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Immunological Characterization of the Natural Antibodies to Human T-Cell Leukemia Virus in Human Sera

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Human T-cell leukemia virus (HTLV) is a unique, exogenously acquired human type C retrovirus. Its isolation, characterization, and evidence for its infectious transmission are presented elsewhere in this volume. Here we will discuss some of the biochemical and immunological properties of HTLV *gag* gene products, and summarize the main features of the natural antibodies to these proteins present in some human sera.

Like all the mammalian retroviruses, HTLV contains several small molecular weight proteins such as p19, p12, p24, and p15. These proteins appear to be homologous to the *gag* proteins p15, p12, p30, and p10 respectively of the prototype mouse type C viruses. We have purified p19, p24, and p15 of HTLV to homogeneity ([3]; Schuepbach J, Kalyanaraman VS, Sarngadharan MG, Blattner WA, Gallo RC, to be published). P19 is believed to be the 5′ terminal *gag* protein of HTLV and, like its homologue in murine viruses, appears to be blocked at its 5′ terminus (Oroszlan, unpublished observations). Extensive amino acid sequence analyses have been done on p24 and p15 ([6]; Oroszlan et al., unpublished). While it is quite clear that HTLV and bovine leukemia virus (BLV) are very different viruses, e.g., lack of immunological cross reactivities and little nucleic acid sequence homology, when the amino acid sequences of HTLV proteins were compared with the sequences of the corresponding *gag* proteins of other mammalian retroviruses, there were significant similarities only with proteins of BLV. Thus, between the amino terminal first 25 residues of BLV p24 and HTLV p24, there was correspondence among nine amino acids. Similar correspondence was observed up to the first 150 residues for which sequence data are available. This has been determined to be a statistically significant correlation, one that cannot be a result of chance alone. Similarly the amino acid sequence of HTLV p15 shares extensive homology with the BLV p12. This homology also includes a conservation of the nucleic acid binding domain characterized by the repeated cystine at positions n, n+3, and n+13 and a histidine at n+8, tryptophan at n+9, and aspartic acid at n+12 (Copeland TD, Morgan MA, Oroszlan S, to be published). These data strongly suggest that these homologous proteins of HTLV and BLV may have evolved from common ancestral molecules in some distant past. The extent of amino acid sequence homology described above also underscores the substantial *dissimilarities* between the two viruses and, in fact, the uniqueness of HTLV among mammalian retroviruses. As predicted, and as noted above, we have observed no immunological cross reactivity between HTLV and any other retrovirus, including BLV in conventional radioimmunoassays in either homologous or several widely cross-reactive heterologous systems ([3, 8]; Schuepbach J, Kalyanaraman VS, Sarngadharan MG, Blattner WA, Gallo RC, to be published).

A major finding that helped in assessing the extent of HTLV spread and its relevance to leukemias and lymphomas was that the initial patients whose cultured cells expressed the virus also had specific antibodies in their sera reactive against the protein components of the virus [4, 7]. It was expedient, therefore, to screen sera of pa-

tients for antibodies to HTLV, rather than attempting to isolate virus from each case. Four different methods were used in this large scale screening. (1) Immunofluorescence on HTLV-producing cells upon incubation with the test serum and an appropriate FITC-antibody conjugate [9]; (2) A solid-phase immunoassay using whole disrupted HTLV [7]; (3) A competitive binding assay using a monoclonal antibody to HTLV p19 and disrupted HTLV [10]; and (4) a specific radioimmunoassay using homogeneous core proteins ([4, 5]; Schuepbach J, Kalyanaraman, Sarngadharan MG, Blattner WA, Gallo RC, to be published). Detailed discussion here will be limited to specific immunoprecipitation studies using purified proteins.

Representative patterns of immunoprecipitation curves for ^{125}I-labeled HTLV proteins obtained with some antibody-positive human sera are shown in Fig. 1. The sera are from some Caribbean patients with T-cell lymphosarcoma cell leukemia (T-LCL) [1], some of their relatives, and some clinically normal Caribbean individuals not known to be related to any of the leukemia patients. The results indicate that all the HTLV *gag* proteins tested (p24, p19, p15) are precipitated by these sera. In all cases, the precipitation has been found to be highly specific. Irrespective of the labeled antigen used, the only competition observed is with either HTLV extracts or extracts of a cell line producing HTLV. None of a large number of other mammalian retroviruses including BLV or cultured normal human T cells showed any effect on this precipitation (Fig. 2). Specificity was also observed within HTLV to the particu-

