doubtful (*12*), particularly in view of their relatively low association constant (2.9×10 liter/mole) calculated from binding studies at equilibrium in vitro (*18*). When histamine is increased as a result of its being released from mast cells or inhaled, it binds to the cell surface and causes an increase in Ca^{2+} influx, which facilitates smooth muscle contraction. An increased capacity for exchange of cellular Ca^{2+} with La^{3+} was observed in cultured heart cells after the specific removal of sialic acid from the cell surfaces with purified neuraminidase (*19*). Moreover, from nuclear magnetic resonance studies, Jaques *et al.* (*20*) concluded that sialic acid forms a complex with Ca^{2+} in the proportion of 1:1 at neutral *p*H. It is reasonable, therefore, to assume that at least one molecule each of histamine and Ca^{2+} can bind per molecule of disialoganglioside, but the ratio may change in the case of trisialogangliosides. It is also possible that Ca^{2+} acts as bridge in the binding process.

2) The change in ganglioside sequence from $GM_3 > GM_1 > GM_2 > GD_{1a} > GD_{1b}$ and GT_1 to $GT_1 > GM_2 > GM_1 > GM_3 > GD_{1b} > GD_{1a}$ in the hyperreactive trachea might result from a defect in the degradation which could be due to decreased enzyme secretion. Earlier observations indicate that when either neutrophils or granulocytes from patients with active atopic eczema are incubated with histamine, the release of the lysosomal enzyme β-glucuronidase is inhibited (*21*).

3) Lung tissue might contribute to bronchoconstriction; however, analysis of the glycosphingolipids of lung tissue indicates that, other than releasing histamine from the mast cells, the contribution of the lung to bronchial hyperreactivity is almost negligeable. Further, Agrawal's data show that $d(V_1 - V_2)/dV_2$ is independent of lung volume because the larger the lung, the larger will be the value of $d(V_1 - V_2)$ for a given change in alveolar pressure and the larger will be the amount of air respired (dV_2) (*9*).

4) Although inhalation of histamine causes bronchoconstriction in susceptible animals, the altered ganglioside pattern is probably not due to the histamine because in each experiment the animals were killed after a 4-day recovery period. Moreover, 8 to 10 percent of guinea pigs have low concentrations of gangliosides in the tracheal tissue.

Clearly, this relation between the levels of gangliosides and bronchoconstriction offers a new approach for studying the mechanisms involved in the coupling of histamine release and bronchoconstriction and has important clinical implications.

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30. I thank K. P. Agrawal of the Institute of Nuclear Medicine and Allied Sciences, Delhi, India, for screening the guinea pigs, D. Alling of the National Institute of Allergy and Infectious Diseases for statistical analysis and computer plotting, V. Ginsburg and H. B. Pollard for critical reading of the manuscript, and J. Mok for preparing the manuscript.

11 August 1982

A New Subtype of Human T-Cell Leukemia Virus (HTLV-II) Associated with a T-Cell Variant of Hairy Cell Leukemia

Abstract. *Human T-cell leukemia virus (HTLV) is a human type-C RNA tumor virus (retrovirus) previously identified in and isolated from several patients with T-cell leukemias or lymphomas. The known virus isolates from the United States and Japan are closely related and are found in adults with an acute malignancy of mature T cells. A related retrovirus has been found in a patient (Mo) with a somewhat different disease (a T-cell variant of relatively benign hairy cell leukemia). Serum from Mo contains antibodies to the major internal core protein (p24) of HTLV. A T-cell line established from the spleen of Mo expresses HTLV antigens. However, HTLV from Mo is significantly different from all previous HTLV isolates in immunological cross-reactivity tests of p24. The usual prototype HTLV isolate is represented as HTLV-I, and the HTLV from Mo is represented as HTLV-II. Individual members of each subgroup may then be identified by subscript initials of the patient [for example, HTLV-$I_{(CR)}$, HTLV-$I_{(MB)}$, and HTLV-$II_{(Mo)}$].*

A novel human retrovirus (RNA tumor virus) designated human T-cell leukemia virus (HTLV) was first isolated from some cases in the United States of human leukemia and lymphoma involving relatively mature T cells (*1, 2*). Several additional isolates of HTLV were subsequently identified in other cases from the United States, England, and elsewhere (*3–6*), and recent evidence has shown that HTLV is endemic in certain geographical areas (*5*). HTLV is different from all the animal retroviruses isolated by comparisons of viral nucleic acids by nucleic acid hybridization (*7*), by immunological analyses of the structural proteins (*8, 9*) and of reverse transcriptase (*10*), and by amino acid sequencing of structural proteins (*11*). That HTLV is acquired by infection is suggested by the findings of high titer antibodies to HTLV structural proteins in serums of patients and in some normal people (*12–16*) and is conclusively shown by the finding of proviral sequences in the DNA of neoplastic T cells, but not in various normal human cells (*7, 17*). HTLV sequences were also absent in normal B cells from

0036-8075/82/1105-0571$01.00/0

patients whose neoplastic T cells contained HTLV proviral sequences (*17*).

