Unveiling the Immunological Mechanism Underlying a Cutaneous Adverse Reaction to Enfortumab Vedotin: A Case Report Based on the Lymphocyte Transformation Test

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Enfortumab vedotin (EV) is an antibody-drug conjugate that targets nectin-4. It has been approved for the treatment of locally advanced or metastatic urothelial carcinoma in cases of progression after treatment with platinum-based chemotherapy and inhibitors of programmed death receptor 1 or programmed death-ligand 1 [1]. Nectin-4, a transmembrane protein, is present on the surface of many urothelial carcinoma cells [2] and other cancer cells. Nectins, including nectin-4, play a crucial role in cell-cell adhesion, working alongside cadherins to form adherens junctions. In healthy human skin, nectin-4 is typically found in the suprabasal layers of the epidermis [3].

Late skin disorders are the most frequent adverse reactions in patients receiving EV. These are varied, ranging from erythema multiforme–like rash [1] and toxic erythema of chemotherapy [4] to Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS-TEN), which may be fatal [1,4,5]. To date, the reactions have been attributed to the mechanism of action of nectin-4, although no underlying immunological mechanisms have been documented.

We present the case of an 83-year-old man with stage IV urothelial carcinoma and retroperitoneal lymph node invasion diagnosed in June 2022. He initially received first-line treatment with carboplatin and gemcitabine, with avelumab as maintenance therapy. A computed tomography scan in February 2023 revealed a lytic lesion in the vertebral body of the third lumbar vertebra and pulmonary nodules in the right upper and lower lobes, indicating progression of his disease. Therefore, second-line treatment was started with EV (1.25 mg/kg on days 1, 8, and 15). Three days after completing a third cycle (dose) of EV, he developed widespread cutaneous erythema followed by blistering on the neckline and upper and lower limbs and desquamation. The laboratory values were normal. His oncologist prescribed antihistamines, oral corticosteroids, and local treatment, which improved the symptoms. As the patient responded well to EV, the oncologist wanted to know if he could continue the drug. Therefore, the patient was referred to the allergology department for further study. A lymphocyte transformation test (LTT) was performed with EV 6 weeks after the reaction. The patient gave his consent for his medical data to be reported here.

The LTT was performed according to Fernández-Lozano et al [6], with minor modifications. Peripheral blood mononuclear cells were isolated from whole blood using Ficoll (LymphoPrep) gradient centrifugation. The cells were resuspended in AIM-V medium (Gibco, Thermo Fisher Scientific Inc., 1×10^6 cells/mL) and cultured in 96-well round (U) bottomed plates (100 µL/well) containing the following stimuli: Dynabeads Human T-Activator CD3/CD28 (1 µL/well) (Gibco) as the positive control, AIM-V medium as the negative control (unstimulated condition), and EV (0.00001, 0.0001, 0.001, 0.01, 0.1, and 1 µg/µL). Cultures were grown in triplicate and incubated for 4 days at 37°C in an atmosphere of 5% CO_2/95% air. On day 4, the culture plates were centrifuged, and 100 µL of each well was replaced by fresh AIM-V medium containing 10 µCi of [3H]-thymidine. On day 6, cells were harvested using a vacuum manifold, and incorporation of radioactivity into DNA was measured using a liquid scintillation counter. Lymphocyte proliferation in cultures was expressed as a stimulation index (SI), which was calculated as the ratio of disintegrations per minute (dpm) of the drug-stimulated T cells and the mean dpm of the unstimulated T cells. An SI ≥3 was considered a positive response [6]. The patient had an SI greater than 3 at 3 concentrations of EV (Figure). This was considered positive according to Pichler and Tilch [7]. To validate our results, we also performed an LTT in 2 control patients with the same diagnosis, both receiving EV treatment, with no skin involvement. In each case, the LTT was negative (Figure).

