of the iNOS inhibitor did not alter the expression of CD86, CD80, CD40, or CCR7 (Figure 5a and b). In chemotaxis, however, the expression of

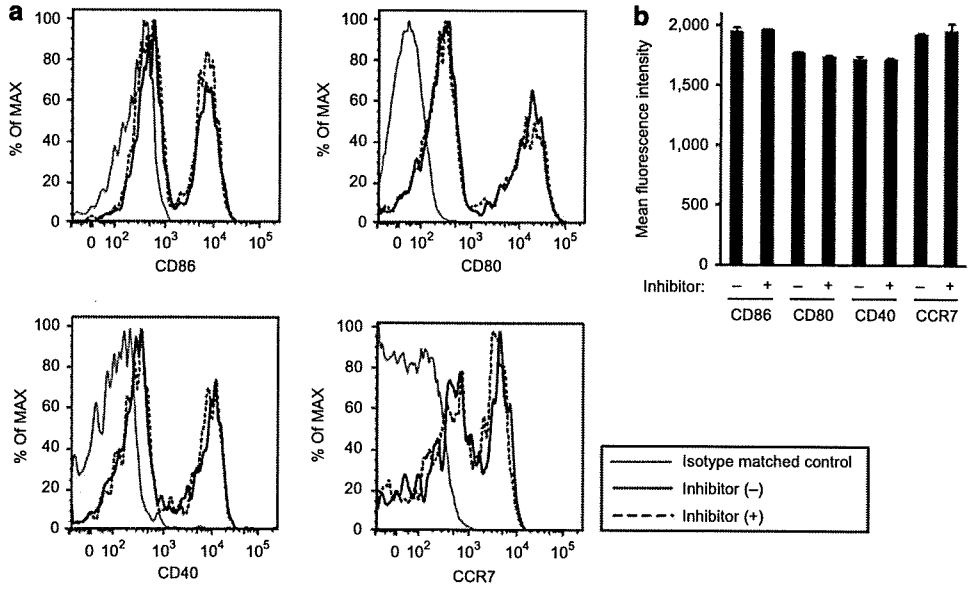

Figure 5. No modulation of CD86, CD80, CD40, or CCR7 expression in LCs by iNOS inhibitor. (**a** and **b**) EC suspensions from naive mice were cultured for 24 hours with or without iNOS inhibitor. The cultured LCs were examined for their expression levels of CD86, CD80, CD40, and CCR7. Data are representative of three independent experiments.

CCR7 in DCs is not sufficient to guarantee its functionality (Sanchez-Sanchez *et al.*, 2006); therefore, it is possible that iNOS alters certain downstream functions of LCs without affecting CCR7 expression.

iNOS inhibitor reduces LPS-induced apoptosis of LC

We then evaluated the effect of endogenous iNOS activity on the viability of LCs. EC suspensions from the earlobes of B6 mice were cultured for 9 hours with or without LPS in the presence or absence of L-NIL, an iNOS inhibitor. LPS stimulation reduced the number of LCs, but this reduction was reversed by the addition of the iNOS inhibitor (Figure 6a). It has been reported that epidermal LCs are unable to proliferate *in vitro* when they are incubated as an EC suspension (Schuler and Steinman, 1985), suggesting that the observed effects of the iNOS inhibitor stem from a survival change.

To examine whether the iNOS inhibitor promotes the survival of LCs, cellular viability was assessed through flow cytometry after Annexin V/propidium iodide staining and 9 hours of culture (Figure 6b). This flow cytometry experiment used anti-MHC class II and anti-CD11c mAbs. The percentage of Annexin V and propidium iodide double-positive cells in samples that had been treated with 100 μM iNOS inhibitor and those that had not was as follows: 100 μM, 1.6 ± 0.4%; no addition, 2.2 ± 1.0% (mean ± SD, $n = 3$). These results suggest that the reduction of apoptotic cells that occurs through iNOS inhibitor treatment is not due to the increment of necrotic cells. We found that LPS-induced apoptosis of LCs was reduced by the addition of the iNOS inhibitor (Figure 6c), suggesting that the iNOS inhibitor promotes DC survival.

DISCUSSION

The results of this study on the effects of an iNOS inhibitor include several major findings about the involvement of NO in the sensitization phase of CHS. First, CHS as a model of acquired skin immune response was enhanced by treatment with the iNOS inhibitor. Second, the iNOS inhibitor markedly increased the number of migrating cutaneous DCs. Accordingly, the chemotactic response of LCs to CCL21 was enhanced by *in vitro* incubation with the iNOS inhibitor. Finally, the iNOS inhibitor was capable of reducing LPS-induced apoptosis of LCs.

It has generally been believed that iNOS is involved in CHS as a producer of NO and a trigger of inflammatory responses (Cals-Grierson and Ormerod, 2004). It has been

