To determine whether the increase resulted from peptide interaction with a specific SP receptor, we used a specific receptor antagonist [D-Pro^2, D-Phe^7, D-Trp^9]-SP (Phe-SP) (*11*). The SP agonist (Sar-SP) increased ganglion TOH by approximately 70 percent in both explants and dissociated cell cultures (Fig. 1D), reproducing the effects of authentic SP. Addition of Phe-SP blocked the effect of Sar-SP, suggesting that elevation of TOH activity by Sar-SP is mediated by interaction with specific SP receptors. Addition of antagonist alone had no significant effect.

We do not know whether the increased TOH activity represented an increased number of enzyme molecules or activation of a preexistent enzyme. Nevertheless, our studies suggest that SP and SS may regulate noradrenergic metabolism, possibly through the mediation of SP receptors and the slow, noncholinergic EPSP. In the rat SCG, where SP (*12*, *13*) and SS (*3*) are localized primarily within the noradrenergic sympathetic neurons and in intraganglionic collateral processes (*14*, *15*), these peptides may subserve a feedback autoregulatory mechanism. By contrast, in the guinea pig inferior mesenteric ganglion where SP is localized in sensory fibers (*1*, *12*), sensory peptide may influence autonomic neurons, thus allowing environmental stimuli to regulate sympathetic noradrenergic function.

Our finding that peptide interactions with sympathetic neurons may modulate autonomic functions suggests that pharmacologic manipulation of peptide systems may offer a novel therapeutic approach to disorders involving autonomic neurons, such as hypertension. In a different perspective, the changes in TOH activity in sympathetic neurons in vitro provide a convenient and quantitative biochemical assay for peptidergic agonist or antagonist activity. Our observations also reinforce the concept that neurotransmitter interactions play an integral role in the maintenance and regulation of neurotransmitter phenotypic characters. Analogous transmitter interactions are well documented in the adrenal gland and elsewhere in the nervous system (*13*, *16*). Consequently, neurotransmitter phenotypic expression is a dynamic process that reflects the physiologic state and milieu of the neuron.

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Antibodies to Human T-Cell Leukemia Virus Membrane Antigens (HTLV-MA) in Hemophiliacs

Abstract. *Along with homosexual men, Haitians, and intravenous drug abusers, hemophiliacs are at high risk of contracting acquired immunodeficiency syndrome (AIDS). An earlier study revealed that 36 percent of a group of the AIDS patients had antibodies to cell membrane antigens associated with the human T-cell leukemia virus (HTLV-MA), whereas only 1.2 percent of matched asymptomatic homosexual controls had these antibodies. In the present experiments, serum samples from 172 asymptomatic hemophiliacs were examined for the presence of antibodies to HTLV-MA. Such antibodies were detected in 5 to 19 percent of the hemophiliacs examined from four geographical locations, but in only 1 percent or less of laboratory workers, normal blood donors, donors on hemodialysis, or donors with chronic active hepatitis.*

Hemophiliac patients, most of whom receive infusions of blood clotting preparations, become exposed to products from thousands of blood donors. Such hemophiliacs may also have abnormal T-lymphocyte profiles (*1*). Along with homosexual men with multiple partners, Haitians, and intravenous drug abusers, hemophiliacs represent one of the major risk groups for developing the acquired immunodeficiency syndrome (AIDS) (*2*).

Most researchers suspect that AIDS may be caused, at least in part, by an infectious agent (*3*). Such an agent would presumably be transmitted only with great difficulty, and in the case of intravenous drug abusers and hemophiliacs it could perhaps be transmitted by blood or blood products. Among the many candidate etiologic agents that have received research attention is the human T-cell leukemia virus (HTLV) (*4*). We recently reported that AIDS patients have substantially increased rates of exposure to HTLV; this conclusion was based on our finding, by means of indirect membrane immunofluorescence (IMI) and radioimmunoprecipitation (RIP), an antibody to HTLV-infected cells in the blood of AIDS patients (*5*). Independently, Gallo and co-workers reported that HTLV had been isolated from one AIDS patient and proviral sequences had been found in two (*6*).

