we have found that when Feti and Dumeti neurons are prevented from reaching their target muscles, they still survive and differentiate. We conclude that specific instructions from the target muscle play little if any part in the development of these neurons, although we cannot exclude the possibility that contact with some muscle, albeit the wrong one, might be a general condition for their survival.

Neurons in the central nervous system of insects are generated by segmental sets of embryonic precursor cells (*18*). In each embryonic segment the set of precursors is similar, yet the final pattern and number of neurons are strikingly different. Segment-specific differences in cell survival have been described (*19*). For example, limb-innervating neurons homologous by cell lineage to Feti and Dumeti are produced in the abdominal segments, but they do not survive (*3*). Our experiments suggest that the differential survival of limb-innervating neurons is independent of the presence (in the thorax) or absence (in the abdomen) of limb muscles. The segment-specific pattern of muscles depends on the pattern of underlying ectoderm (*20*), but this determinative sequence appears not to extend to the nervous system through the muscles. Segment-specific neuron differentiation might be an intrinsic, inherited commitment of neurons, or it might be extrinsically controlled by their segmental environment. Our experiments do not distinguish between these alternatives. However, they rule out one of the possible extrinsic determinants, the target muscles.

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Natural Antibodies to Human Retrovirus HTLV in a Cluster of Japanese Patients with Adult T Cell Leukemia

Abstract. *Human T cell lymphoma leukemia virus (HTLV) is a human retrovirus (RNA tumor virus) that was originally isolated from a few patients with leukemias or lymphomas involving mature T lymphocytes. Here we report that the serum of Japanese patients with adult T cell leukemia, but not the serum of tested normal donors, contains high titers of antibodies to HTLV. These observations, together with data from Japan showing that adult T cell leukemia is endemic in southwest Japan, suggest that HTLV is involved in a subtype of human T cell malignancy, including Japanese adult T cell leukemia.*

A human T cell lymphoma leukemia virus (HTLV) was previously isolated from cells of patients with cutaneous T cell lymphoma and leukemia (CTCL) (*1, 2*) and shown to be unrelated to other retroviruses by nucleic acid hybridization studies (*3*) and by immunologic studies of its major core protein, p24 (*4*), and reverse transcriptase (*5*). HTLV is an acquired, not endogenous, retrovirus as shown by the presence of proviral sequences in DNA from the neoplastic T cells of the patient from whom HTLV was first isolated and the absence of such sequences both in B cells of the same patient (*6*) and in other sources of DNA from normal people (*3*). The previous reports of natural antibodies to HTLV proteins in the serum of some patients with CTCL (*7, 8*) and the absence of similar antibodies in the serum of numerous randomly selected normal donors are consistent with the interpretation that HTLV is acquired by infection and suggest a possible etiologic role of HTLV with human T cell malignancies.

In studying the association of HTLV with human T cell neoplasias we have recently focused on a documented clustering of Japanese patients with adult T cell leukemia (ATL). This leukemia, a subtype of T cell malignancy, occurs with high frequency and in striking clusters on the islands of Kyushu and Shikoku in southwestern Japan (*9, 10*). Among its unique clinical features are adult onset, frequent skin involvement, and common organ involvement. The leukemic cells have convoluted nuclei and ultrastructurally are similar to a small cell variant form of Sézary syndrome, another malignancy of mature T cells (*11*). Adult T cell leukemia differs from classical Sézary T cell leukemia in that people with ATL show no typical Sézary cells, no leukemic cells in the epidermis despite intradermal or subcutaneous infiltration, an infiltration of leukemic cells into the bone marrow, and a shorter survival time (*9*). Nevertheless, because similarities exist between ATL and Sézary syndrome, the leukemic form of CTCL, and because the geographic clustering of Japanese ATL patients also suggests a possible viral etiology, we examined the serum of Japanese patients with lymphoid malignancies for the presence of natural antibodies to HTLV.

Serum samples were surveyed for HTLV-specific antibodies by means of both an indirect membrane immune fluorescence assay and a solid-phase radioimmunoassay (RIA). The latter is one of the methods we previously used to de-

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tect HTLV-specific antibodies in the serum of some CTCL patients (*7*). In the present study we found that the serum of six out of seven patients with ATL showed positive results both with the solid-phase RIA and with the immune fluorescence assay (Table 1). Other serum samples clearly positive in one or both systems included three of 20 samples from patients with acute lymphocytic leukemia, two of six samples from patients with chronic myelogenous leukemia in the blast crisis stage, and one of five samples from patients with acute monocytic leukemia. This last positive serum showed the highest antibody reactivity in both assay systems. In contrast, none of the serum samples from patients with chronic lymphocytic leukemia, acute myelogenous leukemia, or acute myelo-monocytic leukemia were significantly positive. In addition, samples from 41 random healthy Japanese donors had no detectable antibody reactivity in either assay. None of the 39 serum samples obtained from healthy donors living in the ATL endemic region possessed HTLV-specific antibodies.