A significant feature of the various HTLV isolates was the lack of identifiable differences among them by tests of homology of their nucleic acids and by immunological cross-reactivity of their structural proteins. The patients whose malignant T cells expressed HTLV were also generally positive for serum antibodies to HTLV antigens. Apart from some close family members of virus-positive patients and some normal individuals in HTLV endemic areas, only very seldom in randomly selected normal people in the United States antibodies to HTLV were detected (*4, 12–16*). Since serum immunoassays are simple and more rapid than virus isolations, we screened patients with T-cell malignancies for HTLV association by various convenient immunological assays of serums for antibodies to HTLV proteins, especially the major structural protein, p24, of HTLV (*8*). We report on a patient (Mo) with another T-cell malignancy, hairy cell leukemia (a T-cell variant). Mo is a 37-year-old white male from Seattle, Washington, who first developed evidence of T-cell leukemia in 1976 and remains clinically stable (*18*). Like the previous typical leukemia and lymphoma patients who were positive for HTLV, Mo's leukemic cells were OKT-4, positive mature T lymphocytes. In contrast, however, they exhibited typical morphology of hairy cells, contained tartrate-resistant acid phosphatase (typical of hairy cells), and the course of the disease has been relatively benign (*18*). Serum from Mo reacts with HTLV p24 in an immunoprecipitation assay and his cells express retroviral antigens that are highly cross-reactive with HTLV antigens. However, virus from this patient is clearly distinguishable from other HTLV isolates in immunological assays. We propose that the earlier isolates of HTLV (*1–6*), including HTLV later isolated in Japan (*19, 20*), belong to one subgroup (HTLV-I), while virus from Mo belongs to a second (HTLV-II). As new isolates are obtained and analyzed, we suggest that they be named accordingly to these or new subgroups. Individual isolates within the same subgroup can be distingushed by initials of patients, such as, HTLV-$I_{(CR)}$, HTLV-$I_{(MB)}$, and HTLV-$II_{(Mo)}$.

The immunoprecipitation of ^{125}I-labeled p24 of HTLV from Mo serum showed that about 95 percent of the labeled antigen present was precipitated by the Mo serum (Fig. 1), and the pattern of precipitation is similar to those with the control serums, which included one from a patient in the United States with cutaneous T-cell lymphoma (CR) whose cells provided the first isolate of HTLV (*1*), another from a T-cell lymphosarcoma cell leukemia from the Caribbean (*3*), and a third from an adult T-cell leukemia patient from southwestern Japan (*16*). In