Using the same procedures that we described earlier (*5*), we examined serum samples from 172 hemophiliacs that were asymptomatic, two hemophiliacs with AIDS, and one with severe lymphadenopathy. Of the 172 asymptomatic hemophiliacs, 45 were from Atlanta, Georgia; 41 were from Birmingham, Alabama; 39 were from Los Angeles, California; and 47 were from New York City (see Table 1). Only one sample was examined from each individual. Except in the case of the New York hemophiliacs and the control blood donors, all the serum samples were collected in late 1982 or early 1983. Thirty-nine of the samples from New York hemophiliacs were collected in 1978 and 1979; the remaining eight samples were collected in 1976, 1977, or 1981. Also examined were serum samples from 47 healthy workers at the hospitals or laboratories where HTLV-producer cell cultures or tissue samples from patients were handled (the Harvard School of Public Health Laboratory, the Centers for Disease Control, the University of Buffalo, and the University of Alabama Medical

Center). These last samples were collected in 1982 or 1983.

All samples were initially checked at a 1:4 serum dilution in a double-blind, coded way with the use of two standard reference HTLV-producer cell lines, Hut 102 and MT 2. The procedure used has been described (*5, 7*). All samples that reacted with Hut 102 or MT 2, or both, by IMI were subsequently screened on HTLV-negative T- and B-cell lines to confirm specificity, and those that specifically reacted with 40 percent or more of the Hut 102 or MT 2 cells were considered positive.

Overall, about 12 percent of the samples from asymptomatic hemophiliacs gave a positive reaction with at least 40 percent of the Hut 102 or MT 2 cells, and 8.7 percent reacted with at least 50 percent of the cells in one or both of the positive reference lines (Table 1). Samples from the Birmingham hemophiliacs showed the lowest proportion of positive reactions (4.9 percent); samples from the New York hemophiliacs showed the highest proportion of positive reactions (19 percent). Only two samples were available from hemophiliacs with AIDS, and one was positive for HTLV-MA antibody by IMI. Another sample from a 14-year-old hemophiliac with severe lymphadenopathy was also positive.

All ten of the samples from hemophiliacs in Atlanta and Los Angeles that gave a positive reaction by IMI, and six of the nine samples from New York hemophiliacs that gave a positive reaction, were available for testing by RIP of [^{35}S]methionine-labeled Hut 102 cells or [^{35}S]cysteine-labeled cells (Fig. 1). This procedure was also described previously (*5*). Eleven of the 16 showed either clear immunoprecipitation or weak precipitation of proteins that migrate in the same position as those regularly detected with reference sera to HTLV-related proteins. In particular, those antigens detected included glycoprotein 61 (gp61), which is the protein detected most frequently by human antibodies from

Table 1. Presence of antibodies to HTLV-MA in hemophiliacs and related controls. Each sample was tested at a 1:4 dilution on the reference cells (Hut 102 and MT 2) taken during the peak phase of logarithmic growth. The procedure followed was exactly as described earlier (*5*). Each test included positive and negative reference sera, and all samples were tested in a double-blind manner.

Category	Tested	> 50 percent cells positive using			> 40 percent cells positive using		
		Hut 102	MT 2	Either Hut 102 or MT 2	Hut 102	MT 2	Either Hut 102 or MT 2
Asymptomatic hemophiliacs							
Atlanta, Georgia	45	2 (4.4)*	3 (6.7)	3 (6.7)	3 (6.7)	4 (8.9)	5 (11)
Birmingham, Alabama	41	2 (4.9)	0	2 (4.9)	2 (4.9)	0	2 (4.9)
Los Angeles, California	39	1 (2.6)	2 (5.1)	3 (7.7)	4 (10)	5 (13)	5 (13)
New York, New York	47	5 (11)	7 (15)	7 (15)	5 (11)	9 (19)	9 (19)
Total	172	10 (5.8)	12 (7.0)	15 (8.7)	14 (8.1)	18 (10)	21 (12)
Healthy lab or hospital workers	47	0	0	0	0	0	0
Adult blood donors†	137	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)	1 (0.7)
Chronic active hepatitis†	29	0	0	0	0	0	0
Hemodialysis†	21	0	0	0	0	0	0
Hemophiliacs with AIDS or severe lymphadenopathy‡	3	2 (67)	2 (67)	2 (67)	2 (67)	2 (67)	2 (67)

*Number and percentage positive shown in parentheses. †From (*5*). ‡Two had confirmed AIDS and one had lymphadenopathy.

