Impairment of Trk-Neurotrophin Receptor by the Serum of a Patient With Subacute Sensory Neuropathy

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Background: Paraneoplastic peripheral neuropathy is sometimes associated with unidentified neuronal autoantibodies.

Objective: To examine the effects of serum from a patient with subacute sensory axonopathy on the function of the Trk high-affinity nerve growth factor receptor.

Patient: An 86-year-old man with sensory neuropathy exhibiting an autoantibody to Trk.

Methods: Immunoblot analyses of the brain homogenates and immunoprecipitation were performed with human sera. We further examined the effect of sera on nerve growth factor–induced neurite outgrowth and Trk autophosphorylation.

Results: The patient showed sensory nerve axonopathy without well-known paraneoplastic autoantibodies. His serum inhibited nerve growth factor–induced neurite outgrowth and Trk autophosphorylation in PCTrk cells. Moreover, the patient’s serum, but not control serum, immunoprecipitated Trk and recognized Trk in brain homogenates as well as in Trk immunoprecipitates.

Conclusion: These data strongly suggest that an anti-Trk autoantibody might cause subacute sensory neuropathy.

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TRK IS A HIGH-AFFINITY NERVE growth factor (NGF) receptor, 1 of the cardinal growth factors of the neurotrophin family that support neuronal survival and differentiation. Following ligand binding, the Trk receptor is activated by homodimerization. The degree of tyrosine autophosphorylation correlates well with the biological effects in responsive cells.

Paraneoplastic neurological syndromes include a wide variety of disorders that affect the central and peripheral nervous systems. They appear to have an autoimmune pathogenesis, as suggested by the presence of autoantibodies directed against antigens shared by both neurons and cancers. To date, a role for paraneoplastic autoantibodies in the development of neuronal dysfunction has not been established in the majority of cases. The involvement of antigen-specific cytotoxic T lymphocytes has been discussed, but further investigation is still required to identify the mechanisms of cytotoxic T-lymphocyte–induced neuronal dysfunction.

We now describe an anti-Trk autoantibody that was found in a male patient with sensory neuropathy. His serum provoked functional disturbances of NGF–dependent molecular events in PCTrk cells. This sheds light on the possibility of anti-Trk autoantibody–positive sensory neuropathy.

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An 86-year-old man complained of numbness and burning pain in the extremities. He had a history of non-Hodgkin lymphoma on the stomach 5 years previously, and he had been in complete remission since. Five months before admission, he developed sensory disturbances in the lower limbs. On admission, neurological examination revealed nonspecific findings aside from the decreased deep tendon reflexes and sensory disturbances. Nerve conduction examination revealed sensory nerve axonopathy (Table). He had coarse and dry skin as well as bladder dysfunction. Cerebrospinal fluid examination revealed an increased protein content (60 mg/dL).
the normal level is <40 mg/dL) without pleocytosis. Magnetic resonance imaging of the brain revealed minimal lacunar infarctions. Results were normal in extensive hematological evaluations and negative in bone scintigraphy, positron emission tomography with [18F]fluoro-2-deoxy-D-glucose, and gastrointestinal fiberoptic examinations. Examination results of complete blood cell count, coagulation profiles, C-reactive protein, antinuclear antibodies, antineutrophil cytoplasmic antibodies, rheumatoid factor, complement levels for C3 and C4, serum protein electrophoresis, cryoglobulins, plasmic antibodies, rheumatoid factor, complement levels for C3 and C4, serum protein electrophoresis, cryoglobulins, quantitative serum immunoglobulins, vitamins B1, B2, and B12, and folic acid were all normal without anti-Hu and anti-Yo antibodies. The patient was given a large dose of intravenous human immunoglobulin, with an improvement of sensory symptoms and skin manifestations after treatment, and some improvement in sensory nerve action potential (Table).

### RESULTS

#### BONE MARROW ASPIRATION FINDINGS

Examination of bone marrow aspirate revealed a hypocellular marrow with erythroid predominance and normoblastic erythropoiesis. There was a partial aplasia of granulocytic and myeloid series. There were no typical features of acute myeloid leukemia. The bone marrow biopsy revealed a hypocellular marrow with a normal architecture and some improvement in sensory nerve action potential.

#### WESTERN BLOT ANALYSIS

Human brain samples obtained at autopsy by informed consent were suspended in 25 mM Tris-HCl buffer (pH 7.4) containing 100 mM ethyleneglycoltetraacetic acid, 1 mg/mL of leupeptin, and 1 mM phenylmethylsulfonyl fluoride, and then homogenized with a blender (Waring, Torrington, Conn). Equal amounts of protein determined by the method by Bradford were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by immunoblot analysis, and they were probed with diluted serum (0.1% final concentration) in blocking buffer containing 0.1% Nonidet P-40 (Nakatari Teque Inc, Kyoto, Japan) and 1% nonfat milk with appropriate secondary antibody. The positive bands were detected with chemiluminescence (PerkinElmer Inc, Boston, Mass).

