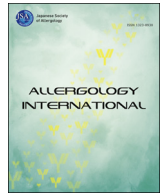




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Letter to the Editor

Multiple fixed drug eruption due to carbocysteine: Presence of circulating interferon- γ -producing CD8⁺ T cells reactive with its night metabolite thiodiglycolic acid

Dear Editor,

Carbocysteine (S-carboxymethyl-L-cysteine, SCLC) is widely used as adjunctive therapy for respiratory disorders with viscous

mucus.¹ Notably, the metabolic pathway of SCLC in humans are different between the daytime and the night. In the daytime, SCLC is metabolized to SCLC S-oxide and S-methyl-L-cysteine due to the daytime increased activity of S-oxidase. At night,

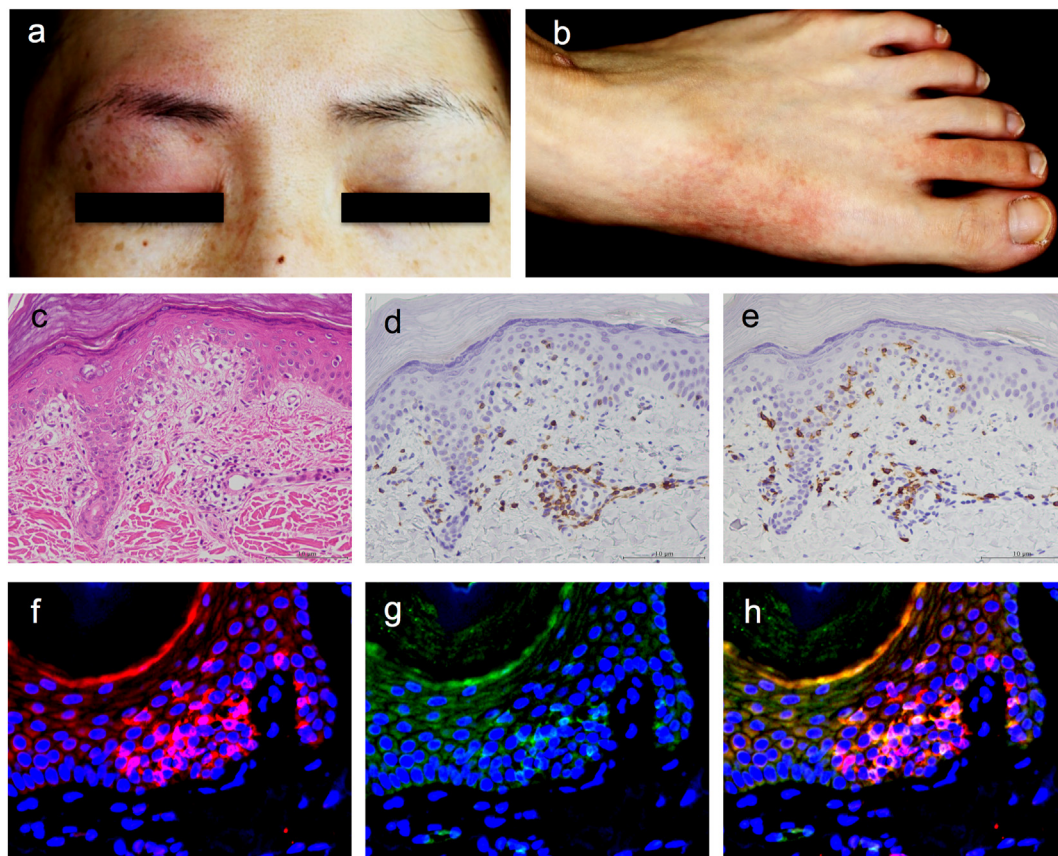


Fig. 1. Clinical and histopathological features. Clinical appearance of multiple fixed drug eruption upon oral SCLC challenge test. Erythematous lesions occurred on the right eyelid (a) and the left dorsum of the foot and the second toe (b). Histopathology, showing lymphocytic infiltration of the upper dermis with interface dermatitis (c). Immunostaining for CD3 (d) and CD8 (e). Immunofluorescence staining for CD8 (f), CD103 (g), and merged image of CD8 and CD103 (h). For use of the image of (a), a permission from the patient has been obtained.

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thiodiglycolic acid (TDA) is the main metabolite yielded through the tricarboxylic acid cycle, because the activity of S-oxidase is decreased at night due to its chronopharmacological properties.^{2,3} TDA is accumulated when SCLC is administered during the night for several days and can cause fixed drug eruption (FDE) as reported in a considerable number of cases.⁴ Here, we report a case of multiple FDE due to SCLC and demonstrate, for the first time, the presence of circulating CD8⁺ T cells that specifically respond to the night metabolite TDA.

A 33-year-old woman was referred to us because of four episodes of transient pruritic erythema during the past two years. The sites of her skin eruption were limited to the right upper eyelid, thenar of the right hand, inner aspect of the left foot, and second toe of the left foot. She noticed repeated occurrence of these four skin lesions approximately two days after oral administration of drugs including SCLC. We tentatively diagnosed her skin lesions as FDE and considered SCLC as the culprit drug.