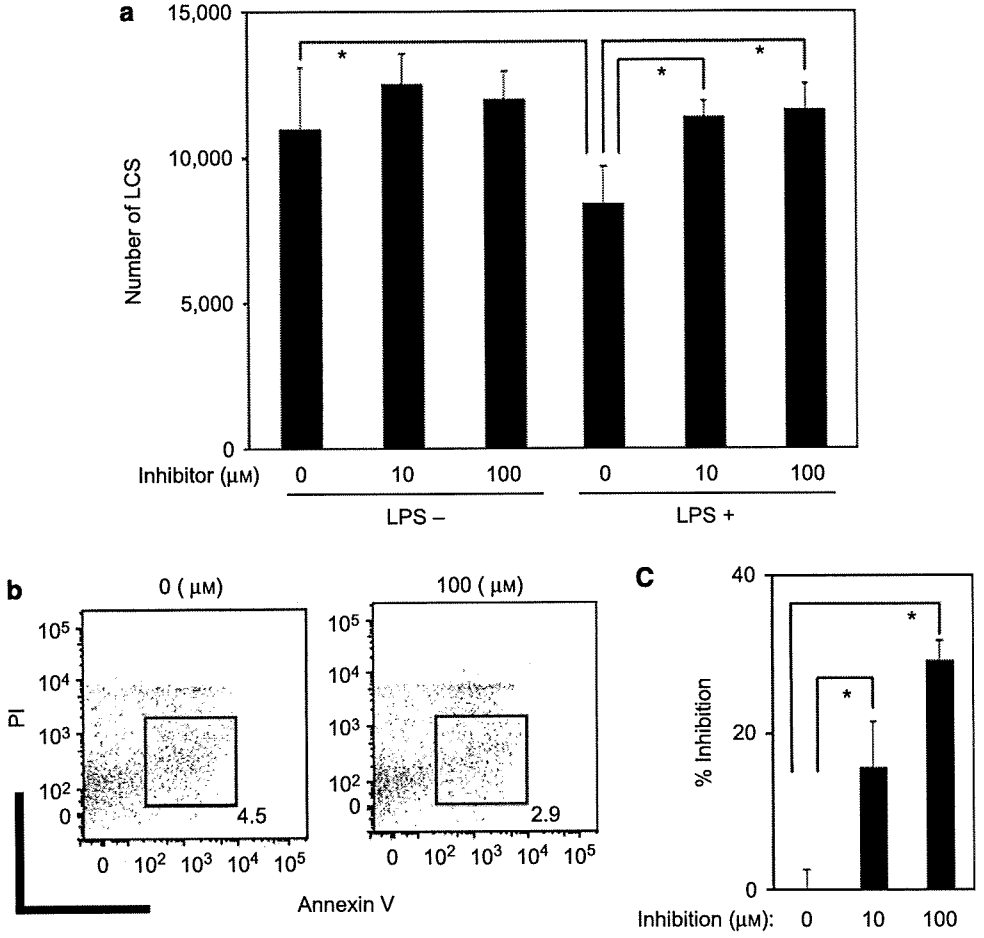

Figure 6. The effect of LPS on LC survival. (**a**) EC suspensions were cultured with or without LPS and iNOS inhibitor for 9–12 hours. iNOS inhibitor dose dependently increased the number of LCs. Columns show the mean ± SD. *$P<0.05$. Data are representative of three independent experiments. (**b**) Apoptosis was determined using annexin V/propidium iodide double staining. (**c**) The percentage inhibition is calculated. Data represent the mean ± SD. *$P<0.05$.

reported that iNOS and NO were produced in human skin subjected to positive patch tests to contact allergens (Cruz *et al.*, 2007; Ormerod *et al.*, 1997) and to the irritant sodium lauryl sulfate; nevertheless, it remains controversial whether iNOS is inhibitory or augmentative in CHS (Ross and Reske-Kunz, 2001). It has also been reported that an iNOS inhibitor exerted a suppressive effect on the CHS response to 2,4,6-trinitrochlorobenzene (TNCB) (Musoh *et al.*, 1998), although this effect was limited to the first few hours of the response, and neither NO production, NO-expressing cells, nor NOS isoenzymes were identified. Thus, the mode of action of iNOS in CHS remains a matter of debate.

It is possible that iNOS first modulates keratinocytes so that they produce cytokines, thereby subsequently modifying LC function. Yet, we found that the production levels of GM-CSF and tumor necrosis factor-α in the culture supernatant of primary keratinocytes of B6 mice cultured for 72 hours were not significantly affected by the presence of the iNOS inhibitor (Supplementary Figure S2). With regard to the effect of iNOS on T cells, we cultured immune $CD4^+$ T cells for 72 hours with varying concentrations of the iNOS inhibitor in the presence of anti-CD3 mAb and found that the iNOS inhibitor was incapable of stimulating T cells *per se* (Supplementary Figure S3).

LCs have traditionally been believed to have a role in the induction of CHS, but three research groups have reported three contradictory findings after applying haptens to transgenic mice deficient in LCs: a diminished reaction (Bennett *et al.*, 2005), an enhanced reaction (Kaplan *et al.*, 2005), and an unchanged response (Kissenpfennig *et al.*, 2005). Moreover, recent findings suggest that dDCs has a critical role in initiating CHS (Fukunaga *et al.*, 2008). In our study, dDCs augmented iNOS expression in response to hapten application more than LCs did. Our findings suggest that iNOS can suppress cutaneous DC migration and survival. Given that, in CHS, dDCs and LCs have positive and regulatory capacities, respectively, our findings on cutaneous DCs seem to be consistent with the observation that iNOS inhibitor induces an enhancement of CHS.

The findings of our study are clinically relevant in two respects. First, iNOS and NO exert immunosuppressive effects on cutaneous inflammation. In this context, the *in vivo* immunosuppressive effect of NO has also been shown in human studies (Kuchel *et al.*, 2003). Second, iNOS reduces cutaneous DC function and survival in the sensitization phase of CHS. The observation that NO directly reduces the number of LCs in the human epidermis supports our conclusion (Mowbray *et al.*, 2008).

MATERIALS AND METHODS

Animals and reagents

Female B6 mice were purchased from Japan SLC (Hamamatsu, Japan). All experiments were conducted on 8-week-old mice. The mice were maintained on a 12-hour light/dark cycle under a specific pathogen-free condition. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Occupational and Environmental Health. L-NIL (a highly selective inhibitor of iNOS enzymatic activity) and LPS were obtained from Sigma-Aldrich (St Louis, MO). CCL21 was purchased from R&D Systems (Minneapolis, MN).

DNFB-induced CHS model

B6 mice were sensitized through the application of 25 μl of 0.5% (v/v) DNFB in 4:1 acetone/olive oil to their shaved abdomens on day 0. They were then challenged on both sides of each ear with 20 μl of 0.3% (v/v) DNFB. Ear thickness change was calculated as follows: (ear thickness 24 or 48 hours after challenge)−(ear thickness before challenge). iNOS was inhibited with L-NIL as described previously (Diefenbach *et al.*, 1998). Briefly, L-NIL was applied by intraperitoneal injection (2.5 mg in 0.5 ml PBS twice daily) for 6 consecutive days starting 1 day before sensitization. We chose his protocol because treatment with L-NIL at this concentration and frequency for 4-13 days is one of the most common methods of blocking *in vivo* activity of iNOS (Diefenbach *et al.*, 1998; Stallmeyer *et al.*, 1999).

