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Original Communication

# Stevens–Johnson syndrome/toxic epidermal necrolysis-like disease in human T-cell leukemia virus type 1 Tax transgenic mice

**Takeo Ohsugi***
Division of Microbiology and Genetics, Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan.

## ABSTRACT

Human T-cell leukemia virus type 1 (HTLV-1) can cause adult T-cell leukemia/lymphoma (ATLL) and inflammatory diseases such as HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). My research group established a transgenic (Tg) mouse model of HTLV-1 infection using the distal promoter of *Lck* to induce *tax* expression in mature thymocytes and peripheral T lymphocytes. The major disease phenotypes in this model were mature T cell leukemia/lymphoma, similar to ATLL, and inflammatory arthropathy. While expanding Tax-Tg mouse colony, my group found that about 2% of the Tg mice developed Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)-like disease. SJS/TEN-like lesions were characterized by a rash and diffuse exfoliation of large areas of the skin, similar to second-degree burns. The pathology of Tg mice with SJS/TEN-like disease included epidermal necrosis and detachment at the dermoepidermal junction. Serum soluble Fas ligand levels were significantly increased in Tax-Tg mice with SJS/TEN-like disease.

**KEYWORDS:** SJS/TEN, HTLV-1, ATLL, Tax, transgenic mice

*Present address: Laboratory of Animal Science, School of Veterinary Medicine, Rakuno-Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan.
ohsugi@rakuno.ac.jp

## ABBREVIATIONS

ATLL, adult T-cell leukemia/lymphoma; FasL, Fas ligand; H&E, hematoxylin and eosin; HAM, HTLV-1–associated myelopathy; HTLV-1, human T-cell leukemia virus type 1; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; Tg, transgenic; TSP, tropical spastic paraparesis; TDT-TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

## INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1) was the first human retrovirus to be isolated [1]. Some individuals infected with HTLV-1 develop adult T-cell leukemia/lymphoma (ATLL), an aggressive T cell malignancy, or inflammatory diseases such as HTLV-1–associated myelopathy/ tropical spastic paraparesis (HAM/TSP) and uveitis [2, 3]. HTLV-1 encodes the oncoprotein Tax, which modulates the expression of several genes that promote T cell transformation and appears to be a key molecule in the development of ATLL or other diseases [4]. Transgenic (Tg) mice expressing HTLV-1 Tax develop T-cell leukemia/lymphoma resembling ATLL or other diseases, such as arthropathy or HAM/TSP-like disease [5-7]. My research group also found that some Tax-Tg mice exhibited severe dermatosis, similar to second degree burns, that also occurs in Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) disease [8]. The author investigated whether the histopathology and etiology of severe

dermatosis in Tax-Tg mice resemble those of SJS/TEN.

## MATERIALS AND METHODS

### Animals

The generation of Tg mice expressing the *tax* gene under the control of the *Lck* distal promoter is described elsewhere [5]. The Tg mice (background strain BDF1) used here were the second- to sixth-generation progeny of a backcross into C57BL/6J mice (B6 mice). All mice with SJS/TEN-like lesions were male. This study was carried out in strict accordance with the Guidelines for Proper Conduct of Animal Experiments of the Science Council of Japan. All procedures involving animals and their care were approved by the Animal Care Committee of Kumamoto University in accordance with the regulations for animal experiments of Kumamoto University.

### Histological analysis

Tissue samples were fixed in 10% neutral-buffered formalin or 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin using standard protocols. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling assays were used to detect apoptotic cells on tissue sections according to the manufacturer's instruction (Merck Millipore, Billerica, MA, USA).

### Measurement of soluble Fas ligand (sFasL) and granulysin concentrations

The serum concentrations of sFasL and granulysin were measured using the following enzyme-linked immunosorbent assays (ELISA): Mouse Fas Ligand/TNFSF6 Quantikine ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) and mouse granulysin GNLY ELISA kit (MybioSource, Inc., SanDiego, CA, USA).