Additional experiments confirmed that the natural antibody reactivities were specific for HTLV antigens. None of the serum samples listed as positive by immune fluorescence on the CTCL-derived HTLV-producing HUT102 cells (Table 1) were significantly positive on similar HUT78 cells, a T cell line also derived from a CTCL patient but not producing virus. Nor were "tumor-associated" antibody reactivities observed because occasional samples which recognized HUT102 and HUT78 cells in the immune fluorescence assay also recognized antigens present on other human T cells, including Jurkat cells (*12*) and phytohemagglutinin (PHA)-stimulated normal human T cells (data not shown). The HTLV antigen recognized in the fluorescence assay is probably an HTLV envelope protein.

Competition experiments in the solid-phase RIA system similarly confirmed the HTLV specificity of the natural antibody reactivities. Representative results are illustrated in Fig. 1. When cell lysates served as competitors only HTLV-producing HUT102 cells were able to compete maximally with the antibody binding reactivities for HTLV (Fig. 1, A to C). Only plateau levels of competition not totally abolishing antibody reactivities for HTLV occurred with lysates of non-HTLV–producing HUT78 cells or PHA-stimulated normal human T cells or

Table 1. Natural antibodies to HTVL antigens in serum samples from Japanese patients with lymphoid malignancies. Lyophilized serum samples were obtained from 53 Japanese patients with various lymphoid malignancies, including six patients with ATL whose birthplace was in regions of endemic ATL, either Kyushu Island or Shikoku Island. Control serum was obtained from 41 random healthy Japanese donors and 39 healthy donors primarily from Shikoku Island. After the serum samples were reconstituted they were screened for HTLV-specific antibodies. A solid-phase RIA was carried out as described (*7*). Briefly, human serum diluted 1:75 in phosphate-buffered saline (PBS) containing 0.5 percent Tween 20 (PBS-Tween) was added to wells of 96-well Immulon microtiter plates previously coated with preparations of either disrupted HTLV or disrupted avian myeloblastosis virus (AMV) as control. After 1 hour of incubation at room temperature, unbound proteins were aspirated and the wells were washed three times with PBS-Tween. The binding of human antibodies to HTLV was detected by adding iodinated (~50,000 count/min), affinity-purified goat antibody to human immunoglobulin G (IgG) to the wells and incubating the mixture for 1 hour at room temperature. Unbound ^{125}I-labeled antibody was removed and the plates were washed with PBS-Tween. The wells were separated and counted in a gamma counter. For the indirect membrane immune fluorescence assay we used as the primary target the HTLV-producing HUT102 cell line (*1*) which was derived from cells of a patient with CTCL by culturing with T cell growth factor (TCGF) (*21, 22*). After multiple passages in culture, the HUT102 cells now grow independently of TCGF. Secondary target cells serving as a specificity control were HUT78 cells, which do not produce HTLV but which were also derived from cells of a patient with CTCL and are also now TCGF-independent. When necessary, additional controls included Jurkat cells, derived from cells of a patient with T cell acute lymphocytic leukemia (*12*), and PHA-stimulated normal human peripheral blood lymphocytes. For the assay, 50 μl of each serum sample diluted in PBS were incubated with 10^6 target cells previously washed with PBS. After incubation for 30 minutes at 37°C the cells were washed three times with PBS. Fifty microliters of rabbit antibody to human IgG conjugated with fluorescein isothiocyanate and appropriately diluted in PBS were added and the cells were incubated again for 30 minutes at 37°C. The cells were washed three times in PBS and suspended in 50 μl of PBS for microscopic examination.