EC preparation and culture

EC suspensions were prepared as described previously (Tokura *et al.*, 1994). Ears of naive mice were split along the plane of the cartilage, which was then removed together with the subcutaneous tissue. These specimens were incubated for 1 hour at 37 °C in a 0.2% solution of trypsin in PBS. After incubation, the epidermis was separated from the dermis and the separated epidermal sheets were rubbed to disperse the ECs in PBS supplemented with 10% fetal calf serum. The cells were filtered and washed twice in PBS. As a culture medium, RPMI-1640 (Sigma-Aldrich) was supplemented with 10% heat-inactivated fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin.

Preparation of dermal cell suspensions

Dermal cells were obtained from normal murine skin from which the epidermis had been removed. Samples were minced and incubated for 2 hours at 37 °C in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with collagenase (2 mg ml^{-1}; Sigma-Aldrich), hyaluronidase (260 U ml^{-1}; Sigma-Aldrich), DNase (0.1 mg ml^{-1}; ICN, Costa Mesa, CA), and 10 mM HEPES (Sigma-Aldrich). The obtained cells were filtered through a 40-μm filter.

Flow cytometry

For flow cytometry, cells were plated at a density of 1×10^6 cells per well in 96-well U-bottomed plates (Falcon, BD Biosciences, San Jose, CA). Cells were then stained for 20 minutes on ice with mAbs in 25 μl of PBS containing 2% fetal calf serum, 1 mM EDTA, and 0.1% NaN_3, and washed twice with 200 μl of this buffer. Data were collected on a FACSCanto system (BD Biosciences) and analyzed with FlowJo software (TreeStar, San Carlos, CA). The mAbs used were as follows: FITC-conjugated anti-CD86 and Annexin V mAbs, PE-conjugated anti-CD80 and CD40 mAbs, PE-Cy5-conjugated anti-MHC class II mAb, APC-conjugated anti-CD11c mAb (all from BD Biosciences), and PE-Cy7-conjugated anti-CCR7 mAb (eBioscience, San Diego, CA). For detection of Langerin and iNOS, anti-Langerin Ab (eBioscience), PE-conjugated anti-iNOS Ab (Santa Cruz Biotechnology, Santa Cruz, CA), and PE-Cy5-conjugated streptavidin were

used after fixation and permeabilization of cells using a Cytofix/Cytoperm Kit (BD Biosciences).

Histology

At 48 hours after the challenge with hapten, the ears of B6 mice were excised and fixed in 10% formaldehyde. Sections of 5-μm thickness were prepared and stained with hematoxylin and eosin.

FITC-induced cutaneous DC migration

The shaved abdomens of the mice were painted with 200 μl of 2% FITC (Sigma-Aldrich) dissolved in a 1:1 (v/v) acetone/dibutyl phthalate (Sigma-Aldrich) mixture, and the iNOS inhibitor was applied through intraperitoneal injection (2.5 mg in 0.5 ml PBS) twice daily for 4 days. Cutaneous DCs migrating into the draining inguinal and axillary lymph nodes were then counted by means of flow cytometry (Kabashima *et al.*, 2007) using Flow-Count Fluorospheres (Beckman Coulter, Fullerton, CA). The principle of Flow-Count Fluorospheres is based on the precise mixing of microparticles whose concentration and volume are known. Before flow cytometric analysis, 10 μl of Flow-Count Fluorospheres were added to each specimen. The percentages of fluorospheres and migrating DCs within each node were then determined using the FACSCanto system (BD Biosciences). To find the number of migrating DCs, the ratio of DCs to fluorospheres was counted using the following formula, based on Reimann *et al.* (2000), with some modifications: number of migrating DCs = (percentage of migrating DCs/percentage of fluorospheres) × number of fluorospheres.

Chemotaxis assay

EC suspensions were incubated for 9 hours with or without the iNOS inhibitor, and then tested for transmigration across uncoated 5-μm transwell filters (Corning Costar, Corning, NY) to CCL21 or medium in the lower chamber for 3 hours. Migrating cells were enumerated by means of flow cytometry (Ngo *et al.*, 1998). The medium used in this assay was RPMI-1640 with 0.5% fatty acid-free bovine serum albumin (Calbiochem, San Diego, CA).

Apoptosis analysis

The EC suspensions from B6 mice were stained with PE-Cy5-conjugated anti-MHC class II mAb for 20 minutes on ice, then stained with FITC-conjugated Annexin V and propidium iodide (BD Pharmingen, Franklin Lakes, NJ), according to the manufacturer's protocol. The number of LCs was assessed by means of flow cytometry with anti-MHC class II and APC-conjugated anti-CD11c mAbs. Apoptosis in LCs was analyzed using a FACSCanto system with FlowJo software.

Statistical analysis

Data were analyzed using an unpaired two-tailed *t*-test. $P<0.05$ was considered to be significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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CASE REPORT

Analysis of CXCL9 and CXCR3 expression in a case of intravascular large B-cell lymphoma

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Intravascular large B-cell lymphoma is a rare disease with multiorgan involvement that also affects the skin. Skin manifestations include purpuric to red macules, plaques, or nodules with occasional edema and tenderness. We report a 68-year-old woman with bilateral leg edema and occasional high fever. A biopsy specimen from a subcutaneous nodule showed that the blood vessels in the dermis and subcutaneous tissue were filled with irregularly shaped chromatin-rich large atypical lymphocytes positive for CD20 and bcl-2, consistent with the diagnosis of intravascular large B-cell lymphoma. In addition, immunohistochemical analysis showed expression of CXCR3 in the atypical lymphocytes; its ligand, CXCL9, was detected in blood vessels. Although limited to a single case, our study could provide a possible new clue to the pathogenesis of intravascular large B-cell lymphoma by virtue of the characteristic expression of CXCL9-CXCR3. (J Am Acad Dermatol 10.1016/j.jaad.2009.02.007.)

Key words: chemokine; chemokine receptor; CXCL9; CXCR3; intravascular large B-cell lymphoma; skin.

Intravascular large B-cell lymphoma (IVLBCL) is a rare and highly aggressive disease that is usually fatal and affects the skin and many other organs, including the central nervous system, lungs, heart, kidneys, and eyes.[1,2] Cutaneous manifestations include purpuric to red macules, plaques, or nodules with occasional edema and tenderness. Aggregation of atypical large B cells confined within the vascular lumen is characteristic histologically, but the mechanism underlying this vessel-associated infiltration remains unexplained.