Given that the skin reaction was a late event and SJS/TEN is a type IV hypersensitivity reaction mediated by T-cell activation [8], we performed in vitro testing, in this case LTT. The patient was offered patch testing with EV and skin biopsy but refused both. Although not validated, the LTT has been shown to serve as a valuable tool in clarifying the hypersensitivity mechanism of nonimmediate drug

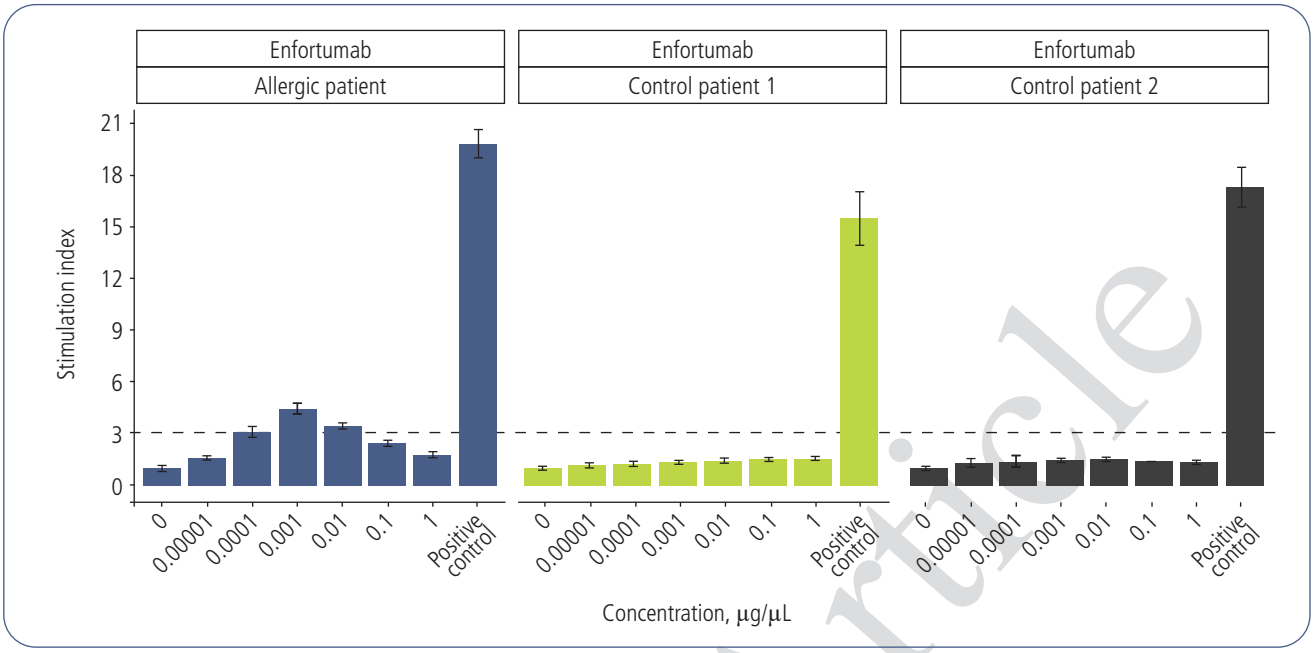

Figure. Lymphocyte transformation test. The stimulation index (SI) was calculated as the ratio of [3H]-thymidine incorporated by drug-stimulated cultures and basal [3H]-thymidine uptake by unstimulated cells. As standard criteria, an SI ≥3 in at least 1 concentration (above the dotted line) was considered positive. The positive control corresponds to cells treated with Dynabeads Human T-Activator CD3/CD28. All results are expressed as mean (SEM).

hypersensitivity reactions [9,10], especially in patients in whom skin testing or controlled exposure testing is not possible either because the techniques are not available or the reaction is too severe [10]. The LTT alone does not confirm T-cell hypersensitivity reactions. However, when combined with a relevant clinical history, it provides sufficient evidence to suggest a T-cell activation mechanism. Consequently, there may be underlying immunological mechanisms that could trigger adverse reactions, and not all EV reactions can be attributed to activity against nectin-4. Since the LTT result was positive, the treating oncologist suspended EV. As the patient's condition is now stable, he no longer receives chemotherapy. Moreover, his treating physician was advised that if he wished to prescribe any medication containing vedotin in the future, he should refer the patient back to allergology for further study. Cross-reactivity between vedotin-conjugated products has not been reported to date, and vedotin has not been studied in isolation. This could be interesting in the case of future treatment with vedotin-related products, where an LTT or drug provocation test should be performed to ensure the safety of re-exposure to this compound. To our knowledge, we report the first case of a positive LTT result with EV. Our findings highlight the potential of LTT as an encouraging and innovative approach for the diagnosis of T cell–mediated allergies to EV.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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