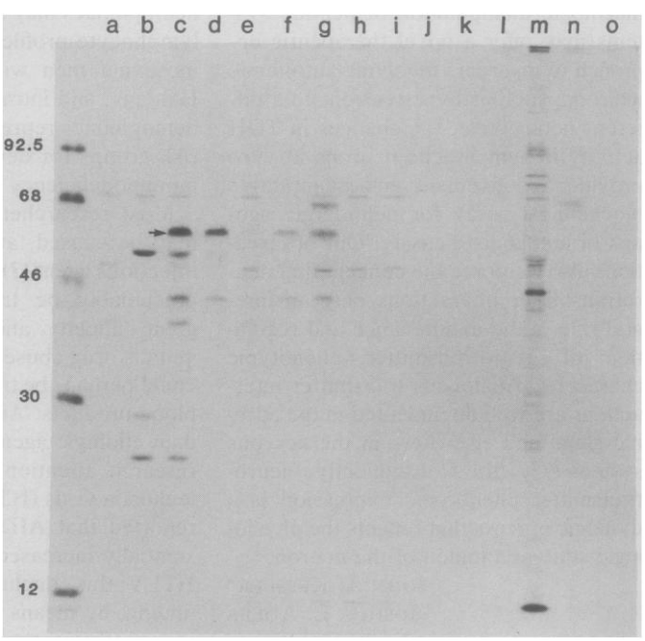

Fig. 1. Reactivity of serum samples from asymptomatic hemophiliacs positive for antibody to HTLV-MA as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Hut 102 cells (lanes a to i) and T8402 cells (lanes j to o) at their peak log phase of growth were harvested and exposed to [^{35}S]cysteine (100 μCi/ml; specific activity 1070.3 Ci/mmole; New England Nuclear), for 4 to 10 hours. A soluble cell lysate was obtained after disruption with RIPA buffer (0.15*M* NaCl, 0.05*M* tris-HCl, *p*H 7.2, 1 percent Triton X-100, 1 percent sodium deoxycholate, and 0.1 percent SDS) and this lysate was centrifuged for 1 hour at 100,000*g*. The lysate supernatant was cleared once with 50 μl of Protein-A Sepharose CL-4B (Protein-A beads) before portions were reacted with the following sera preabsorbed with Protein-A beads: (a) representative serum negative for antibodies to HTLV-MA from a healthy resident of Kyushu, Japan (10 μl); (b) reference goat antiserum to purified p24 of HTLV (3 μl); (c and j) representative serum positive for antibodies to HTLV-MA from a healthy resident of Kyushu, Japan (3 μl); (d and k) representative serum sample positive for antibodies to HTLV-MA from a Japanese ATL patient (5 μl); (e and l) representative serum positive for antibodies to HTLV-MA from an AIDS patient (10 μl); (f and m) a representative sample positive for antibodies to HTLV-MA from an asymptomatic hemophiliac (10 μl); (g and n) another representative sample positive for antibodies to HTLV-MA from an asymptomatic hemophiliac (10 μl); (h and o) a representative serum sample negative for antibodies to HTLV-MA from an asymptomatic hemophiliac (10 μl); (i) another representative serum sample negative for antibodies to HTLV-MA from an asymptomatic hemophiliac (10 μl). Immunoprecipitates were eluted in a sample buffer containing 0.1*M* Cleland's reagent, 2 percent SDS, 0.08*M* tris-HCl, *p*H 6.8, 10 percent glycerol, and 0.2 percent bromophenol blue by boiling at 100°C for 2 minutes. Samples were analyzed in a 12.5 percent acrylamide resolving gel with 3.5 percent stacking gel according to the discontinuous buffer system of Laemmli (*11*). The molecular weight markers, purchased from New England Nuclear, were ^{14}C-labeled phosphorylase b (92,500), bovine serum albumin (68,000), ovalbumin (46,000), carbonic anhydrase (30,000), and cytochrome *c* (12,000). Arrow indicates the 61,000-dalton glycoprotein.