Decreased expression of CD29 and CD54 on the surface of IVLBCL cells, which is necessary for adhesion to endothelial cells and for transmigration into tissues, might explain how the IVLBCL cells remain in the vascular lumen.[3] However, it is uncertain how atypical B cells aggregate inside the vascular lumen. Chemokine-chemokine receptor engagement may be involved.

Chemokines mediate cell migration through the binding of their receptors, and their expressions are differentially regulated in lymphocyte subsets. The expression profiles of chemokine receptors on B-cell proliferative disorders have been studied.[4,5] Among chemokine receptors, CXCR3 has been intensely studied. CXCR3 expression is highly variable on normal B cells and on cells from B-cell lymphoproliferative disorders, possibly because of their lability during processing.[4] CXCR3 is expressed in chronic lymphocytic leukemia, mucosa-associated lymphoid tissue type lymphoma, and other B-cell non-Hodgkin lymphomas,[6-8] but rarely or never in hairy cell leukemia, mantle cell lymphoma, and peripheral blood B cells.[9] In contrast, high chemotactic responses to its ligand, CXCL9 (monokine-induced by interferon-γ; Mig), have been observed in atypical B cells of chronic lymphocytic leukemia and mucosa-associated lymphoid tissue type, but not in B cells of hairy cell leukemia, mantle cell lymphoma, or healthy subjects.[6] However, these responses have not been studied in IVLBCL. Here we report a case of IVLBCL with an immunohistochemical study of CXCR3 and CXCL9 expression.

CASE REPORT

A 68-year-old woman was referred to our clinic and hospitalized for evaluation of bilateral leg

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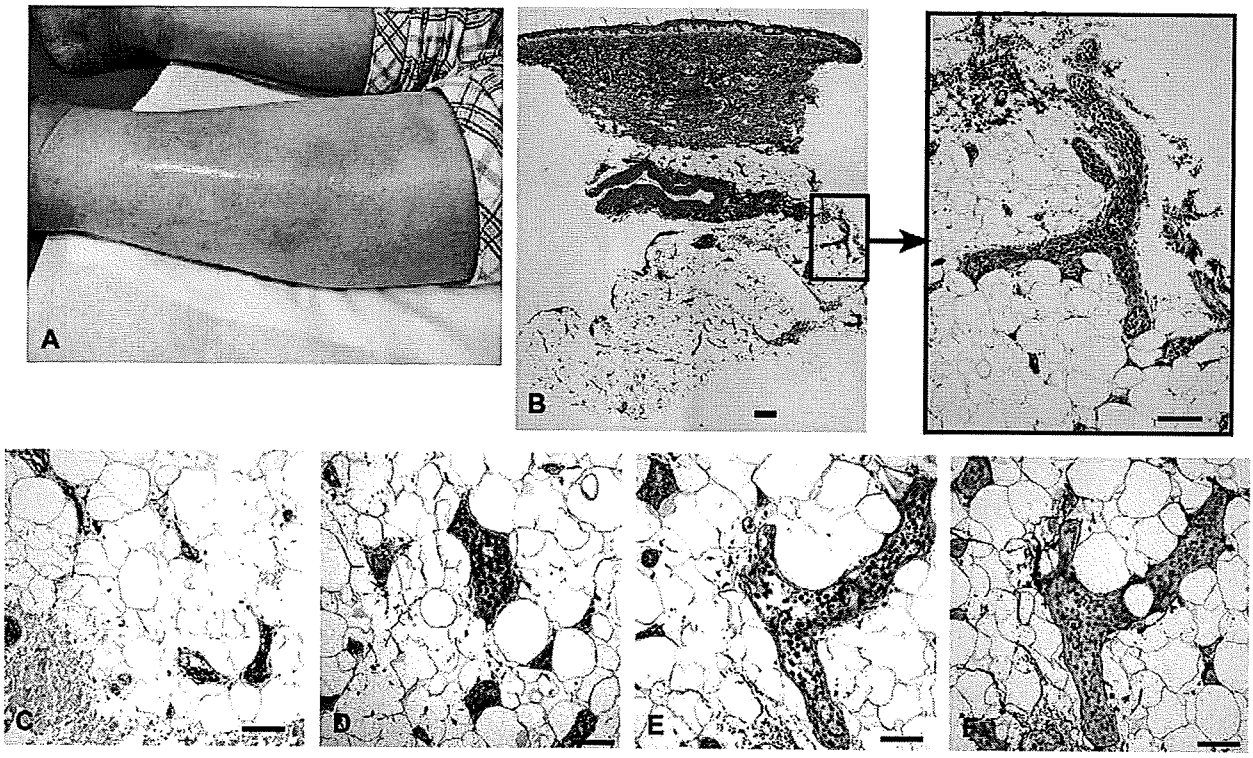

Fig 1. Clinical appearance and histologic analysis. **A**, Erythema and edema of leg. **B**, Biopsy specimen from left leg, showing irregularly shaped chromatin-rich large atypical lymphocytes in blood vessels of dermis and subcutaneous tissue (hematoxylin-eosin stain). Scale bars, 500 μm (*left*) and 100 μL (*right*). Immunohistochemical staining showed that these cells were positive for CD20 (**C**) and bcl-2 (**D**), and negative for CD5 with DAB (**E**) and CD10 (**F**). Scale bars, 100 μm.

edema. She had a history of occasional febrile episodes. During her hospitalization, leg edema became more prominent with palpable, tender, subcutaneous nodules underneath the edematous skin (Fig 1, *A*). Otherwise, her mental status was clear, and no other organ involvement such as splenomegaly was noted on computed tomography scan. Bone-marrow biopsy specimen showed no atypical lymphocytes, and complete blood cell counts were within normal range (leukocyte counts, 6400 cells/μL; neutrophils, 66.4%; monocytes, 18.5%; and lymphocytes, 13.8%). Blood serum tests revealed elevated levels of lactate dehydrogenase (2191 IU/L; normal, 120-240 IU/L), C-reactive protein (4.82 mg/dL; normal, <0.3 mg/dL), ferritin (710 ng/mL; normal, 10-200 ng/mL), and soluble interleukin-2 receptor (9970 U/mL; normal, 220-530 U/mL).

