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TECHNICAL NOTE

A novel mouse model of adult T-cell leukemia cell invasion into the spinal cord

Takeo Ohsugi[1] | Shuhei Tanaka[1] | Keigo Iwasaki[1] | Yusuke Nagano[1] | Tomohiro Kozako[2] | Kazuya Matsuda[3] | Takuya Hirose[4] | Kazushige Takehana[4]

[1]Department of Laboratory Animal Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[2]Faculty of Pharmaceutical Sciences, Department of Biochemistry, Fukuoka University, Fukuoka, Japan

[3]Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[4]Department of Veterinary Microanatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

Correspondence
Takeo Ohsugi, Department of Laboratory Animal Science, School of Veterinary Medicine, Rakuno-Gakuen University, Ebetsu, Hokkaido, Japan.
Email: ohsugi@rakuno.ac.jp

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Abstract

Adult T-cell leukemia (ATL) is a mature T-cell malignancy caused by human T-cell leukemia virus type I infection, and 10%-25% of patients show central nervous system (CNS) involvement. CNS involvement significantly reduces survival and there are no effective treatments for CNS involvement. Therefore, an appropriate animal model is required to evaluate the inhibitory effects of novel drugs on the progression of ATL with CNS involvement. Here, we established a mouse model of ATL with CNS involvement using NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ mice inoculated with ATL cells intramuscularly in the postauricular region, and these mice showed paraparesis. Of the 10 mice inoculated with ATL cells intramuscularly (I.M.) at 5 weeks of age, 8 (80%) showed paraparesis, whereas none of the 10 mice inoculated with ATL cells subcutaneously (S.C.) showed paraparesis. In the I.M. group, PCR detected HTLV-1-specific genes in the thoracic and lumbar vertebrae; however, in the S.C. group, the vertebrae were negative for HTLV-1 genes. Histological analysis revealed a particularly high incidence of tumors, characterized by accumulation of the injected cells, in the thoracic vertebrae of mice in the I.M. group. Tumor cell infiltration was relatively high in the bone marrow. Spinal cord compression caused by invasion of the tumor mass outside the pia mater was observed in the thoracic vertebrae of the spinal cord. In conclusion, we have reported a mouse model of tumor growth with paraparesis that may be used to assess novel therapeutic agents for ATL with CNS involvement.

KEYWORDS

adult T-cell leukemia (ATL), central nervous system (CNS), human T-cell leukemia virus type I (HTLV-1), mice, NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ mice

1 | INTRODUCTION

Adult T-cell leukemia (ATL) is a mature T-cell malignancy caused by human T-cell leukemia virus type I (HTLV-1) infection, which is endemic to Japan, the Caribbean islands, and Central and South America.[1,2] ATL is a lethal disease for which there is no effective treatment.[3] Central nervous system (CNS) involvement in ATL may occur during systemic progression of the disease, with an estimated frequency of 10%-25%.[4,5] Although CNS involvement significantly reduces survival, the mechanism of CNS involvement is unclear. Furthermore, despite good descriptions of animal models of ATL, including those involving organs other than the CNS, an animal model of ATL with CNS involvement has not yet been described.[6]

In the present study, we established a mouse model of ATL with CNS involvement using NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice inoculated with ATL cells intramuscularly (I.M.) in the postauricular region, and these mice showed paraparesis.

2 | METHODS

NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) female mice, 4 weeks of age, were obtained from Charles River Japan (Tokyo, Japan). The mice were housed in autoclaved polycarbonate cages with paper chip bedding (Japan SLC, Inc., Hamamatsu, Japan) covered with filter caps (CLEA Japan, Inc. Tokyo, Japan), placed in isolation cabinets (BBH Unit, Seobit Inc., Tokyo, Japan), and fed a sterile irradiated diet (CLEA Japan) with free access to acidified autoclaved water. The animal room was maintained under barrier-sustained conditions and controlled temperature (23 ± 2°C) and lighting (12-hour light/dark cycle). After 1 week of preliminary care, the mice were used for experiments. This study was carried out in strict accordance with the Guidelines for Proper Conduct of Animal Experiments, Science Council of Japan (http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2e.pdf). All animal procedures and care were approved by the Animal Care and Use Committee of Rakuno-Gakuen University in accordance with the Guide for the Care and Use of Laboratory Animals. NSG mice (5 weeks old) were inoculated with 5×10^6 S1T cells (HTLV-1-infected leukemic CD4$^+$ T cells derived from an ATL patient)[7] subcutaneously (S.C.) or intramuscularly (I.M.) in the postauricular region (Figure 1A). For S.C. inoculation, the injection needle was inserted almost parallel to the body axis. For I.M. inoculation, the injection needle was inserted at an angle of 45° to the body axis. Animals were euthanized by isoflurane inhalation by 4 weeks after inoculation. The mice were autopsied and their organs collected for histopathological studies.