Diagnosis of serum samples	Number positive out of number tested	Positive serum No.*	Detection of antibody to HTLV		
			By solid-phase RIA†		By immune fluorescence‡
			Maximum bound	Titer	Titer
Adult T cell leukemia	5/6	1	17,700	1,000	32
		2	17,800	1,000	32
		15	13,100	400	32
		42	5,300	500	512
		45	4,200	500	128
Adult T cell leukemia (chronic)	1/1	10	8,500	350	16
Acute lymphocytic leukemia of T cells	0/2				
Acute lymphocytic leukemia	1/6	SSS62	8,200	1,000	512
Null-cell acute lymphocytic leukemia	2/12	4			128
		5	11,400	1,000	
Chronic lymphocytic leukemia of B cells	0/4				
Chronic lymphocytic leukemia	0/1				
Acute myelogenous leukemia	0/5				
Acute monocytic leukemia	1/5	26	19,500	4,800	1,024
Acute myelo-monocytic leukemia	0/1				
Chronic myelogenous leukemia in the blast crisis stage	2/9	31	5,400	500	64
		33	13,500	1,000	32
Healthy donors, endemic region	0/39				
Random normal donors	0/41				

* The numbers refer to the codes used by the Japanese collaborators for identifying serum samples and patients. † Natural antibody binding was calculated by subtracting the radioactivity (counts per minute) of the antibody bound to AMV from the radioactivity of the antibody bound to HTLV. Serum was scored positive only if the antibody reactivity was subsequently shown to be HTLV-specific by competition in the solid-phase RIA (see Fig. 1). Titers reflect the serum dilution at which the natural antibody binding to HTLV was 50 percent of the maximum. ‡ Scoring was based on the intensity of fluorescence and the percentage of cells fluorescing. Only HTLV-specific serum samples as shown by studies with non-HTLV–producing target cells were marked positive. Titers reflect the serum dilution at which 50 percent of HUT102 cells fluoresced weakly.

with antigens present in fetal calf serum. Experiments with retroviral lysates further showed that only HTLV could compete (Fig. 1, D to F). These results not only demonstrate the specificity of the natural antibodies for HTLV but also confirm the lack of immunological relationship between HTLV and other known retroviruses (*4*, *5*). In addition, the data indicate that the natural antibodies are not merely recognizing common antigens acquired by retroviruses during passage through a variety of tissue culture cells.

We have previously shown that natural antibodies in the serum of some patients with CTCL recognize at least two antigens of the HTLV core, p19 and p24 (*7*), suggesting the presence of replicating HTLV in these patients. Similar analyses of radioimmune precipitates were carried out here. All serum samples positive by the solid-phase RIA precipitated both p24 and p19 with the exception of serum 5 (Table 1), in which p19 precipitation was barely detectable (data not shown). The serum with the highest titer (serum 26 from a patient with acute monocytic leukemia) precipitated p15 in addition to p24 and p19 (data not shown). The p24 of HTLV has been unambiguously identified as a viral protein, both by its presence in the HTLV core (*4*) and by amino acid analysis showing sequences typical of other retroviral major core proteins (*13*). The serum samples positive in the solid-phase RIA also show the presence of natural antibody to HTLV p24 when tested with the homogeneously purified protein labeled with ^{125}I (*14*). In contrast to serum samples from antibody-positive patients, the serum of healthy donors did not precipitate any low molecular weight HTLV proteins.

Analysis of immune precipitates showed that the natural antibodies are directed against more than one HTLV antigen, and, clearly, more than one antibody reactivity was monitored here (see serums 4 and 5 in Table 1). The positivity of the serum from six of the seven patients with ATL is striking. The only negative sample was obtained from a patient that received chemotherapy, whereas all the positive samples were from untreated individuals. It is possible that the chemotherapy suppressed an immune response to HTLV in this one case. As HTLV is apparently involved with some malignancies of mature T cells, the positivity of the serum samples from patients with leukemias other than ATL is difficult to explain. Perhaps these results merely reflect a greater prevalence of HTLV in southwestern Japan, or they could indicate that HTLV is also involved in some leukemias other than those of mature T cells. These questions will require serologic and epidemiologic surveys of patients, family members, and other healthy individuals in regions where diseases such as ATL are endemic.