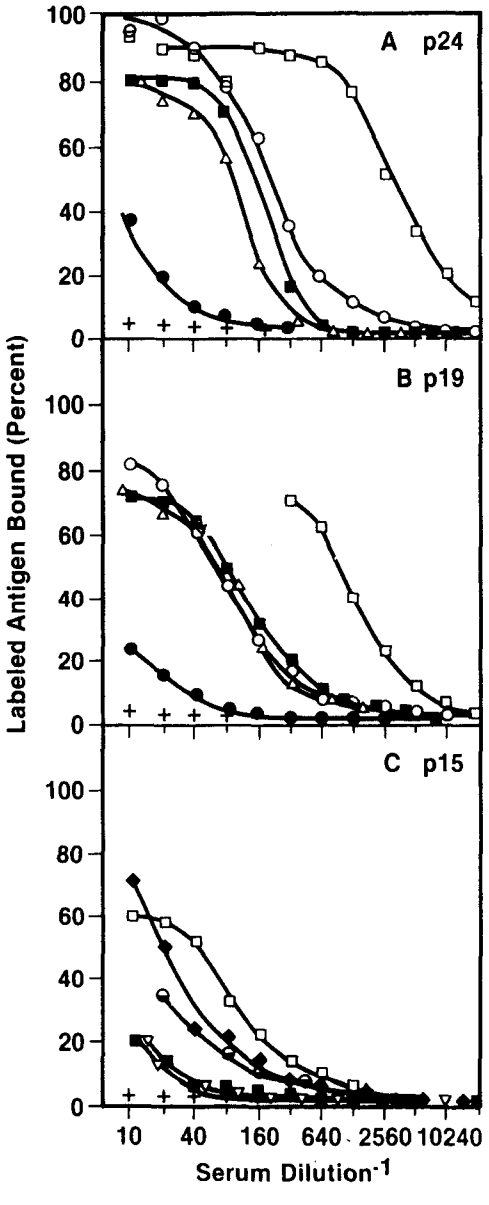

Fig. 1A–C. Representative radioimmune precipitations of purified and labeled HTLV proteins p24 **A**, p19 **B**, and p15 **C** by serum samples of TLCL patients (○, 23.1; ●, 24.1; △, F0706; +, F1123), their family members (■, F0781), and healthy individuals from the Caribbean (□, #81-001; ▽, C-579; ◆, C-581; ◒, C-585). Serial dilutions of serum were incubated with 8,000–10,000 cpm of the lapeled protein. A 30-fold excess of goat anti-human IgG antibody was added, and the percentage of labeled antigen bound in the precipitation was determined

lar protein being precipitated. Thus, when a mixture of ^{125}I-labeled p24, p19, and p15 was immunoprecipitated and the immune complex analyzed by polyacrylamide gel electrophoresis in the presence of SDS, radioactive peaks corresponding to all three proteins were observed (Fig. 3A). Anti-p15 titer was considerably lower than titers against p24 and p19 and, therefore, an aliquot of the serum precipitated less p15 than p24 and p19 (Fig. 3A). Increasing the serum concentration did increase the amount of p15 precipitated (Fig. 3E). When the above precipitation of the protein mixture was repeated in the presence of nonradioactive p24, p19, or p15 in successive experiments, the unlabeled antigens specifically blocked the precipitation of the corresponding radioactive proteins (Fig. 3B–D). Therefore, the serum contained specific and separate antibodies to all these internal viral proteins.