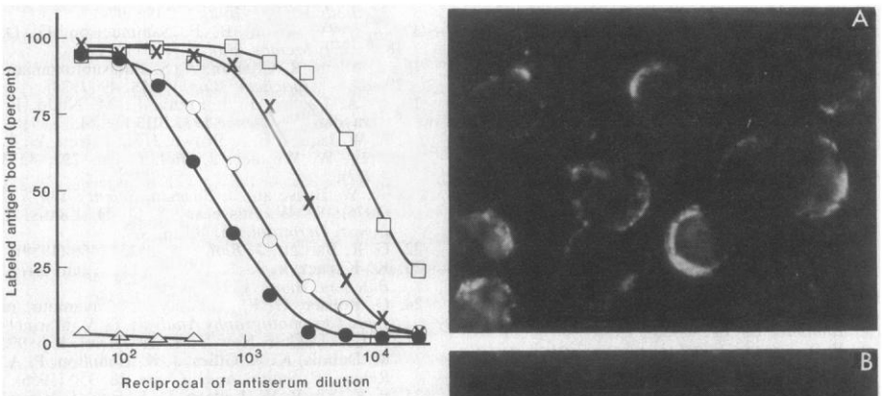

Fig. 1 (left). Immunoprecipitation of ^{125}I-labeled HTLV p24 by human serums. The labeled p24 (8,000 to 10,000 count/min) was mixed with twofold serial dilutions of human serum in 200 μl of buffer 1 [20 m*M* tris-HCl (*p*H 7.5), 200 m*M* NaCl, 1 m*M* EDTA, 0.3 percent Triton X-100, 0.1 m*M* phenylmethylsulfonyl fluoride, and bovine serum albumin (2 mg/ml)]. The reaction mixture was incubated for 2 hours at 37°C and overnight at 4°C. A 20-fold excess of goat antiserum to human IgG was then added and the volume was made up to 1 ml with buffer 1. The samples were incubated for 1 hour at 37°C and an additional 2 hours at 4°C and then centrifuged at 2500 rev/min for 15 minutes (Beckman centrifuge). The supernatants were aspirated, and the radioactivity in the sediment was counted (LKB Ultro-Gamma counter). ●—●, Serum from patient Mo; ○—○, serum from a Caribbean patient with T-cell lymphosarcoma cell leukemia; □—□, serum from a Japanese adult T-cell leukemia patient; and △—△, normal human serum. Fig. 2 (right). Indirect immune fluorescence assay with monoclonal antibody in HTLV p19. Cells were washed and fixed for 10 minutes with 50 percent methanol and 50 percent acetone as described (*9*). The indirect immunofluorescent assay with the use of mouse ascites fluid containing monoclonal antibody to p19 and FITC-conjugated F(ab')$_2$ fragment of sheep antibody to mouse IgG has also been described (*9*). (A) HUT 102, clone A9 cells; (B) Mo cells.

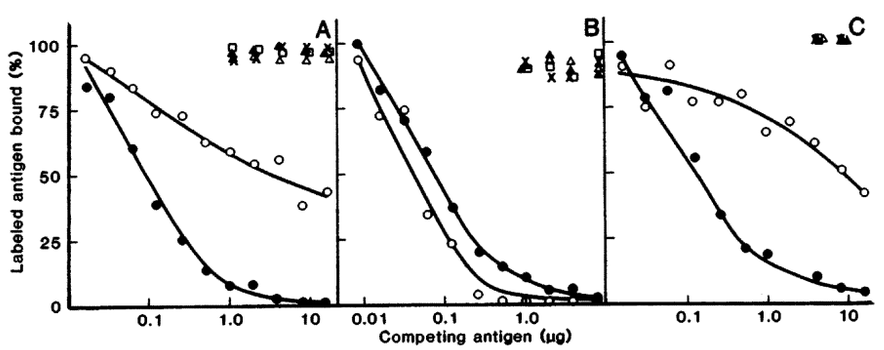

Fig. 3. Homologous and heterologous competition radioimmunoassays of HTLV p24. Competition radioimmunoassays were carried out with ^{125}I-labeled HTLV p24 and a limiting dilution of hyperimmune rabbit antibody to HTLV or serums from patients Mo and MJ. Serial dilutions (100 μl) of the solubilized cells in buffer 1 (see legend to Fig. 1 for description) were incubated with the appropriate serum for 1 hour at 37°C. Labeled HTLV p24 (8000 count/min in 50 μl of buffer 1) was then added, and the mixture was further incubated at 37°C for 2 hours and at 4°C overnight. A 20-fold excess of goat antiserum to rabbit IgG or goat antiserum to human IgG was then added, and the volume was made up to 1 ml in buffer 1. The samples were further incubated at 37°C for 1 hour and at 4°C for 2 hours and then centrifuged at 2500 rev/min for 5 minutes in a Beckman centrifuge. The supernatants were aspirated, and the radioactivity in the sediment was determined in an LKB Ultro-Gamma counter. (A) Competition radioimmunoassay (RIA) with rabbit antiserum to HTLV; (B) Competition RIA with Mo serum; (C) competition RIA with MJ serum. Virus extracts used for competition were as follows: ●—●, HTLV; ○—○, Mo particles; X—X, Mason Pfizer monkey virus; △—△, bovine leukemia virus; □—□, Rauscher murine leukemia virus; and ▲—▲, simian sarcoma virus.

contrast; the normal serum had no reactivity with HTLV p24. The specificity of these immune reactivities was indicated by the fact that the precipitation was only blocked by extracts of HTLV or cells producing HTLV (*12, 16*) (data not shown).