A biopsy specimen from a subcutaneous nodule on the left leg showed that blood vessels in the dermis and subcutaneous tissue were filled with irregularly shaped chromatin-rich large atypical lymphocytes (Fig 1, *B*). Immunohistochemically, these cells were positive for CD20 (Fig 1, *C*) and bcl-2 (Fig 1, *D*), and negative for CD3 (data not shown), CD5 (Fig 1, *E*), and CD10 (Fig 1, *F*), stained using diaminobenzidine (DAB). Based on these clinicopathologic features, she was given the diagnosis of IVLBCL.

Staging computed tomography scans and histologic examination of the bone marrow disclosed no extracutaneous involvement. Chemotherapy was initiated with cyclophosphamide, doxorubicin, vincristine, and prednisolone. However, several days later, the patient noted acutely progressive severe shortness of breath with pancytopenia and hypotension, and died 1 month later. No autopsy was performed.

DISCUSSION

IVLBCL is a systemic lymphoma often involving the skin and is characterized by aggregation of large atypical lymphocytes within vessels in the subcutaneous tissue. Currently, the mechanism of this aggregation remains unknown. To this end, an immunohistochemical study using several monoclonal antibodies for chemokines-chemokine receptors, CXCR3 (R&D Systems, Minneapolis, MN) and CXCL9

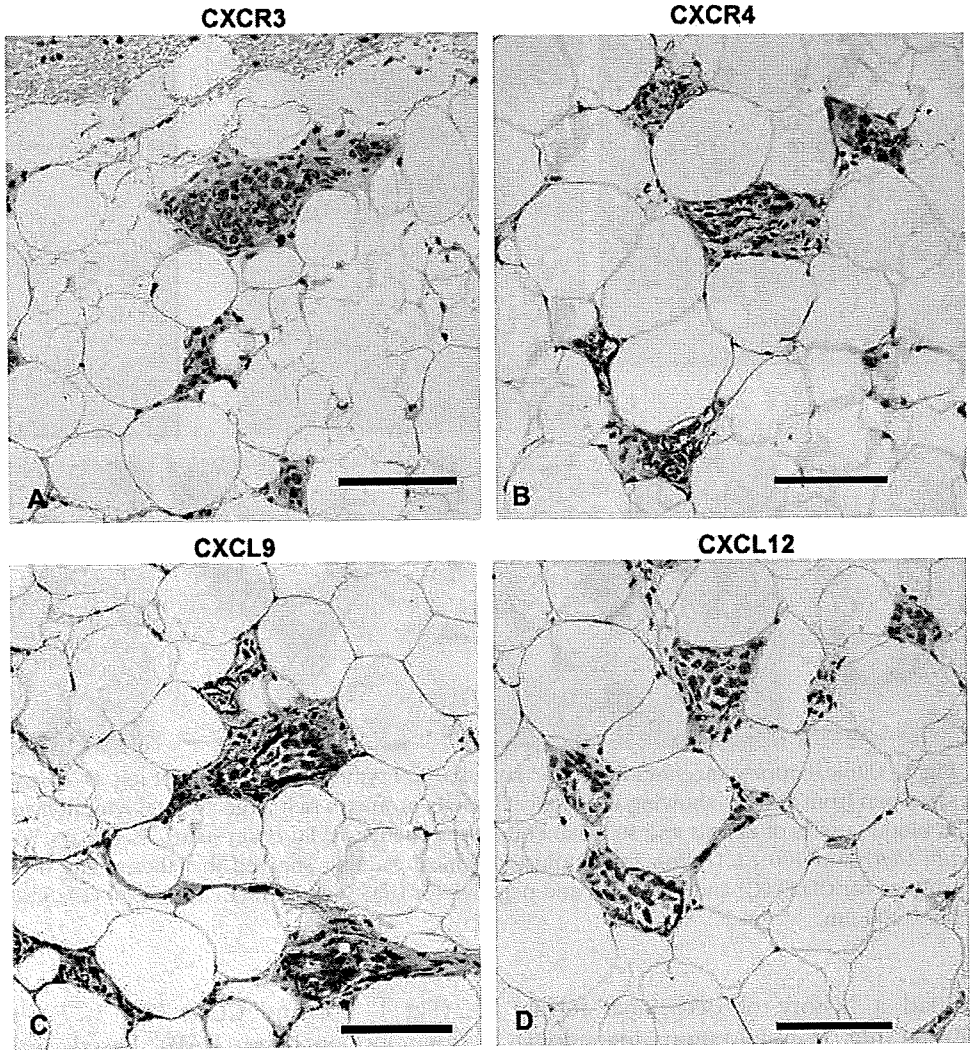

Fig 2. Chemokine-chemokine receptor expression. Immunohistochemical analyses of leg specimen from patient with intravascular large B-cell lymphoma performed with anti-CXCR3, CXCR4, CXCL9, and CXCL12 antibodies. Scale bars, 100 μm. Atypical lymphocytes are positive for CXCR3 (**A**) but negative for CXCR4 (**B**). Blood vessels are strongly stained with CXCL9 (**C**), but not with CXCL12 (**D**). In addition, CXCL9 (with DAB staining) is mildly positive in some atypical lymphocytes (**C**).

(R&D Systems), was performed. It is known that normal B cells express CXCR4, but not CXCR3, and that some B-cell lymphomas induce CXCR3 expression.[4] Therefore, as references, CXCR4 (R&D Systems) and its ligand CXCL12 (stroma cell–derived factor-1α; SDF-1α) (R&D Systems) were also analyzed as described previously.[5]

Atypical lymphocytes were positive for CXCR3 but negative for CXCR4 (Fig 2, *A* and *B*). CXCR3 positivity was stronger in IVLBCL than our previous cases of diffuse large B-cell lymphomas.[8] On the other hand, blood vessels, especially the endothelial cells, were strongly stained with CXCL9, but not with CXCL12 (Fig 2, *C* and *D*). We have previously reported that CXCL9 was positive in fibroblasts but not or much less in the blood vessels in patients with diffuse large B-cell lymphoma.[8]

Although limited to a single case, our study could provide a possible new clue to the pathogenesis of IVLBCL by virtue of the characteristic expression of CXCL9-CXCR3. CXCR3 was expressed in IVLBCL and its ligand, CXCL9, was expressed in blood vessels, which might explain the aggregation of atypical lymphocytes in the vascular lumen. Certainly, we cannot rule out a possibility that chemokine-chemokine receptor engagements other than CXCL9-CXCR3 are involved in the pathogenesis of IVLBCL. More reports and precise observations with thorough chemokine panels might further reveal the pathomechanisms underlying IVLBCL.