## RESULTS

SJS/TEN is characterized by a rash, bullae, and diffuse exfoliation of large areas of skin, similar to second-degree burns [8]. The severe dermatosis, as seen in second-degree burns, in these Tg mice mainly occurred in males derived from a C57BL/6 background ≥ 12 months of age. These mice showed a skin detachment rate exceeding 30% of the total skin area (Figure 1a). Histological analysis of skin sections revealed that the enlarged lesions from Tg mice displayed full-thickness epidermal necrosis and contained abundant necrotic keratinocytes (Figure 1b). Based on these results, the lesions with confluent epidermal necrosis can be differentiated from staphylococcal scalded skin syndrome [9], which is characterized by separation of the superficial layer of the epidermis. Because of the severity of the lesions, it was not possible to determine whether the early death of keratinocytes was due to apoptosis. Therefore, my research group performed pathologic studies of healthy skin and lesions near the severe lesions. Histological analysis of these areas revealed numerous apoptotic keratinocytes within the dermoepidermal junction of the epidermis (Figure 1c). These results suggest that the skin lesions with confluent epidermal necrosis in Tax-Tg mice closely resemble those of SJS/TEN [8].

Several soluble mediators contribute to keratinocyte death. In particular, secretory sFasL and granulysin were reported to be key mediators in the extensive keratinocyte death in SJS/TEN [10, 11]. Therefore, the serum levels of sFasL and granulysin were measured in Tg mice (Table 1). Of note, the serum sFasL level was significantly greater in Tg mice with SJS/TEN-like disease than in non-Tg mice. By contrast, the serum granulysin level was not significantly different between Tg mice with SJS/TEN-like disease and non-Tg mice.

## DISCUSSION

The results of this study indicate that Tg mice with severe dermatosis were clinically and histopathologically similar to SJS/TEN [8]. As in humans, the onset of SJS/TEN-like disease was rare in mice, occurring in just 10 of approximately 500 mice. However, the disease was only found in those Tg mice bred in my facility and not found in any wild-type mice or other genetic models. There are no reports describing a direct relationship between HTLV-1 infection and SJS/TEN. However, in the clinical course of ATLL, the skin is the most common extranodal site of the disease. In addition, Ohtani *et al.* [12] reported erythema-multiforme-like eruptions, very similar to SJS/TEN, after radiotherapy in a patient with ATLL. More recently, it has been reported that patients with ATLL developed SJS/TEN during treatment with