#### IMMUNOBLOT ANALYSIS

Although there was some nonspecific reaction between the proteins, a specific 140-kilodalton band against brain homogenates was only present when probed with the patient’s serum (compare lanes 2 and 3 of Figure 2A with lanes 2 and 3 of Figure 2B). Taken together, we hypothesized that this band corresponds to Trk. To test this hypothesis, Trk was immunoprecipitated with α-Trk and subjected to immunoblot using the patient’s serum. The patient’s serum clearly detected the presence of Trk (Figure 3A, lane 2), and no reaction was observed with control sera (Figure 3A, lane 1). Then, we performed an immunoprecipitation using the patient’s serum, and we

<table>
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<th>Nerve</th>
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<th>CMAP, mV</th>
<th>SCV, m/s</th>
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Abbreviations: CMAP, compound muscle action potential; MCV, mean corpuscular volume; NA, not applicable; SCV, subclavian vein; SNAP, sensory nerve action potential.

### CELL CULTURE AND IMMUNOPRECIPITATION

Rat pheochromocytoma-derived PC12 cells and their stable transfectants of human trk complimentary DNA (Pctrk cells) were cultured as described. Trk was immunoprecipitated with an anti-Trk antibody (α-Trk; Santa Cruz Biotech Inc, Santa Cruz, Calif.). To test whether sera can immunoprecipitate Trk, the sera were used for immunoprecipitation as the first antibody with recovery by L-agarose protein. To examine the effect of sera on NGF-induced morphological differentiation, Pctrk cells were cultured in 24-well dishes with serum-free Dulbecco modified Eagle medium (Nissui Pharmaceutical Co Ltd, Tokyo, Japan). Photographs of each representative area were taken.

#### EFFECT OF THE PATIENT’S SERUM ON NGF-INDUCED TRK AUTOPHOSPHORYLATION

Cells were preincubated with serum-free medium containing human sera (0.2% final concentration) for 1 hour at 37°C, and they were stimulated with 50 ng/mL of NGF for 5 minutes. After stimulation, the cells were collected with chilled phosphate-buffered saline and lysed with lysis buffer. The cell-free lysates were normalized for protein (1 mg/mL) and immunoprecipitated with α-Trk. The Trk immunoprecipitates were subjected to electrophoresis on sodium dodecyl sulfate–10% acrylamide gels, which was followed by blotting on polyvinylidene difluoride membranes. Tyrosine phosphorylation of Trk was detected with an antiphosphotyrosine antibody (α-PY; Upstate Biotechnology Inc, Waltham, Mass).

#### BIOLOGICAL EFFECTS OF THE PATIENT’S SERUM ON THE TRK-MEDIATED SIGNALING CASCADE IN PCTRK CELLS

Nerve growth factor clearly induced morphological differentiation in the presence or absence of control sera within 2 days, whereas the patient’s serum inhibited NGF-induced neurite outgrowth (Figure 1B). We examined the effect of the patient’s serum on NGF-induced Trk autophosphorylation and found that the patient’s serum inhibited NGF-induced Trk autophosphorylation (Figure 1E, compare lanes 2, 3, and 4).

#### IMMUNOBLOT ANALYSIS

Photographs of each representative area were taken.
followed this with an immunoblot analysis with α-Trk. The serum from the patient clearly immunoprecipitated Trk (Figure 3B, lanes 1 and 2) whereas all of the control sera failed to recognize Trk (Figure 3B, lane 3). Moreover, the serum taken after the treatment with intravenous immunoglobulin immunoprecipitated much less protein than did the serum taken before the treatment (compare lanes 1 and 2 of Figure 3B). The patient’s serum did not recognize TrkB or TrkC prepared from NIH3T3 cells expressing trkB or trkC complimentary DNA (data not shown).

Survival and differentiation of the sensory neurons of the dorsal root ganglia are dependent on the neurotrophin receptor–mediated signaling cascade. Pain and temperature afferents depend on NGF. Therefore, loss of in-
puts from or inhibition of the neurotrophin-dependent signaling pathway might result in a significant functional disturbance in sensory neurons. In fact, mutation of the trk protooncogene causes congenital insensitivity to the pain and anhidrosis that is characterized by severe autonomic and peripheral nervous system dysfunction, suggesting the importance of Trk-mediated intracellular signaling in the maintenance of the autonomic and peripheral nervous systems. The present patient showed sensory axonopathy with autonomic dysfunction. These clinical pictures seem to fit the spectrum of Trk-dependent neuronal systems well.

The patient’s serum can provoke the functional disturbance of the Trk-mediated signaling cascade in PCtrk cells. Moreover, it can recognize Trk whereas the patient’s serum that was precleared with L-agarose protein (data not shown) cannot, suggesting the presence of an autoantibody to Trk. Therefore, it seems reasonable to assume that this autoantibody might cause the development of sensory axonal neuropathy. This assumption was supported by the fact that intravenous immunoglobulin treatment resulted in clinical and neurophysiologic improvement and, concomitantly, a marked reduction of his serum in the capacity of immunoprecipitating Trk, although it is not clear why he developed an abnormal production of an autoantibody to Trk.

Thus, although this article deals with a single case, the results of the present patient strongly suggest the presence of a new type of sensory neuropathy associated with an anti-Trk autoantibody.

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REFERENCES