NATURAL ANTIBODIES TO THE HUMAN T CELL LYMPHOMA VIRUS IN PATIENTS WITH CUTANEOUS T CELL LYMPHOMAS

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Type C RNA tumor viruses are known to cause lymphomas or leukemia in lower mammals and subhuman primates (1, 2). In certain animals, e.g., cats, antibody against virus-associated proteins apparently protects against virus-induced neoplasias (3, 4). Nucleic acids and proteins related to components from animal retroviruses have sometimes been found in fresh and cultured human cells, but the detection and isolation of discrete particles is a rare event (5–7). Mammalian type C retroviral proteins can be immunogenic in man (8), and human antibodies reactive with retroviral proteins have been reported by several investigators (9–14). The major reactivity reported has been against the viral glycoprotein coat (gp70), and there has been a consistent lack of correlation between antibody titer and a specific pathologic state. Recently, the existence of human antiviral reactivity has been questioned because many of the natural antibodies described have been shown to lack specificity and to cross-react with antigenic determinants present in fetal calf serum and nonhuman cells (15–17). The lack of a well-defined human retrovirus has obviously impeded progress on this issue.

In animals, retroviruses often infect T cells. It was therefore of interest to examine human T cells for the presence of these viruses. To carry out such analyses, the ability to grow these cells in culture is essential. After the discovery of T cell growth factor (TCGF; 18, 19)[1] it became possible to grow in vitro relatively mature human T cells. Several novel types of normal and neoplastic T cell lines were established (20, 21). Two interesting observations emerged from these studies: (*a*) many of the neoplastic T cells from some T cell leukemias and lymphomas respond directly to TCGF, whereas normal T cells require initial lectin or antigen activation (20, 22, 23), and (*b*) the use of purified TCGF (24) is superior to crude fractions in maintaining the growth of these cells because of the removal of inhibitors as well as the removal of the lectin

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[1] *Abbreviations used in this paper:* AMV, avian myeloblastosis virus; BaEV, baboon endogenous virus; BoLV, bovine leukemia virus; BSA, bovine serum albumin; CBC, coating buffer; CTCL, cutaneous T cell lymphoma and leukemia; DTT, dithiothreitol; FCS, fetal calf serum; FeLV, feline leukemia virus; $GALV_{SF}$, San Francisco strain of the gibbon ape leukemia virus; HTLV, human T cell lymphoma (leukemia) virus; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; RD114, feline endogenous virus; RIP, radioimmunoprecipitation; RLV, Rauscher murine leukemia virus; SSV, simian saroma virus; TCGF, T cell growth factor.

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that activates normal T cells. Purified TCGF can therefore be used to culture neoplastic T cells in the absence of contaminating normal T cells (22).

Three cell lines established a year apart from two patients with cutaneous T cell lymphoma and leukemia (CTCL), have expressed type C retrovirus particles called HTLV, strains CR and MB (25).[2] Analysis of these isolates showed that they are not significantly related to previous animal retrovirus isolates. These analyses included the lack of significant nucleic acid homology between HTLV sequences and those of animal retroviruses (26), lack of detectable immunologic cross-reactivity of the major core protein (p24) of HTLV with that of other retroviruses (27), and lack of significant antigenic relatedness of the reverse transcriptase purified from HTLV and the reverse transcriptase of other retroviruses (28).

The availability of frozen sera from patient CR, whose lymph node-derived cell line, HUT102, gave rise to $HTLV_{CR}$ (25), provided a unique system with which to readdress the question of natural antibodies. In this paper, data obtained using two different assay systems demonstrate: (*a*) the presence of natural antibodies in the serum of patient CR directed against the major core proteins of $HTLV_{CR}$, and (*b*) the specificity of these antibodies for the proteins of HTLV. Data reported elsewhere have shown the presence of the major core protein (p24) and nucleotide sequences of $HTLV_{CR}$ in the primary uncultured leukemic cells of a patient with Sezary T cell leukemia.[2] Cells obtained from the blood of this patient were those from which $HTLV_{MB}$ was isolated. The results reported here extend the finding of HTLV information in primary leukemia cells by providing strong evidence that the virus was present in patient CR during the course of his disease and that viral core proteins were important in the antibody response.

Materials and Methods

Sera. Serum samples from patients with CTCL were collected at the Washington Veteran's Administration Hospital during 1979. Normal sera were obtained from 55 random donors, including some laboratory workers.