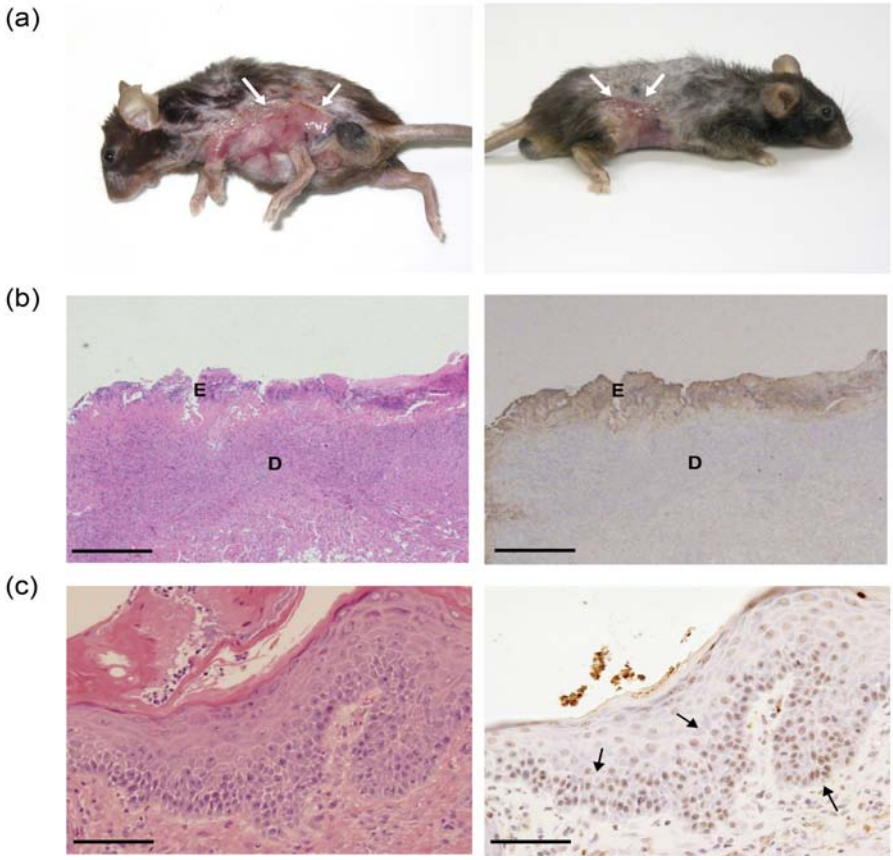


**Figure 1.** Transgenic mice expressing Tax (Tg mice) develop Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN)-like disease. (a) The mice had SJS/TEN-like skin lesions similar to second-degree burns (white arrows). (b) Histological analysis of a skin section with a SJS/TEN-like lesion from a Tg mouse. Hematoxylin and eosin (H&E) staining (left) and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TDT-TUNEL; right) revealed considerable skin necrosis similar to that seen in SJS/TEN. E, epidermis; D, dermis. Scale bars: 500 μm. (c) Histological analysis of healthy skin samples from near the SJS/TEN-like lesion. H&E staining (left) and TDT-TUNEL staining (right). Arrows indicate apoptotic keratinocytes. Scale bars: 100 μm.

**Table 1.** Serum levels of sFasL and granulysin in transgenic mice expressing Tax.

| Disease phenotype | n | sFasL (pg/ml) | Granulysin (ng/ml) |
|---|---|---|---|
| SJS/TEN-like | 3 | 43.1 ± 11.6* | 3.7 ± 0.4 |
| Leukemia | 7 | 28.9 ± 9.9 | 5.1 ± 1.3 |
| Arthritis | 9 | 22.3 ± 4.0 | 7.1 ± 0.9 |
| HAM-like | 1 | 17.5 | n.d. |
| Healthy Tax (+) | 3 | 14.3 ± 4.0 | 5.5 ± 1.3 |
| Tax (−) | 3 | 7.9 ± 0.6 | 4.4 ± 0.7 |

Values are expressed as the mean ± standard error.
*$P < 0.05$ versus Tax(−) mice.
sFasL, soluble Fas ligand; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; HAM, human T-cell leukemia virus type 1–associated myelopathy; n.d., not done.

mogamulizumab, a humanized anti–C-C chemokine receptor type 4 monoclonal antibody [13-15].

The Tg mice with SJS/TEN-like disease had elevated serum sFasL levels but normal granulysin levels. Patients with HAM/TSP are well known to have high expression of sFasL mRNA in $CD8^+$ T cells and increased levels of sFasL in the cerebrospinal fluid [16]. Therefore, FasL may contribute to the pathogenesis of HAM/TSP. I also speculated that Fas–FasL interactions directly induce the apoptosis of epidermal keratinocytes in Tg mice with SJS/TEN. My research group is now studying whether this Tg mouse model is a good animal model for the analysis of skin disorders that occur during the clinical course of ATLL.

## CONCLUSION

This study indicates that Tg mice with severe dermatosis were clinically and histopathologically similar to SJS/TEN. The Tg mice with SJS/TEN-like disease had elevated serum sFasL levels, and therefore, Fas-FasL interactions may contribute to the pathogenesis of the disease.

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## CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interests.

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