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LETTER TO THE EDITOR

Antihistaminic drug olopatadine downmodulates CCL17/TARC production by keratinocytes and Langerhans cells

Dear Editor,

Olopatadine hydrochloride (OLP; [Z]-11-[3-dimethylaminopropylidene]-6,11- dihydrodinenz [b,e] oxepin-2-acetic acid monohydrochloride) is a histamine H_1-receptor-blocking agent that possesses both acidic and basic residues.[1] This H_1 blocker also suppresses the production by epithelial cells or mast cells of various chemical mediators and cytokines, such as leukotrienes, arachidonic acid, interleukin (IL)-6, IL-8 and tumor necrosis factor-α (TNF-α),[2,3] and inhibits intracellular adhesion molecule 1 (CD54) expression on conjunctival cells[4] and activity/migration of eosinophils.[5] Based on these findings, OLP is now widely used for the treatment of allergic rhinitis, urticaria and various itchy skin diseases including eczematous dermatitis.[6]

It has been reported that OLP has a unique anti-allergic property, which may provide implications for the mechanisms underlying its therapeutic actions. Thymus and activation-regulated chemokine (CCL17/TARC) is one of the T-helper (Th)2-associated chemokines, and an important regulator of Th2 cell recruitment into the skin.[7] Serum CCL17 level is proportional to the disease activity of atopic dermatitis (AD), and OLP inhibits CCL17 production by peripheral blood mononuclear cells from AD patients.[7] Serum CCL17 level is also related to the disease activity of bullous pemphigoid, mycosis fungoides, chronic actinic dermatitis and papuloerythroderma.[8,9] In the skin, CCL17 is secreted by keratinocytes (KC) and Langerhans cells (LC). LC are professional antigen-presenting cells in the epidermis, and we have recently shown that they are the main source of CCL17 among epidermal cells.[10] These findings urged us to investigate whether OLP induces inhibition of CCL17 production by KC or LC *in vitro*. To examine the effects of OLP on KC, we used human KC cell line HaCaT cells. LC-enriched epidermal cells (LC-EC) and bone marrow-derived dendritic cells (BMDC) were prepared from BALB/c mice. Our results suggest that OLP exerts its therapeutic effectiveness by inhibiting CCL17 production by both KC and LC.

First, to explore whether olopatadine suppresses CCL17 production by KC, we added olopatadine into the culture medium of KC cell line HaCaT cells. Three-day culture supernatants from HaCaT cells were collected, stored at −80°C and measured for CCL17, CCL22/MDC, monokine induced by γ-interferon (IFN-γ) (CXCL9/Mig) and IFN-γ-inducible protein 10 (CXCL10/IP-10) using enzyme-linked immunosorbent assay (ELISA) kits (Genzyme/Techne, Minneapolis, MN, USA) according to the manufacture's directions. It has been reported that the concentrations of OLP at 10^{-5} to 10^{-7} mol/L suppresses *in vitro* activities of both KC and LC.[11] Therefore, we followed the protocols to examine the inhibitory activity of olopatadine in our experiments. As shown in Figure 1, the IFN-γ/TNF-α-augmented production of CCL17 was suppressed significantly by the addition of olopatadine at a concentration of 10^{-6} or 10^{-5} mol/L. The concentrations of OLP in this *in vitro* study were chosen on the basis of the therapeutic dose of this drug.[12]

To see the effects of olopatadine on CCL17 production by LC-EC, epidermal cell (EC) suspensions freshly isolated from naïve BALB/c mice were subjected to Ficoll gradient separation of LC-EC as described previously.[13] The percentage of LC in LC-EC fraction was 15–20%, as assessed by flow cytometric analysis with anti-I-A^d phycoerythrin (PE)-labeled monoclonal antibody (BD PharMingen, San Diego, CA, USA). OLP

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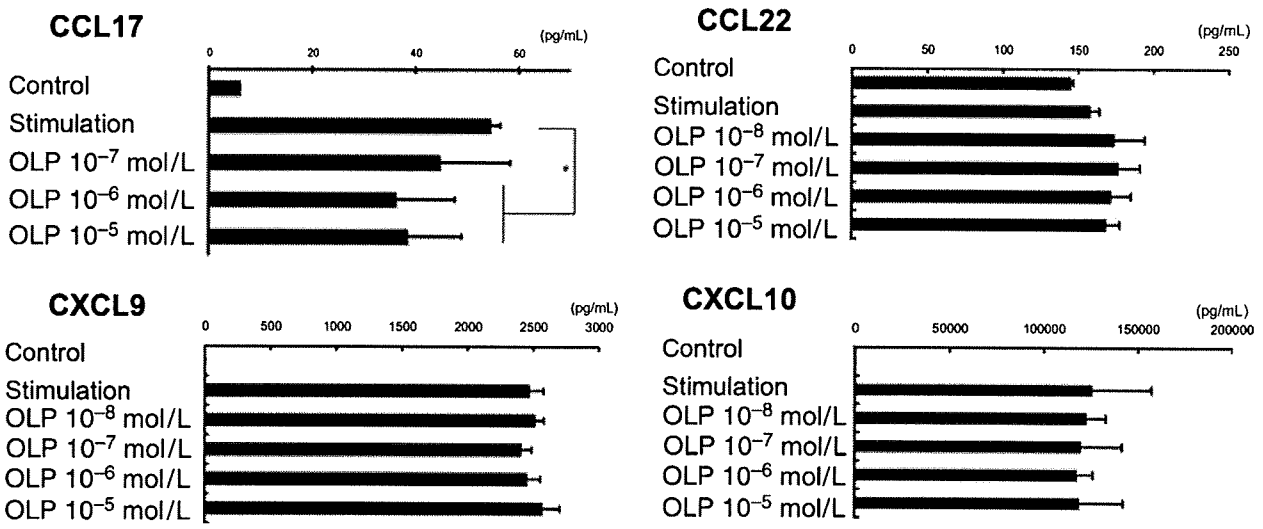