HTLV-infected T-cell leukemia patients of Japanese origin or from AIDS patients (*5, 8*). Whether the gp61 is virus-encoded or an HTLV-activated cellular protein has not been resolved. It appears, however, to be distinct from the T-cell growth factor–receptor protein which migrates to a different position as detected by a reference monoclonal antibody (provided by T. Waldmann, National Cancer Institute).

Figure 1 illustrates the presence of antibodies to gp61 in representative hemophiliacs from Atlanta (lane f) and New York (lane g). The same reaction was seen with serum from a representative healthy Japanese individual from the HTLV-endemic region (lane c), serum from a representative Japanese patient with the HTLV-related adult T-cell leukemia (ATL) (lane d) (*8*), and with serum from a representative AIDS patient (lane e). All of the latter individuals also reacted in a positive manner by IMI. No precipitation of the gp61 was observed with sera from two hemophiliacs that reacted negatively by IMI (lanes h and i). Similarly, no reaction occurred with a serum from an IMI-negative healthy Japanese individual from the HTLV-endemic area (lane a). Neither the IMI-positive sera from hemophiliacs nor the IMI-positive sera from the Japanese individuals reacted with comparable proteins on T8402, an HTLV-uninfected human T-cell line (lanes j to o).

To demonstrate that the reactivity seen by RIP with the gp61 band was common to both the Japanese IMI-positive sera and the IMI-positive hemophiliac sera, we conducted adsorption studies (Fig. 2). The prior incubation of [^{35}S]methionine-labeled Hut 102 lysate with IMI-positive sera from either a Japanese ATL patient (lane d) or a healthy Japanese individual from the endemic area (lanes c and g) resulted in the loss of reactivity when the same lysate was subsequently exposed to IMI-positive hemophiliac sera. This reaction could not be blocked by incubating with IMI-negative sera from Japanese individuals (lane b); thus, the pattern of reactivity with sera from hemophiliacs was similar to that seen with IMI-positive sera from Japanese individuals or AIDS patients.

Although increased rates of exposure to HTLV are known to occur among Caribbean islanders and southern Japanese, hemophiliacs represent the first group in the continental U.S. population other than AIDS cases (*5*), homosexual males with lymphadenopathy (*5*), or selected groups of leukemia patients (*7*) that has elevated rates of exposure to HTLV. We did not detect any individuals that were HTLV-MA antibody–positive among 47 laboratory or hospital workers, 29 patients with chronic active hepatitis, or 21 patients on hemodialysis (Table 1). Among 137 healthy blood donors from Philadelphia in 1977, only one was positive. By the same procedure, Gallo *et al.* found only 4 of 538 normal U.S. blood donors to be positive (*7*).

Hemophiliacs represent one of the major risk groups for development of AIDS (*2, 3, 10*). They may receive blood elements from tens of thousands of donors in a relatively brief period of time and they frequently receive both blood and blood products (*10*). The likelihood that blood from healthy HTLV-carrier individuals may be present in the donor pool thus seems high. Although the current results are compatible with our earlier observations that AIDS patients have rates of exposure to HTLV above those seen in matched controls (*5*), they do not necessarily add evidence to a hypothesis that would etiologically associate HTLV and AIDS. For example, hemophiliacs may have abnormalities of the immune system prior to exposure to HTLV (*1*). The infusion of HTLV-carrier blood or blood products into a preconditioned host might then enhance the possibility of infection.

As a group, hemophiliacs probably have more frequent contact with major medical centers than the other subpopulations at risk for developing AIDS. Because of this, they may provide one of the most appropriate populations for prospective studies to determine if HTLV-exposed people have a relative increase in risk for disease development.