3 | RESULTS

Of the 10 mice inoculated with S1T cells I.M. at 5 weeks of age, 8 (80%) showed spastic paraparesis, whereas none of the 10 mice inoculated with S1T cells S.C. showed spastic paraparesis (Figure 1B, Video S1). The body weight of mice in the I.M. group decreased 2 weeks after inoculation, whereas that of mice in the S.C. group increased continuously during the observation period (Figure 1C). Serial changes in the subcutaneous tumor volume in mice inoculated with cells S.C. or I.M. are shown in Figure 1D. Tumors in both groups of mice appeared within 2 weeks of inoculation and showed the same growth patterns during the observation period. The tumor was restricted to the cervical skin in the S.C. group, whereas the tumor had invaded into the cervical vertebrae in the I.M. group (Figure 2A). To detect infiltration of the inoculated cells into the vertebrae, genomic DNA extracted from the vertebrae was subjected to PCR analysis using primers specific to the tax gene sequence of HTLV-1 (Figure 2B, upper panel).[8] In the I.M. group, the injected cells were present in the thoracic and lumbar vertebrae; however, in the S.C. group, the vertebrae were negative for the HTLV-1 tax gene (Figure 2B, lower panel). Hematoxylin and eosin staining of tissue sections from I.M. group mice revealed a particularly high incidence of tumors, characterized by accumulation of the injected cells, in the thoracic vertebrae. Tumor cell infiltration was relatively high in the bone marrow. Spinal cord compression caused by invasion

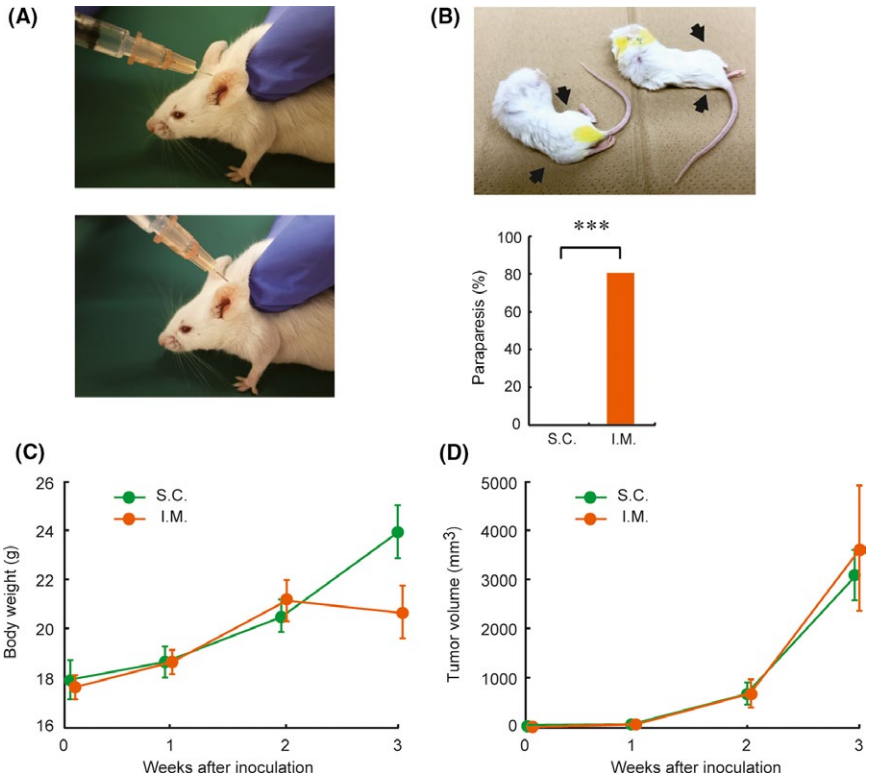

FIGURE 1 Onset of paraparesis in NSG mice inoculated with S1T cells intramuscularly (I.M.). A, Subcutaneous (S.C.; upper panel) and intramuscular (I.M.; lower panel) administration of S1T cells. B, Paraparesis in NSG mice inoculated with S1T cells I.M. (upper panel, arrows). Eight of ten animals (80%) in the I.M. group showed paraparesis, whereas none of the 10 mice inoculated with S1T cells S.C. showed paraparesis (lower panel). ***$P < 0.001$ by Fisher's exact test. C, Serial changes in body weight in the S.C. and I.M. groups. Data represent means ± SEM. D, Serial changes in subcutaneous tumor volume in the S.C. and I.M. groups