To examine the patient's cells for HTLV antigens, we used a cell line established from the patient's spleen (*18*). Fixed cells were reacted with a monoclonal antibody against HTLV p19 in an indirect immune fluorescent assay (*9*), which is very specific for cells expressing HTLV (*4, 9*). A battery of HTLV-negative cells that included human normal and neoplastic T and B cells and a number of human and animal cell lines infected with or producing different animal retroviruses are consistently negative in this assay (*4, 9*). Mo cells were positive, 40 percent of the cells showing bright fluorescence (Fig. 2). The range of p19-positive cells in known HTLV-producing cells is 5 to 95 percent, the highest percentage being seen with clones A-9 and B-2 of HUT 102 cell line.

The cell-free supernatant from Mo cell culture was examined for retrovirus particles related to HTLV. The clarified culture medium (500 ml) was centrifuged at 50,000*g*, and the sedimented particles were separated on a sucrose gradient (25 to 60 percent). The gradient fractions were analyzed by a competition radioimmunoassay for HTLV p24 (*8*). Fractions banding at 1.16 to 1.18 g/ml, characteristic of retroviral particles, were positive for HTLV p24 in the competition assay. These fractions containing HTLV antigens were pooled and subjected to electrophoresis in a cylindrical gel in the presence of sodium dodecyl sulfate in order to estimate the size of the competing protein. The gel was divided into 2-mm segments and eluted overnight in separate tubes with 10 m*M* sodium phosphate (*p*H 7.5) containing 100 m*M* NaCl, 0.1 m*M* phenylmethylsulfonyl fluoride, and bovine serum albumin (1 mg/ml). The eluted proteins were assayed in a homologous radioimmunoassay for HTLV p24 (*8*). A specific peak of competition was observed in the gel region corresponding to a molecular weight of 24,000, indicating that the immune cross-reactivity was resident in processed viral p24. The antigenic cross-reactivity of the particles was analyzed from particles sedimented through a 25 percent sucrose cushion from 200 ml of the culture medium. A homologous competition radioimmunoassay was set up, using ^{125}I-labeled HTLV p24 and a rabbit hyperimmune serum to disrupted HTLV (*8*) (Fig. 3A). Compared with extracts of HTLV-I the competition obtained with Mo particles was only partial (about 60 percent). Further, the slopes of the two competition curves were different, indicating that the antigenic cross-reactivity between Mo and HTLV was not type-specific. This phenomenon is similar to the cross-reactivities between protein p30 of baboon endogenous virus and RD114 virus (*21*), between protein p26 of Mason-Pfizer monkey virus and p36 of squirrel monkey retrovirus (*22*), and between p28 of the different strains of mammary tumor viruses (*23*). In all these examples, homologous competition assays detect strain differences between members of the respective groups, but such differences disappear when competition is measured in heterologous assays within the cross-reactive groups (*21–23*). To verify this non-type–specific interaction between HTLV and Mo, a heterologous radioimmunoassay was set up between ^{125}I-labeled HTLV p24 and antibodies in the serum of patient Mo. The rationale for this design was that the antibodies in the Mo serum would be type-specific only to the Mo antigens and therefore the determinants seen in such an assay will be the widely cross-reactive group specific ones. Such determinants should be common for different strains of the HTLV virus groups and the competition curves for Mo particles and HTLV should be similar. The results shown in Fig. 3B confirm this prediction. Serum antibodies of another patient (MJ) whose cells expressed retrovirus particles, indistinguishable from the original HTLV isolate (*6*), behaved similarly to the rabbit hyperimmune serum against HTLV in competition assays for p24 (Fig. 3C). In this system, Mo particles showed only partial competition like in the homologous assay (Fig. 3A) and the slopes were not identical.

Our data indicate that patient Mo with a T-cell variant of hairy cell leukemia has serum antibodies reactive with the structural protein (p24) of HTLV and that a cell line derived from this patients' spleen expresses antigens cross-reactive with HTLV proteins. Thus, this disorder joins a growing list of HTLV positive forms of lymphoid malignancies of adults. All involve mature T lymphocytes, and it may be that they represent a spectrum of the same disease. However, the pattern of immune cross-reactivity shows that the extracellular virus particles of Mo culture are not identical with HTLV-I from patient CR but are closely related retroviruses belonging to the same virus group. We have suggested that these be distinguished as HTLV-I and HTLV-II and that the initials of patients be used as subscripts to identify individual isolates within a given subgroup [for example, HTLV-I_{CR}].

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24. We thank Lillian Bruch and Emily Shepard for technical assistance.

23 April 1982; revised 6 August 1982