Scratch-patch test with 10% SCLC in petrolatum was negative. Outsourced lymphocyte transformation test (LTT) with SCLC, as assessed by ³H-thymidine uptake, was under the cut-off level

of positivity. On challenge test, oral loading of SCLC, 500 mg three times a day for two consecutive days (days 0 and 1), evoked the eruption at the four sites in the early morning on day 2 (Fig. 1a, b). A punch biopsy specimen taken from the dorsum of left foot showed lymphocytic infiltration in the upper dermis, with vacuolar degeneration of the epidermal basal cells (Fig. 1c). Most of lymphocytes infiltrating in the epidermis were positive for CD3 (Fig. 1d) and CD8 (Fig. 1e), but negative for CD4. The diagnosis of FDE to SCLC was thus confirmed.

We have performed immunofluorescence staining of resident memory T (T_{RM}) cells in the active lesion of FDE (Fig. 1f–h), as reported previously.⁵ There were CD8⁺ T cells infiltrating in the epidermis (red; Fig. 1f), a half (52%) of which also expressed CD103 (green; Fig. 1g), indicating T_{RM} cells.^{5,6} A merged image (yellow; Fig. 1h) showed that CD8⁺CD103⁺ T_{RM} cells were almost exclusively found in the epidermis. As compared with psoriasis lesional skin, where almost all epidermal CD8⁺ T cells express CD103,^{5,6} the half-and-half infiltrates of T_{RM} and Tc1 populations might imply that T_{RM} cells were interacted with another CD8⁺ population.