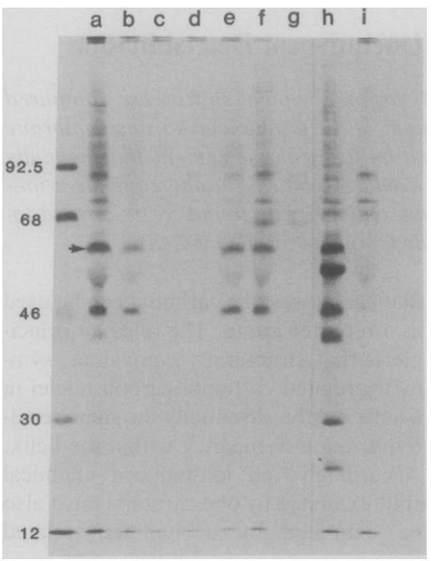

Fig. 2. Blocking of antibody reactivity to HTLV-related antigens present in serum samples of two representative asymptomatic hemophiliacs. Equal portions of cell lysate from Hut 102 cells, prepared by the same procedures as described in Fig. 1, except substituting the [^{35}S]cysteine with [^{35}S]methionine, were first reacted with 10 μl of the following sera preabsorbed to Protein-A beads: (b) reference human serum negative for antibodies to HTLV-MA [same as sample (a) in Fig. 1]; (c) and (g) reference human serum positive for antibodies to HTLV-MA [same as sample (c) in Fig. 1]; (d) reference Japanese ATL serum sample [same as sample (d) in Fig. 1]; (e) reference goat antiserum to p24 [same as sample (b) in Fig. 1]; (a) and (f) phosphate-buffered saline only. After incubation for 90 minutes at 4°C, preabsorbed cell lysate was withdrawn from each tube and centrifuged at 100,000*g* for 15 minutes. The lysate supernatant was then reacted with 10 μl of the following sera preabsorbed to Protein-A beads: (a to e) a representative serum sample positive for antibodies to HTLV-MA from a hemophiliac [same as sample (f) in Fig. 1] and (f) and (g) another representative serum sample positive for antibodies to HTLV-MA from a hemophiliac [same as sample (g) in Fig. 1]. Lane (h) shows the reactivity of a reference human serum [same as sample (c) in Fig. 1] positive for antibodies to HTLV-MA which was used to preabsorb with cell lysate. Lane (i) shows the reactivity of a representative serum negative for antibodies to HTLV-MA from a hemophiliac [same as sample (h) in Fig. 1]. Immunoprecipitates were analyzed in the same way as described in Fig. 1. The arrow indicates the 61,000-dalton glycoprotein.

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Is the Gramicidin A Transmembrane Channel Single-Stranded or Double-Stranded Helix? A Simple Unequivocal Determination

Abstract. *Thallium ion–induced carbonyl carbon chemical shifts were compared for all of the* L*-residue-peptide carbonyl carbons of the gramicidin A transmembrane channel. Molecular structures were deduced by using the argument that helically equivalent and equally proximal carbonyls would exhibit essentially equivalent ion-induced chemical shifts. The transmembrane channel was found to be a head-to-head dimer with the structure of a left-handed, single-stranded* β*-helix.*

It is well-accepted that the gramicidin transmembrane channel is a dimer (*1–6*). However, actively concerned research groups differ in their opinions about the structure of the ion-conducting dimer (*7–13*): is the structure one of single-stranded helices dimerized end to end, or is it a double-stranded helix? The gramicidin channel was the first ion-selective transmembrane channel to be characterized. Knowledge of the channel structure and ionic mechanism provides an understanding of lipid membrane permeation in terms of the basis for selectivity between anions and cations, between monovalent and divalent ions, and among monovalent ions; it provides fundamental information on the repulsion between ions at fixed distances and the relative solvation power of peptide carbonyls and water; it demonstrates that in the selective passage of ions through a channel the largely dehydrated ion is used, and it shows the relative importance of lipid (positive image force) and partial dehydration barriers to passage through the lipid bilayer. Unambiguous data presented in this report exclude double-stranded helices as possible structures of gramicidin in phospholipid and further identify the ion-binding sites within the channel. These data, when combined with previous results on the same system, demonstrate that the channel is specifically the left-handed, single-stranded β-helix, dimerized head to head.