Figure 1. To examine chemokine production, semiconfluent HaCaT cells in 24-well plates were stimulated with 2000 units/mL of recombinant γ-interferon (IFN-γ) (Biogamma; Maruho Pharmaceutical, Osaka, Japan) and 4000 units/mL of tumor necrosis factor-α (TNF-α) (Invitrogen, Carlsbad, CA, USA) for the first 2 h, followed by 200 units/mL IFN-γ and 400 units/mL TNF-α thereafter. Olopatadine (OLP) was added at the starting of culture. Three-day culture supernatants were measured for CCL17, CCL22, CXCL9 and CXCL10 by enzyme-linked immunosorbent assay. Data represent the mean ± standard deviation. $^*P < 0.05$.

downregulated the expression of mRNA for CCL17 but not CCL22 (Fig. 2a). Because KC coexist with LC in LC-EC fraction, we also investigated the production of CCL17 by BMDC, a mimicry of pure LC. Murine immature DC were generated from bone marrow according to standard protocols.[14,15] Minor modification included feed culture medium on day 3 containing granulocyte-macrophage colony-stimulating factor (10 ng/mL). On day 6, BMDC (5×10^6/well) were cultured for 24 h with the two indicated concentrations of OLP. As shown in Figure 2(b), OLP decreased the mRNA expression of CCL17 and CCL22 in mature BMDC. Three independent series of experiments confirmed the result. CCL17 in culture supernatants was quantified by ELISA. OLP significantly suppressed the production of CCL17 by 37%, while the production of CCL22, CXCL9 or CXCL10 was not inhibited (data not shown). The above findings suggested that OLP directly downregulates Th2 chemokine production by DC and LC.

It has been reported that PAM 212 cells, a murine KC cell line, and normal human KC produce CCL17 after stimulation with TNF-α and IFN-γ.[16,17] Consistent with these *in vitro* data, CCL17 is expressed in the lesional KC of AD skin, suggesting that KC is one of the main sources of CCL17.[17] CCL17-transgenic mice showed enhanced Th2 type contact hypersensitivity and reduced Th1 type reactivity.[18] In this study, we demonstrated that OLP downmodulates the production of CCL17 by epidermal KC.

We have previously demonstrated that the ability of LC to present hapten to prime T cells was reduced by OLP with decreased expression of major histocompatibility complex class II and co-stimulatory molecules.[11] LC are capable of producing a high level of CCL17 constitutively during culture even without exogenous stimuli,[19] and we have recently shown that LC are responsible for the production of CCL17 by epidermal cells.[10] The present study showed that OLP inhibits the production of CCL17 by LC-EC. Thus, OLP is effective for the treatment of Th2-associated skin disorders not only by suppressing antigen-presenting ability but also by inhibiting CCL17 production. In our experiment system using LC-EC, KC coexisted with LC, raising the possibility that OLP alters CCL17 production by LC indirectly by modulating bystander KC. Therefore, another DC population without contamination of KC was tested for the modulatory effect of OLP on the chemokine production. Because Th2 chemokines including CCL17 was