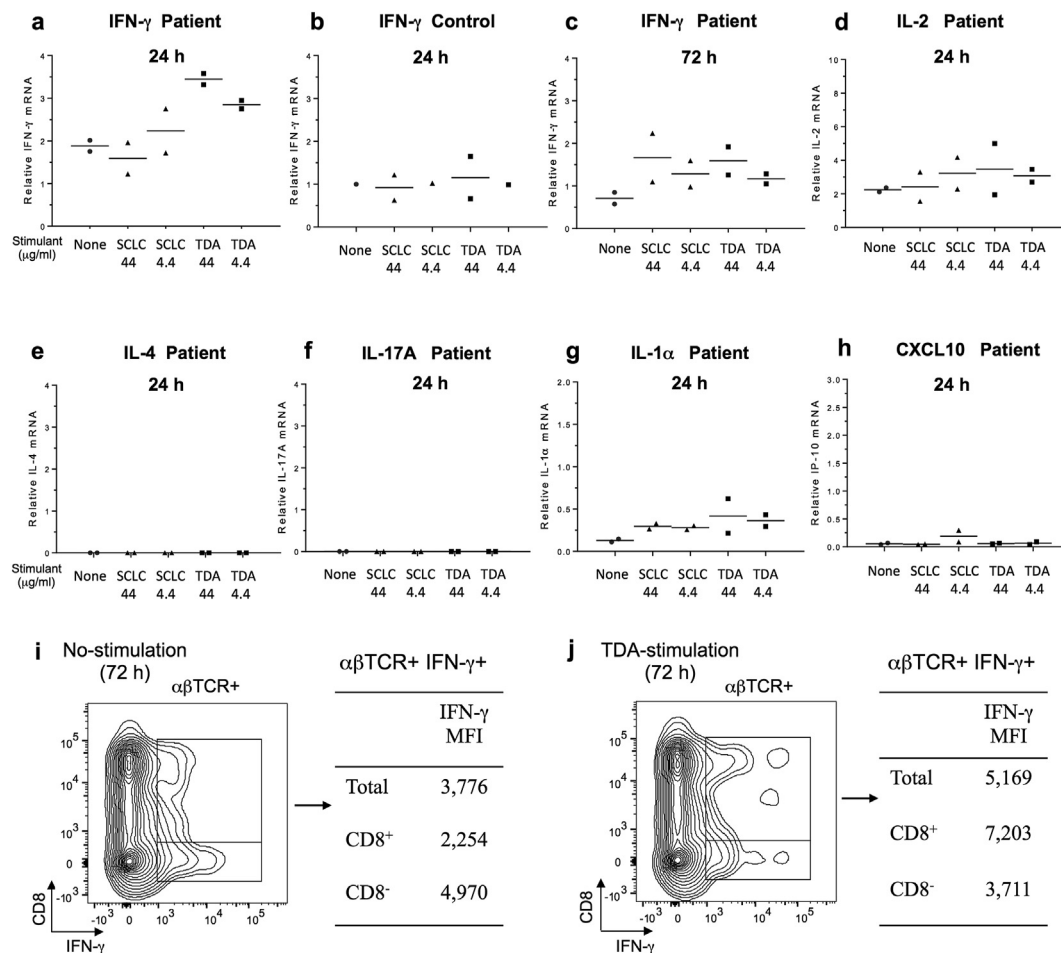


Fig. 2. *In vitro* IFN- γ expression of patient's PBMCs (CD8⁺ T cells) in response to TDA, but not SCLC. (**a–h**) Patient's PBMCs were cultured with the indicated concentration of SCLC or TDA in RPMI-1640 medium supplemented with 10% fetal calf serum at 37 °C for 24 h or 72 h. Total RNA was purified from PBMCs and reverse transcribed by using PrimeScript RT reagent Kit with gDNA Eraser. Real-time PCR analysis was carried out to compare the levels of gene expression using Thermal Cycler Dice Real Time System II. Expression levels were normalized by GAPDH mRNA, which was amplified by using ROX-labeled probe (5'-CATCAGCAATGCCTCTGCACCACCA-3') and a pair of specific primers (forward, 5'-GAACCATGAGAAGTATGACAACAGC-3' and reverse, 5'-TGGGTGGCAGTGATGGCA-3'). TaqMan probes for human IFN- γ , IL-2, IL-4, IL-17A, IL-1 α , and CXCL10 were obtained from Thermo Fisher Scientific. Relative cytokine mRNA represents the expression level relative to that of unstimulated PBMC from healthy control. (**i, j**) Patient's PBMCs were cultured with TDA at 44 μ g/ml for 72 h. Expression of IFN- γ in CD8⁺ and CD8⁻ cells was determined by using FACSscanTM. Data were analyzed by FlowJo software.

To examine the *in vitro* cytokine expression of patient's peripheral blood mononuclear cells (PBMCs), heparinized blood was taken one year after the oral challenge test. PBMCs were cultured in duplicate with the indicated concentrations of SCLC (FUJIFILM Wako Chemical Corporation, Miyazaki, Japan) or TDA (Sigma, St. Louis, MO). Total RNA was purified from PBMCs stimulated with or without SCLC or TDA for 24 h or 72 h. On real-time PCR analysis, the expression of IFN- γ by the patient's PBMCs was not increased by 24-h stimulation with SCLC, but enhanced by incubation with TDA at 44 μ g/ml (corresponding to 10 times of Cmax) and 4.4 μ g/ml (Cmax) in a dose-dependent manner (Fig. 2a). In a normal control subject, such an increase of IFN- γ expression by TDA was not found (Fig. 2b). In 72-h culture, both SCLC and TDA increased the IFN- γ expression to lesser degrees (Fig. 2c), raising a possibility that SCLC was metabolized to TDA or another cross-reactive substance. There were slight increases of mRNA levels of IL-2 (Fig. 2d) and IL-1 α (Fig. 2g) by 24-h cultivation with TDA at 44 mg/ml. Meanwhile, neither SCLC nor TDA increased the expression of IL-4 (Fig. 2e), IL-17A (Fig. 2f), or CXCR10 (Fig. 2h). The 72-h culture did not elevate the expression of all cytokines except for IFN- γ . The 72-h culture supernatants were subjected to IFN- γ ELISA, but the concentration of IFN- γ was under detection level in all samples.

For flow cytometric analysis, PBMCs were cultured with TDA at 44 μ g/ml for 72 h and subsequently incubated with 10^{-8} M phorbol 12-myristate 13-acetate and 10^{-6} M calcium ionophore in the presence of 5 μ g/ml Brefeldin A at for 4 h. Cells were stained with fluorescence-conjugated antibodies against $\alpha\beta$ T-cell receptor (TCR) and CD8, and fixed, permeabilized, and stained with phycoerythrin-labeled anti-IFN- γ antibody. T cells were gated as $\alpha\beta$ TCR $^{+}$ cells, and the expression of IFN- γ was assessed by mean fluorescence intensity (MFI) in the CD8-positive and negative cells. As compared with the no-TDA control (Fig. 2i), total T cells (CD8 $^{+}$ and CD8 $^{-}$) in PBMCs cultured with TDA expressed a high level of the IFN- γ MFI (Fig. 2j). The IFN- γ MFI was greatly higher in CD8 $^{+}$ T cells cultured with TDA than in those without TDA, but not in CD8 $^{-}$ T cells (Fig. 2i,j). Thus, the high expression of IFN- γ was attributable to CD8 $^{+}$ T cells.

This is the first report that circulating T cells in SCLC-FDE are reactive with the SCLC metabolite. Similarly to the reported cases,³ LTT was negative in our patient. LTT is tended to be positive when drug-reactive T cells are Th2 cells.⁷ FDE is induced by Tc1 cells or cytotoxic molecule-producing resident memory T (T_{RM}) cells.^{8,9} By real-time PCR analysis, we found that TDA, but not SCLC, stimulated the patient's CD8 $^{+}$ T cells to produce IFN- γ , suggesting that the metabolite is responsible for the drug eruption.

Of importance is the finding that drug-reactive CD8 $^{+}$ T cells exist even in the circulation of our patient. Skin T_{RM} cells play a role for the development of FDE.^{8,9} Provided that drug-specific, circulating CD8 $^{+}$ T cells are the first responder, they might interact with T_{RM}

cells to form FDE. Our patient strongly suggests that stimulation of circulating Tc1 cells with the SCLC metabolite participate in the mechanism of FDE to SCLC.

Conflict of interest

The authors have no conflict of interest to declare.

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