The approach involves eight separate syntheses of gramicidin A. In each synthesis, one different L-residue carbonyl is 90 percent enriched with carbon-13. The eight syntheses are required in order that each L-residue carbonyl can be used as a reporter group. The relevant principle is that structurally equivalent, symmetry-related carbonyl carbon nuclei in a helix exhibit essentially the same sensitivity to ion occupancy within the helix. Accordingly, an ion-induced chemical shift exhibited by one carbonyl must also be exhibited by a symmetry-related (symmetrically equivalent) carbonyl that is equally proximal to the ion. The present communication is a report of thallium ion–induced carbonyl carbon chemical shifts for [(1-^{13}C)L-Val^{1}]gramicidin A, [(1-^{13}C)L-Ala^{3}]gramicidin A, [(1-^{13}C)L-Ala^{5}]gramicidin A, and [(1-^{13}C)L-Val^{7}]-gramicidin A. These results combine with previously determined (*11*) thallium ion–induced carbonyl carbon chemical shifts of [(1-^{13}C)L-Trp^{9}]gramicidin A, [(1-^{13}C)L-Trp^{11}]gramicidin A, [(1-^{13}C)L-Trp^{13}]-gramicidin A, and [(1-^{13}C)L-Trp^{15}]gramicidin A to define the helical structure required by the data.

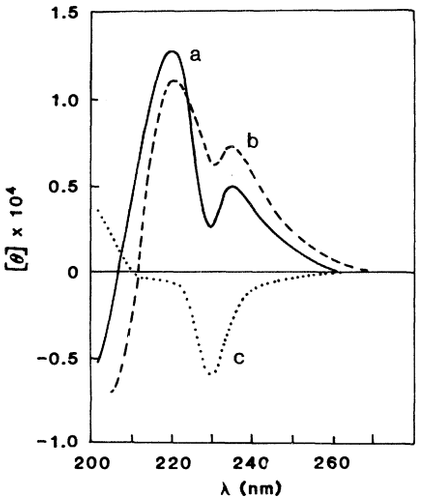

Fig. 1. Unique circular dichroism spectrum of gramicidin A incorporated into lysolecithin membranes, and the effect of thallium ion binding. The molar ratio of gramicidin A to lysolecithin was 1:15. (Curve a) The channel reference spectrum with 0.5 m*M* NaCl; (curve b) effect of 83 m*M* thallium acetate; (curve c) the circular dichroism spectrum of gramicidin A in association with lysolecithin micelles before heat incorporation results in the formation of gramicidin-lysolecithin bilayers (*18*). Incorporation results in a unique circular dichroism spectrum which is only slightly perturbed by thallium ion binding; [θ], mean molar residue ellipticity; λ, wavelength.

The primary structure of gramicidin A is HCO-L-Val^{1}-Gly^{2}-L-Ala^{3}-D-Leu^{4}-L-Ala^{5}- D -Val^{6}- L -Val^{7}- D -Val^{8}- L - Trp^{9} - D -Leu^{10}- L -Trp^{11}- D -Leu^{12}- L -Trp^{13} - D - Leu^{14}-L-Trp^{15}-NHCH$_2$CH$_2$OH as shown by Sarges and Witkop (*14*). The carbonyl carbons of the L-residues at positions 1, 3, 5, and 7 were labeled with carbon-13, and the synthetic gramicidins were produced as reported for [(1-^{13}C)D-Leu^{12,14}]-gramicidin A (*15*). The syntheses of gramicidin A's with labeled carbonyl carbons in L-residues 9, 11, 13, and 15 was verified (*16*). Each gramicidin A was separately incorporated into lysolecithin phospholipid structures (*17*). This heat-induced incorporation results in the formation of bilayer sheets and vesicles (*18*). The evidence that this system contains the channel state is extensive. During the many-hour time course of heat incorporation of gramicidin A into the phospholipid, the motions of the lipid aliphatic carbons become slowed, and a unique circular dichroism pattern develops (Fig. 1). Until the unique circular dichroism pattern is obtained, there is little sodium-23 interaction as measured by nuclear magnetic resonance (NMR) longitudinal (T_1) and transverse (T_2) relaxation studies. The ion interaction that does develop is competitively blocked by silver ion and thallium ion (*17, 19*), which competitively block alkali metal ion transport through the channel (*20, 21*). The energy of activation for sodium-23 interaction (*17, 19, 22*) and for channel transport of sodium ion are essentially the same (*23*). The interaction of the lysolecithin-packaged gramicidin A with Ca^{2+} and Ba^{2+} has the same binding constants (1 to 10M^{-1}) (*24, 25*) as were determined from the effect of these divalent ions on channel transport (*26*). Four rate constants determined by sodium-23 NMR resonance T_1 and T_2 studies can be used with Eyring rate theory to calculate the sodium ion currents through the channel over substantial ranges of ion activity (three decades) and of trans-