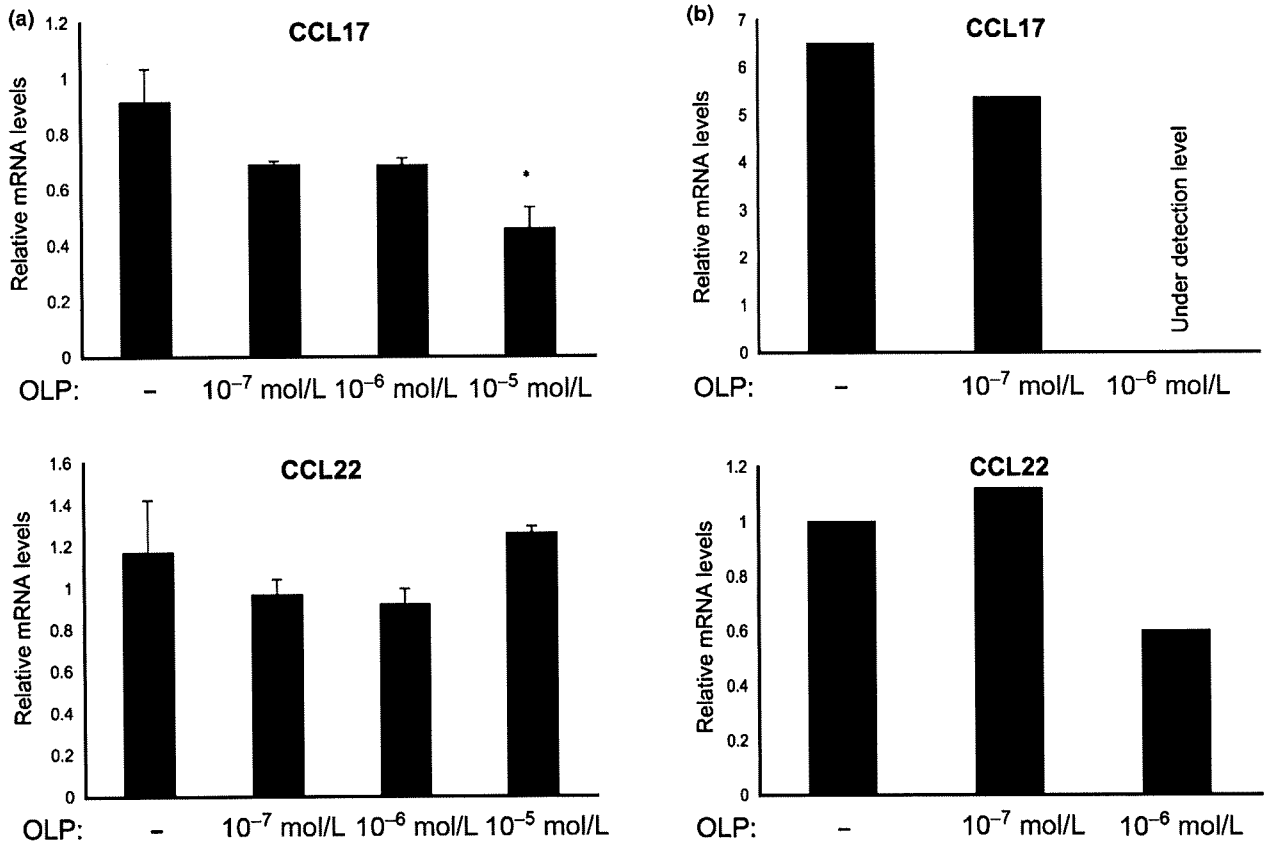

Figure 2. Total cellular RNA was extracted with an RNA extraction kit (Promega, Madison, WI, USA) from cultured Langerhans cell-enriched epidermal cells (LC-EC) and bone marrow-derived dendritic cells (BMDC). RNA was then reverse-transcribed and amplified by random hexamer in single-tube assay using the TaqMan Reverse Transcription Reagants (Applied Biosystems, Foster City, CA, USA) with gene-specific sense and antisense primers and a detection probe labeled on the 5′-end with the reporter dye 6-FAM. Primers and probes were obtained from TaqMan Gene Expression Assays Inventories (accession numbers: CCL17, Mm00516136-m1; CCL22, Mm00436439-m1; β-actin, 4352933E; all for Applied Biosystems). Using an ABI Prism 7000 Sequence Detection Systems (Applied Biosystems), samples were reverse-transcribed and amplified. Quantification of gene-specific message levels was determined by comparing fluorescence intensity from unknown RNA samples to the fluorescence intensity of standard curve generated from control mRNA levels. Amplification of the gene for mouse β-actin was performed on all samples to control interspecimen variations in RNA amounts. (a) mRNA expression for chemokines in LC-EC. LC-EC from naïve mice were cultured with or without olopatadine for 24 h. The cultured cells were subjected to real-time polymerase chain reaction analysis for CCL17 and CCL22. Data are expressed as the mean ± standard deviation of triplicate culture. *$P < 0.05$, compared with the olopatadine non-added one. (b) mRNA expression for chemokines in BMDC. BMDC were cultured for 24 h with or without olopatadine. The cultured cells were subjected to real-time polymerase chain reaction analysis for CCL17 and CCL22. The data are from a representative experiment out of three.

expressed in a subset of BMDC, we investigated the effect of OLP on CCL17 production by BMDC. OLP downregulated both CCL17 and CCL22 production by BMDC. Besides the effects of OLP on KC and LC, another study has shown that antihistamines regulate immune responses by affecting the interaction between DC and $CD4^+$ T cells.[20]

In summary, OLP suppresses the production of CCL17 by KC and DC. This suggests that OLP may exerts its therapeutic effect at least partly by down-modulating Th2 chemokine production by epidermal cells.

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CONFLICT OF INTEREST

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CLINICAL REPORT

Drug-induced Papuloerythroderma: Analysis of T-cell Populations and a Literature Review

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Papuloerythroderma of Ofuji is characterized by coalescent solid papules that spare the skin folds. Although cutaneous lymphomas and internal malignancies are known associated conditions, the causative agents are unclear in most cases. A number of recent reports have documented that drugs can induce papuloerythroderma. We reviewed the reported cases and our own cases of drug-induced papuloerythroderma, together with our data from lymphocyte transformation tests and T-cell subsets of peripheral blood. All of the 9 patients were male, and the causative drugs were various. Provocation tests were positive in all 6 patients examined. Whereas drug patch tests were negative in all 5 cases tested, the patients' peripheral blood lymphocytes responded well to the culprit drug in 4 of 5 patients tested. The patients had higher percentages of circulating $CCR4^+CD4^+$ T helper (Th) 2 cells than $CXCR3^+CD4^+$ Th1 cells. Drug-induced papuloerythroderma seems to be mediated by Th2 cells reacting with the causative drug. ***Key words: drug eruption; allergy; T cells.***

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Papuloerythroderma was first described in 1984 by Ofuji (1). This distinct disorder is characterized by coalescence of solid papules, which typically spare the skin folds, presenting the so-called "deck-chair" sign. Although its association with cutaneous T-cell lymphoma as well as visceral carcinomas has been documented in a considerable number of cases, the aetiology of the condition is unclear in the vast majority of patients (2). However, recent reports have indicated that drugs are causative agents for papuloerythroderma (3, 4) and have suggested that drug-reactive T-helper (Th) 2 cells play an important role in the pathogenesis (5).

The populations of circulating T cells can be skewed upon occurrence of T-cell-mediated drug eruptions (6, 7). In the usual development of drug eruption, peripheral T cells react with a causative drug, migrate to the skin, and trigger dermatitis as a consequence of immunological or inflammatory responses (8). In this article, we review the clinical, laboratory, and histological features and the clinical outcome of drug-induced papuloerythroderma in the literature, including our own 4 published cases. We also characterize circulating T-cell populations in patients with drug-induced papuloerythroderma, focusing on the *in vitro* T-cell response to the causative drug and the Th1/T cytotoxic (Tc)1 or Th2/Tc2 polarization.

MATERIALS AND METHODS

Blood samples were taken from our patients with drug-induced papuloerythroderma (cases 5, 6, 8 and 9 in Table I), with informed consent before treatment. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood of

Table I. *Reported cases of drug-induced papuloerythroderma*

Case (Ref)	Age (years)	Sex	Country	Past or associated diseases	Causative drugs	Other drugs	Latency period
1 (15)	60	M	Spain	Allergic rhinitis, conjunctivitis	Nicardipine hydrochloride, nifedipine	Trichlormethiazide	2 weeks
2 (3)	31	M	Japan	AIDS	Dideoxyinosine	ND	1 month
3 (16)	66	M	Japan	None	Cellulose	ND	Obscure
4 (17)	75	M	Japan	Right eye injury	Isoniazid	Rifampicin, streptomycin, ethambutol	1 week
5 (5)	70	M	Japan	Cerebral infarction	Aspirin	Carvedilol	10 years[a]
6 (9)	79	M	Japan	Chronic eczema, heart failure	Furosemide	ND	4 months
7 (13)	80	M	Japan	Pneumonia, diabetes mellitus	Ranitidine hydrochloride	Codeine phosphate, trapidil	2 weeks
8 (18)	78	M	Japan	Hypertension, angina pectoris	Eperisone hydrochloride	ND	2 years and 5 months[a]
9 (14)	82	M	Japan	Hypertension, prostate cancer	Leuprorelin acetate	Diltiazem hydrochloride	5 years[a]

[a]Intermittently administered for the period.
ND: not described.