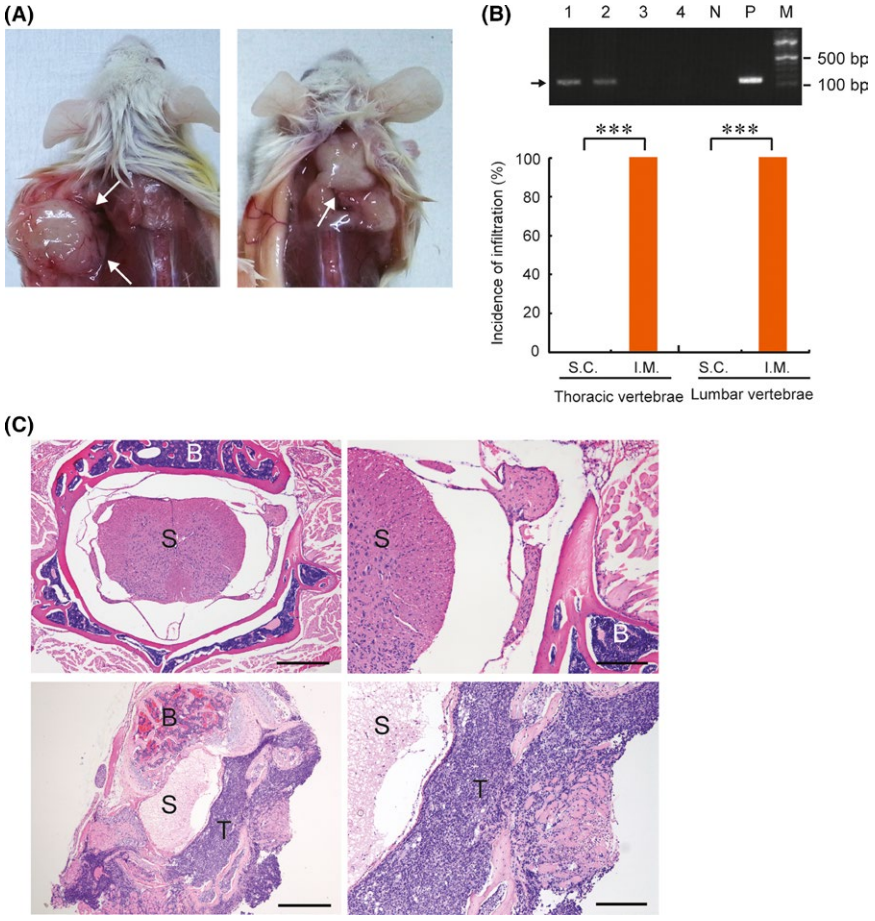

FIGURE 2 Pathological findings in NSG mice inoculated with S1T cells subcutaneously (S.C.) or intramuscularly (I.M.). A, Gross tumor formation in NSG mice in the S.C. (left panel, arrow) and I.M. groups (right panel, arrow). B, The upper panel shows agarose gel electrophoresis of the PCR products obtained from the vertebrae of representative mice from the S.C. and I.M. groups. PCR detection of HTLV-I tax (159 bp PCR product) was performed to assess infiltration of the inoculated cells into the vertebrae. Lane 1, thoracic vertebrate from I.M. group; lane 2, lumbar vertebrate from I.M. group; lane 3, thoracic vertebrate from S.C. group; lane 4, lumbar vertebrate from S.C. group; N, no template DNA; P, S1T cells; M, 100 bp ladder marker. Infiltration of S1T cells into the thoracic and lumbar vertebrae in the S.C. and I.M. groups was detected by PCR using primers specific to the tax gene of HTLV-1 (lower panel). ***$P < 0.001$ by Fisher's exact test. C, Histological findings (hematoxylin and eosin staining) in the vertebrae of mice from the S.C. group (upper) and I.M. group (lower). The right panel shows a high-power view of the left panel. Scale bars: 500 μm (left) and 200 μm (right). B, bone marrow; S, spinal cord; T, tumor

of the tumor mass outside the pia mater was observed in the thoracic vertebrae of the spinal cord (Figure 2C, lower panel). No lesions were found in the spinal cord in the S.C. group (Figure 2C, upper panel).

4 | DISCUSSION

Currently, the method used to generate a mouse model of ATL involves S.C. inoculation of ATL cells into the postauricular region of immunodeficient mice.[6] This model is useful for determining therapeutic strategies for ATL.[9] However, it is difficult to establish an animal model of ATL with CNS involvement. Here, we showed the feasibility of generating a mouse model of ATL with CNS involvement by injecting cells I.M. I.M. administration is a common parenteral route used in large animals and humans but is often avoided in smaller species because of their reduced muscle masses.[10] Ishihara et al reported that one of two severe combined immunodeficiency mice injected with HTLV-1-infected cells I.M. showed paresis of the left hind leg on the injection side.[11] Similarly, our study showed spastic paraparesis, a more severe form of paresis, in NSG mice injected with HTLV-1-infected cells I.M. in the postauricular region. Injection of cells in the postauricular-to-dorsal region may be important for development of paraparesis because of the potential for invasion of the injected cells into the vertebrae. I.M. injection results in uniform and rapid absorption of substances because of the rich vascular supply and nerve abundance.[10]

In conclusion, here we report a mouse model of tumor growth with paraparesis that may be used to assess novel therapeutic agents for the treatment of ATL with CNS involvement.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

TO designed and coordinated the overall study; ST, KI and YN carried out the experimental work. KM and TH conducted pathological examinations. TO, TK and KT analyzed the data. All authors discussed the results and wrote the paper.

ORCID

Takeo Ohsugi https://orcid.org/0000-0002-7818-6840

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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