Since antibodies to HTLV occur only rarely in American patients with CTCL (*7*, *8*, *15*), the high percentage of positive serum from ATL patients is remarkable. One explanation for the contrast between the results from the American CTCL and the Japanese ATL patients may be that the particular subtype of T cell malignancy called ATL is difficult to diagnose, and that rare CTCL patients

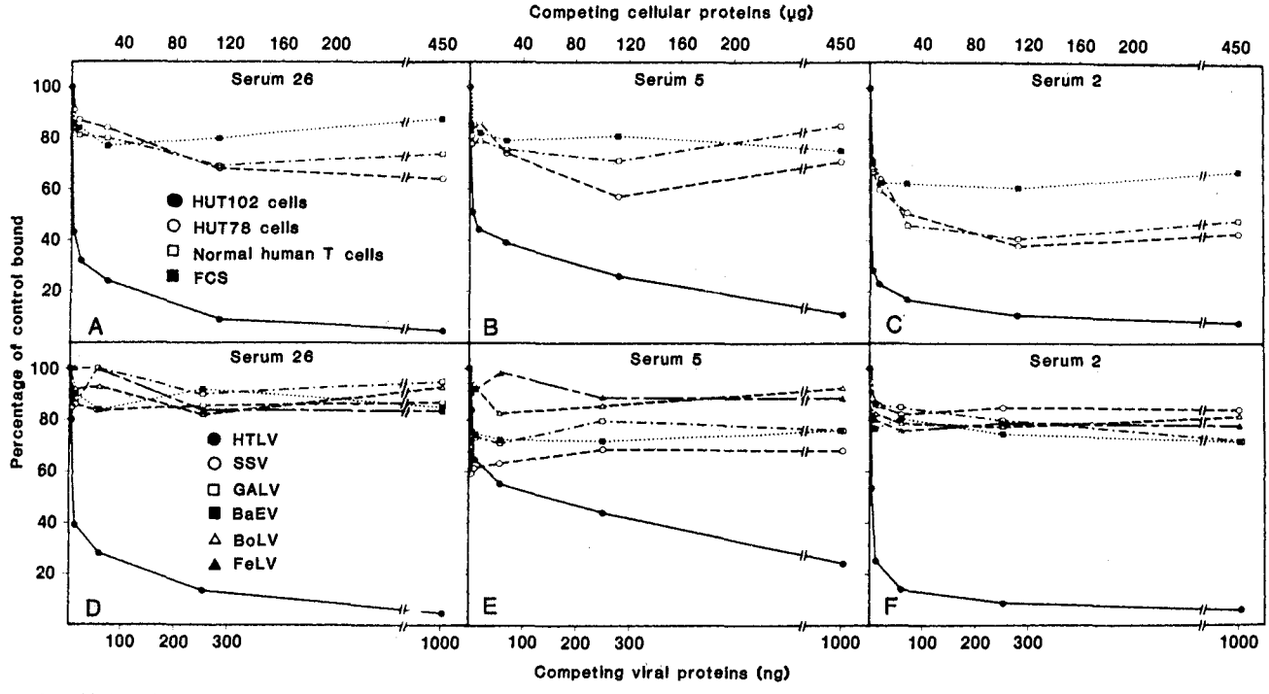

Fig. 1. Specificity of serum antibodies for HTLV antigens by solid-phase competition RIA. The solid-phase competition RIA was carried out as described (*7*). Briefly, competing antigens were incubated for 1 hour at room temperature with human serum appropriately diluted in PBS-Tween. The solid-phase RIA was then carried out as described in Table 1 with microtiter plates coated with disrupted HTLV. Background values of antibody binding to bovine serum albumin were subtracted before calculating the percentage of antibody bound; the radioactivity (in counts per minute) of ^{125}I-labeled goat antibody to human immunoglobulin G bound in the absence of any competitor was counted as 100 percent. Cell lysates used as competitors were prepared as described (*7*) and included HTLV-producing HUT102 cells; a non-HTLV–producing CTCL line, HUT78; and normal peripheral blood lymphocytes stimulated with PHA for 72 hours. Fetal calf serum (*FCS*) was used as an additional control. Viruses disrupted as previously described (*7*) and used as competing antigens included the simian sarcoma virus (*SSV*) grown in the 71AP1 marmoset cell line; gibbon ape leukemia virus (*GALV*) grown in gibbon lymphoblasts; the M7 strain of the baboon endogenous virus (*BaEV*) grown in the human rhabdomyosarcoma cell line A204; the feline leukemia virus (*FeLV*) grown in feline lymphoblasts; the bovine leukemia virus (*BoLV*) grown in fetal lamb kidney cells; and HTLV produced in HUT102 cells. (A, B, and C) The antibody reactivities in serum samples 26, 5, and 2 (see Table 1) were tested for competition with cell lysates of HUT102 cells, HUT78 cells, normal human T cells, and fetal calf serum. (D, E, and F) The antibody reactivities of serum samples 26, 5, and 2 were tested for competition with the following disrupted retroviruses: HTLV, simian sarcoma virus, gibbon ape leukemia virus, baboon endogenous virus, bovine leukemia virus, and feline leukemia virus.

with positive serum actually have the Japanese type of disease (ATL), or a disease closely related to it. In this regard, a study of the leukemic cell type in Japanese ATL will help elucidate the appropriate HTLV-susceptible cell.