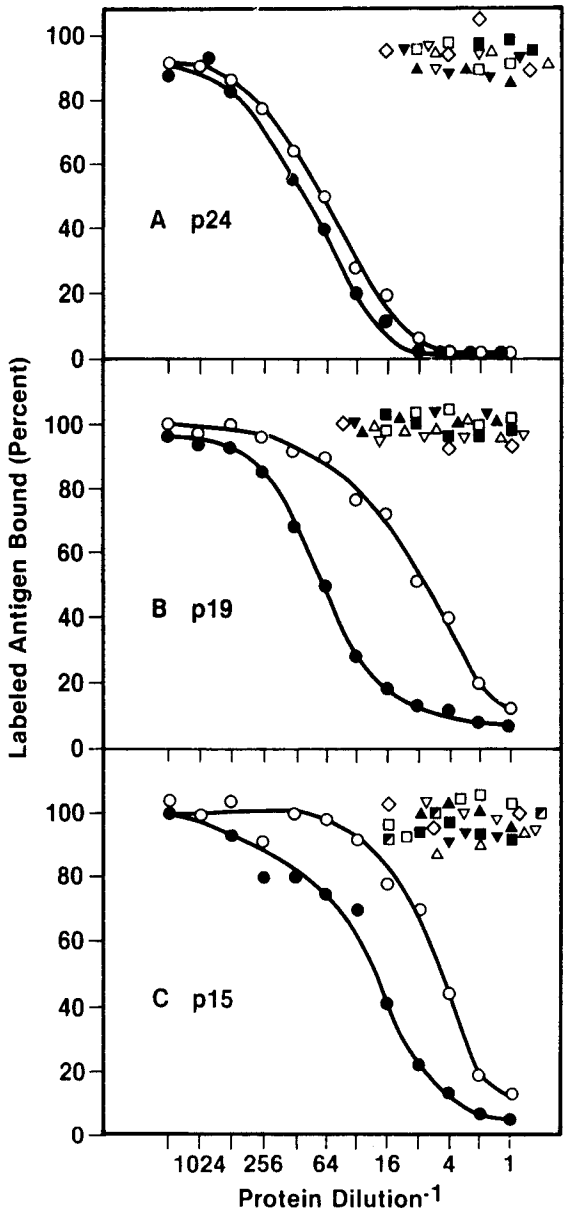

Fig. 2A–C. Viral and cellular competition in the precipitation of HTLV proteins p24 **A**, p19 **B**, and p15 **C**. Competition radioimmunoassays were performed with ^{125}I-labeled HTLV p24, p19, or p15, and limiting dilution of the Caribbean normal serum #81-001. Serial dilutions of the labeled antigens starting with 100 ng protein for viral and 50 μg of protein for cellular extracts were preincubated with the serum of 1 h at 37° C. Then 8,000–10,000 cpm of labeled proteins was added and precipitations were performed as under "Methods". ○, HUT102; □, HUT78; ◪, normal human T cells; ●, HTLV; ■, SSV; △, BaEV; M7; ▽, MPMV; ▲, BLV; ▼, FeLV; ◇, R-MuLV

A summary of the results of immunological screening of sera of patients with cutaneous T-cell leukemias/lymphomas (CTCL) in the United States, T-LCL in the Caribbean, and adult T-cell leukemia (ATL) in Japan are given in Table 1. Sera of normal relatives of HTLV-positive patients along with unrelated normal donors were also analyzed for antibodies. HTLV-related T-cell malignancies were found only very rarely in the United States and Europe. Accordingly, antibodies to HTLV proteins were only detected in 2 of 245 sera of CTCL patients that we analyzed. Both the positive cases were also positive for virus isolation. We should note, however, that there may be a significantly higher number of HTLV-positive cases of CTCL than indicated by these numbers because C. Saxinger in our group has found HTLV antigens in sera of some patients included in the antibody-negative CTCL group. In addition, there was a few other United States patients of miscellaneous diagnosis that were positive for antibody to HTLV ([2]; Gallo et al., to be published). So far only rare (< 0.5%) random normal donor in the United States has been found to be antibody positive to HTLV. In contrast, 100% of all the T-LCL sera from the Caribbean and 85% of the ATL sera from Japan have antibodies to HTLV. In Japan, ATL appears in geographical clusters. In these endemic areas about 10% of the normal population carry serum antibodies while only 2% were positive in nonendemic areas of Japan. In the Caribbean islands, the proportion of the random healthy donors positive was about 4%. Among the healthy

