RAPID COMMUNICATION Jpn. J. Cancer Res. 84, 831–833, August 1993

HTLV-1-associated Myelopathy/Tropical Spastic Paraparesis-like Rats by Intravenous Injection of HTLV-1-producing Rabbit or Human T-Cell Line into Adult WKA Rats

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We intravenously injected Ra-1 cells or MT-2 cells into female adult WKA rats. Spastic paraparesis mainly in the hind-limbs was observed in 1 out of 2 Ra-1 cell-injected WKA rats and in 3 out of 8 MT-2 cell-injected WKA rats 20–27 months after injection. The main neuropathological finding was symmetrical white matter degeneration with mononuclear cell infiltration of the spinal cord, similar to that of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients, and degeneration of nerve roots and peripheral nerves. Antibodies against HTLV-1 antigens were detected in plasma and cerebrospinal fluid from these HAM/TSP-like rats. HTLV-1 provirus was detected from the peripheral blood mononuclear cells of one of these rats 20 months after injection. Interestingly, spastic paraparesis was not observed in F344 rats.

Key words: HTLV-1 — HAM/TSP — MT-2 — Ra-1 — Animal model

Human T cell leukemia virus type-1 (HTLV-1) is considered to be the causative virus of adult T-cell leukemia (ATL).[1-3] Recently, HTLV-1 was also claimed to be associated with tropical spastic paraparesis (TSP)[4] and HTLV-1 associated myelopathy (HAM),[5] which are considered to be the same clinical entity. However little is known about how HTLV-1 infection is involved in the manifestation of HAM/TSP.

An animal model of ATL or HAM/TSP should be useful in studies to clarify the pathogenesis, and to find means to prevent the manifestation and to develop effective therapy of these diseases. As the first step, we have established an HTLV-1 carrier rat model by intravenous administration of HTLV-1-producing T-cell lines.[6] We injected 4×10^7 Ra-1 cells, an HTLV-1-producing rabbit cell line,[7] intravenously into 2 female WKA rats of 4 weeks old. During the follow-up of these rats one showed spastic paraparesis of the hind-limbs 27 months after injection. We next injected 2×10^6 MT-2 cells, an HTLV-1-producing human T-cell line,[8] twice intravenously into either 8 female WKA rats or 8 female F344 rats aged 3 weeks and 4 weeks. Spastic paraparesis was observed in 3 of 8 WKA rats 20 months after MT-2 cell injection, but not in any of the F344 rats.[9]

The major neuropathological finding was symmetrical white matter degeneration with mononuclear cell infiltration of the cervical and thoracic segments of the spinal cord, which was most prominent in an Ra-1-injected rat (Fig. 1A). This finding was quite similar to that in human HAM/TSP. However, infiltrating mononuclear cells in the rats were macrophages and not lymphocytes (Fig. 1B), which is different from the reported human HAM/TSP cases,[11] and resembles the spinal cord changes in AIDS cases.[12] In addition, the nerve fibers of spinal nerve roots (Fig. 1C), cauda equina and sural nerves showed active axonal degeneration with foamy macrophages. Although HAM/TSP is a disease entity involving the spinal cord, there are some reports of peripheral nerve involvements in HAM/TSP.[13,14]

The antibody titers against HTLV-1 antigens, assayed by the particle agglutination method (Fujirebio, Tokyo), were in the range of 16- to 64-fold in plasmas isolated 20–30 months after injection in the 4 rats with neurological symptoms. The antibody titers in the cerebrospinal fluid were 32-fold in 2 MT-2-injected HAM/TSP-like rats and below 8-fold in 1 Ra-1-injected HAM/TSP-like rat 20–30 months after cell injection. The cerebrospinal fluid of the other MT-2-injected HAM/TSP-like rat was not