The RNA tumor viruses have been linked to leukemias and lymphomas of animals since the pioneering studies of Gross (*16*). Subsequently, Jarrett and colleagues (*17*) were the first to associate the feline leukemia virus with a naturally occurring mammalian leukemia. Their studies, combined with those of Essex *et al.* (*18*), clearly demonstrated that FeLV causes feline leukemia. Other retroviruses cause some naturally occurring leukemias in chickens, wild mice, gibbon apes, and cattle (*19, 20*). The results described here are the first to our knowledge that demonstrate a human RNA tumor virus linked to a human neoplasia. This association and the known involvement of animal retroviruses in certain animal leukemias suggest that HTLV may be involved in causing these unusual human lymphoid malignancies.

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Role for the Adenosine Triphosphate–Dependent Proteolytic Pathway in Reticulocyte Maturation

Abstract. *As reticulocytes mature into erythrocytes, organelles and many enzymes are lost. Protein degradation during reticulocyte maturation was measured by monitoring the release of tyrosine from cell proteins. Proteolysis in rabbit red blood cells was directly proportional to the number of reticulocytes and was low in erythrocytes. This process was inhibited by blockers of cellular adenosine triphosphate production and by agents, such as* o*-phenanthroline,* N*-ethylmaleimide, and hemin, which inhibit the soluble adenosine triphosphate–dependent proteolytic system. The breakdown of endogenous proteins in reticulocyte extracts was also inhibited by these agents and required adenosine triphosphate. Inhibitors of lysosomal function, however, did not affect proteolysis. Thus, the proteolytic system that degrades abnormal proteins also catalyzes the elimination of proteins during red cell development.*

When a mammalian reticulocyte matures into an erythrocyte, mitochondria, ribosomes, and many cytosolic enzymes disappear (*1, 2*). Since the loss of these cellular components from reticulocytes can occur in vitro (*3*), this process does not require other organs and apparently does not result from secretion of these structures out of the cell. Reticulocytes contain lysosomal proteases in membrane-bound form (*4*) and also a soluble, adenosine triphosphate–dependent (ATP-dependent) proteolytic system (*4–6*). One function of the nonlysosomal pathway is to hydrolyze proteins whose structures are highly abnormal (*4–8*), as may arise by mutation, biosynthetic errors, or incorporation of amino acid analogs. This pathway also seems to be involved in the breakdown of certain normal cell proteins; in adult hepatocytes, for example, the breakdown of short-lived proteins is not affected by inhibitors of lysosomal function such as chloroquine or leupeptin, which reduce the breakdown of more long-lived proteins in the same cells (*9*). In addition, in growing fibroblasts and myotubes, degradation of most cell proteins is not sensitive to inhibitors of lysosomal proteolysis, but does require ATP (*10, 11*).

We investigated whether the ATP-dependent or the lysosomal pathway is involved in the maturational breakdown of protein during reticulocyte development. Most studies of proteolysis in reticulocytes (*5–8*) have been limited to measurements of the breakdown of newly synthesized proteins. Since over 95 percent of the protein synthesized in these cells is hemoglobin, this approach is not useful for studying maturational loss of reticulocyte components, which were synthesized at earlier stages of the cell's development. To monitor their degradation, we used a fluorometric method to measure the net generation of free tyrosine from preexistent proteins (*12, 13*). We confirmed that there is no synthesis or oxidation of tyrosine in red blood cells (*1*) by establishing that no $^{14}CO_2$ or [^{14}C]hydroxyphenylpyruvic acid was produced from [^{14}C]tyrosine by rabbit reticulocytes and that no increase in tyrosine occurred when such cells were incubated with phenylalanine. Therefore, when protein synthesis is blocked by addition of cycloheximide, the tyrosine accumulating in the cells and medium results from the breakdown of cell proteins.

Reticulocyte production was induced in rabbits (males, 8 to 10 pounds) by subcutaneous injections of phenylhydrazine (25 mg/day) for 5 days. Two or more days after injections were stopped, sam-

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