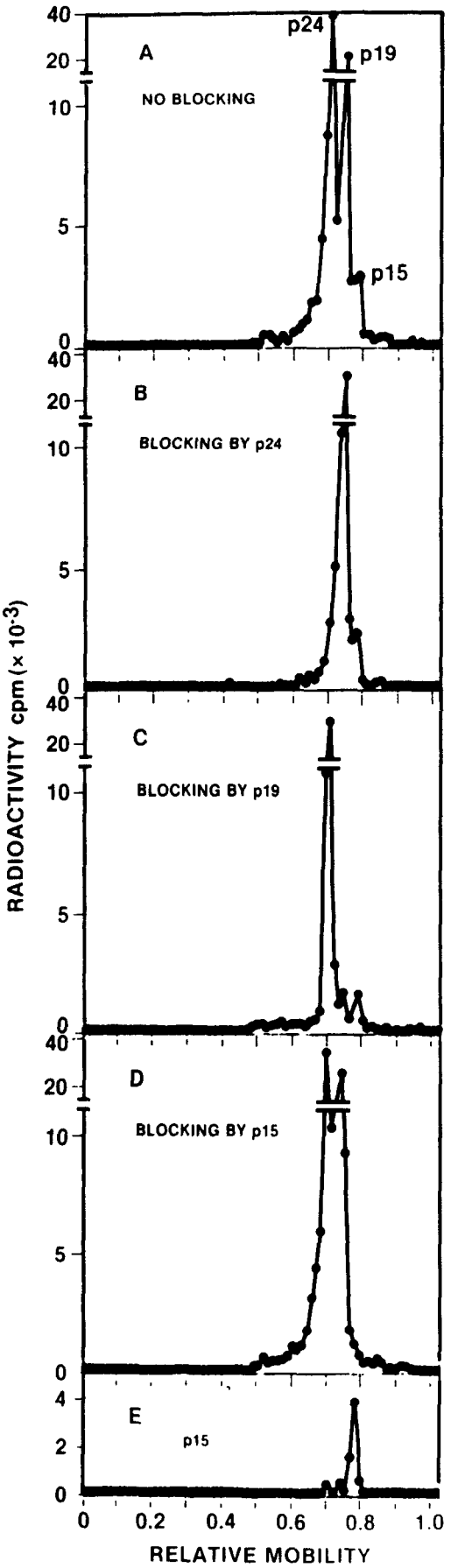

Fig. 3A–E. SDS-polyacrylamide gel electrophoretic analysis of purified and labeled HTLV proteins precipitated by serum #81-001. 100,000 cpm each of p24 p19, and p15 were mixed and incubated with 10 µl serum #81-001, and precipitations were made as described in Fig. 1. The precipitate was washed twice in 5 ml Buffer C, and analyzed by 12% SDS-polyacrylamide gel electrophoresis **A**. To determine the specificity of individual human antibodies for p24, p19, or p15, the serum was also preabsorbed for 3 h at 37° C with 100 ng purified unlabeled p24 **B**, 500 ng p19 **C**, or 500 ng p15 **D** before the mixture of the labeled proteins was added, and precipitations were performed and analyzed as above. A separate precipitate of labeled p15 by 20 µl serum #81-001 was analyzed in **E**, to give additional proof for the existence of the p15 peak in **A–D**. These data show that the purified protein preparations are free of major contaminants and that p24, p19, and p15 do not crossreact

Serum donors	Antibodies to HTLV	
	No. positive/ No. tested	% positive
United States CTCL patients	2/245[a]	<1
Healthy relatives of CTCL patients	1/8	13
Random healthy donors, United States	0/181	0
Caribbean Sézary and T-LCL patients	8/8	100
Healthy relatives of Caribbean patients	3/16	19
Random healthy donors, Caribbean	12/337	4
Japanese ATL patients	29/34	85
Healthy relatives of ATL patients	13/31	42
Random healthy donors, nonendemic areas	9/509	2
Random healthy donors, endemic areas	39/404	10

[a] Recently, C. Saxinger et al. (unpublished) found HTLV antigen in sera of some of the CTCL antibody-negative patients

Table 1. Prevalence of natural antibodies to HTLV in sera of patients with malignancies of mature T cells, their healthy relatives, and random normal donors

donors, the group that has the highest incidence of serum antibodies to HTLV was the relatives of leukemic patients who are known to be virus positive. Thus out of eight relatives of the two CTCL patients in the United States that were screened, one was positive. Similarly, 42% positivity was found among relatives of Japanese ATL patients and 19% among relatives of Caribbean T-LCL patients. These values are significantly higher than the normal incidence in the population in the respective geographical locations. Among these relatives who were seropositive were spouses, parents, children of either sex, brothers, and sisters, indicating a mode of horizontal transmission of the virus. While those living in the endemic areas could have been exposed to the virus outside the family, the significantly elevated incidence among relatives of virus-positive leukemic patients is a clear reflection of the increased exposure to the virus within the family over the background exposure in the respective neighborhood.

There have been a few cases of non-T-cell malignancies in which antibodies to HTLV have been detected [2]. At least some of them are known to be from ATL-endemic areas, while precise information is lacking on some other caces. Fortuitous infection with HTLV cannot be ruled out in these cases. The available evidence points to a correlation between HTLV infection and a group of adult T-cell malignancies.

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