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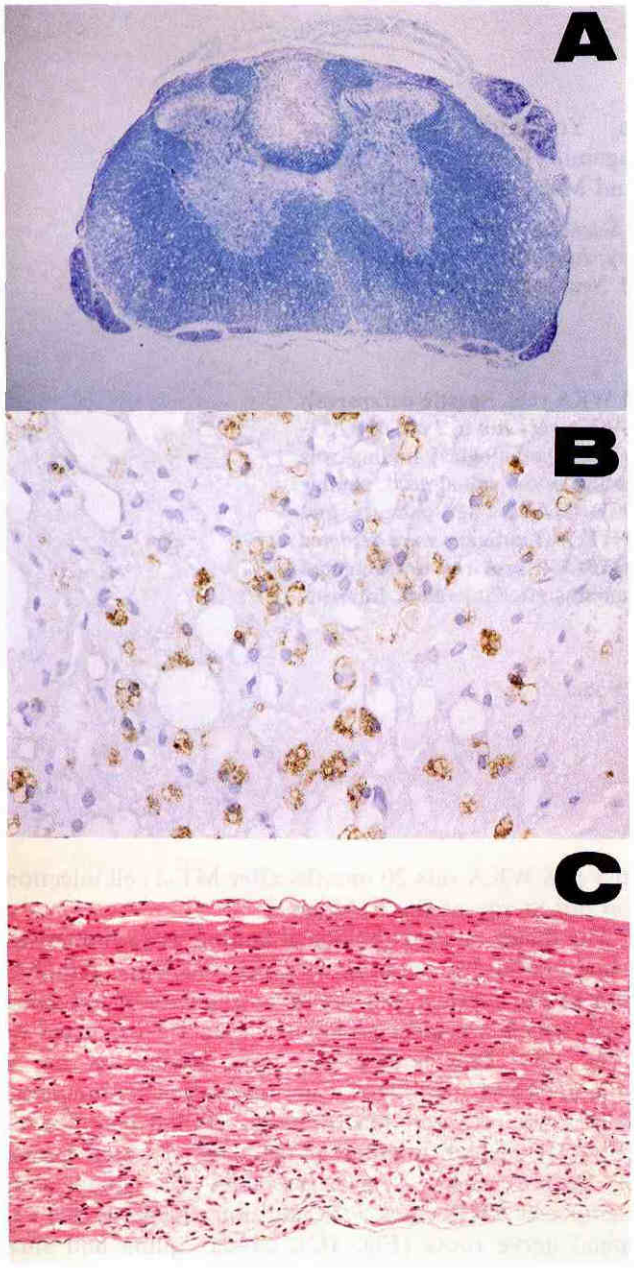

Fig. 1. Neuropathological changes in HAM/TSP-like rats injected with HTLV-1-producing cell lines. A: symmetrical degeneration of the lateral and posterior funiculi of the thoracic cord of an Ra-1 cell-injected rat. Klüver-Barrera stain,[10] ×7.5. B: infiltration of macrophages in the lateral funiculus of the thoracic cord of the Ra-1 cell-injected rat. Immunostaining with antibody against rat macrophages (ED-1), ×100. C: active axonal degeneration with foamy macrophages in the posterior nerve root of lumbar cord of an MT-2-injected rat. H-E, ×50.

analyzed. There is no significant difference of the antibody titers in plasma between HAM/TSP-like WKA rats and other WKA rats without symptoms. The HTLV-I provirus in the peripheral blood mononuclear cells was detected by polymerase chain reaction in 1 of 3 MT-2-injected HAM/TSP-like rats after they had been killed. The lowest number of target molecules detectable by this method is 1–2 molecules of HTLV-1 provirus in 10^5 rat peripheral blood mononuclear cells.[6]

HAM/TSP have been reported to occur in HTLV-1 carriers with certain haplotypes of HLA.[15] Our findings of the presence of neurological symptoms in WKA rats with *k* haplotype of MHC[16] but not in F344 rats with *lv1* haplotype might be consistent with this notion. In addition, it was reported that a significant proportion of HAM/TSP patients have a past history of blood transfusion,[17] and 50% of HAM/TSP patients with a history of blood transfusion had developed clinical manifestations within 3.3 years after the transfusion.[18] Intravenous administration of HTLV-1-carrying cells into adult rats seems to be a reasonable model of human HAM/TSP associated with blood transfusion. Recently, Ishiguro *et al.*[19] reported the appearance of HAM/TSP-like myelopathy in three seronegative WKA rats about 16 months after intraperitoneal injection of MT-2 cells at the neonatal stage. There is a clear difference in the presence of the antibody against HTLV-1 in HAM/TSP-like rats between the present work and that by Ishiguro *et al.*[19] It is quite interesting that HAM/TSP-like symptoms were observed in the same strain of rats. Our work also demonstrated that HAM/TSP-like symptoms occurred following injection of not only an HTLV-1-producing human cell line, MT-2 cells, but also an HTLV-1-producing rabbit cell line, Ra-1 cells. This might suggest that HTLV-1 and not the antigen of a different species other than rat is involved. In addition, we wish to stress that neuropathological changes in the peripheral nerves were found in our HAM/TSP-like rats.

Although there are some differences of the neuropathological findings between our HAM/TSP-like rats and human HAM/TSP, our rat model system using HTLV-1-producing rabbit or human cells should be useful to clarify the pathogenesis of the wide spectrum of neuropathological findings associated with HAM/TSP.

We are grateful to Dr. Kunitada Shimotohno for his encouragement. This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for the Comprehensive 10-Year Strategy for Cancer Control, Japan.

(Received March 31, 1993/Accepted May 20, 1993)

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