Viruses and Cells. Retroviruses used included $HTLV_{CR}$ grown in HUT102 cells; $HTLV_{MB}$, the second independent viral isolate from a human Sezary syndrome-derived T lymphoblastic cell line; the M7 strain of the baboon endogenous virus (BaEV) grown in A204 cells (human rhabdomyosarcoma) or in A7573 cells (dog thymus); the simian sarcoma virus (SSV) grown in A204 cells; the Rauscher murine leukemia virus (RLV) grown in JLSV-10 cells (mouse fibroblasts); the cat endogenous virus RD114, grown in RD cells (human rhabdomyosarcoma); the feline leukemia virus (FeLV), grown in FL-74 cells (feline lymphoblasts); the San Francisco strain of the gibbon ape leukemia virus ($GALV_{SF}$), grown in NC37 cells (normal human B lymphoblasts); the bovine leukemia virus (BoLV) grown in fetal lamb kidney cells; and the avian myeloblastosis virus (AMV) purified from chick plasma provided by Dr. Joseph Beard, Life Sciences, Inc., Gulfport, Fla. These viruses were disrupted in 50 mM Tris-HCl, pH 7.9, 0.25% Triton X-100, 0.2 mM dithiothreitol (DTT), 1 mM EDTA, and 1 mM phenylmethyl sulfonyl fluoride (PMSF), and centrifuged at 80,000 *g* for 90 min. The solubilized viral proteins were mixed end over end at 4°C for 2 h with SM-2 biobeads (Bio-Rad Laboratories, Richmond, Calif.) to remove the detergent (29). Cells used included normal human T cells obtained by phytohaemagglutin stimulation of peripheral blood leukocytes for 72 h; the standard human T cell lines, Molt-4 and CEM; the Daudi human B cell line; the $HTLV_{CR}$-producing HUT102 line; and the CTCL-derived transformed but non-virus-producing HUT78 cell line. Cell lysates were prepared by incubating cell pellets for 1 h at 4°C in 20 mM sodium phosphate buffer, pH

[2] Poiesz, B. J., F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, and R. C. Gallo. Evidence for nucleic acids and antigens of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T cell leukemia and isolation of the virus. Manuscript submitted for publication.

7.5, containing 0.5% Triton X-100, 0.8 M NaCl, and 1 mM PMSF. Particulate material was removed by centrifugation at 42,000 *g* for 30 min. Protein concentrations of perchloric acid-precipitated samples of solubilized viruses and cell lysates were measured by the method of Lowry et al. (30).

Solid-Phase Radioimmunoassay. A modified version of a previously published solid-phase assay was used for screening the sera (31, 32). For each serum sample to be tested, 100 ng of each solubilized viral preparation, diluted in 50 μl of 15 mM $NaHCO_3$, 35 mM Na_2CO_3, and pH 9.6 coating buffer (CBC), were added in duplicate to wells of a 96-well Immulon plastic microtiter plate (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, Va.). Control wells of 50 μl of 1% bovine serum albumin (BSA) in CBC and 50 μl of undiluted fetal calf serum (FCS) were included for each complete serum assay. After overnight absorption at 4°C, unbound protein was aspirated and the wells were coated with 1% BSA in CBC for 2 h to block naked binding sites. The plates were rinsed three times with 0.1% BSA in phosphate-buffered saline (PBS), and 50 μl of a 1:75 dilution of a human serum sample diluted in PBS containing 0.5% Tween 20 (PBS-Tween) was added to each well for 1 h at 27°C. Unbound proteins were aspirated and the wells were washed three times with 0.1% BSA in PBS. Goat anti-human IgG (N. L. Cappel Laboratories, Inc., Cochranville, Pa.) was purified by affinity chromatography through a column of human IgG coupled to Sepharose 4B and then iodinated by the chloramine T method (33) to a specific activity of 800,000 cpm/μg protein. 50,000 cpm of ^{125}I-goat anti-human IgG diluted in 50 μl of PBS-Tween were added to each well for 1 h at 27°C. Unbound radiolabeled detector was removed and the plates were washed 7–10 times with tap water and dried. The wells were separated on a band saw and counted for 1 min in an LKB 1280 Ultrogamma counter (LKB Instruments, Inc., Rockville, Md.)

Solid-Phase Immunocompetition Assay. Disrupted viral protein (50 ng of $HTLV_{CR}$ or 100 ng of BaEV, $GALV_{SF}$, or FeLV) in 50 μl CBC was absorbed to wells of a microtiter plate as for the solid-phase radioimmunoassay. Sera to be tested were diluted in PBS-Tween to give ~30% of maximal binding to the virus on the plate. 50-μl aliquots of the diluted sera were preincubated with the indicated amounts of competing protein in 50 μl PBS-Tween for 1 h at room temperature. Subsequently, 50-μl aliquots of the preincubation mixture were added in duplicate to the virus-coated microtiter wells. The remainder of the assay proceeded as described for the solid-phase radioimmunoassay. In the case of competition by cell lysates, a control competition using cell lysis buffer alone was carried out to correct for possible removal of adsorbed $HTLV_{CR}$ from the microtiter wells by the Triton X-100. The percent antigen binding was determined by dividing the value obtained in the presence of competing cellular protein by the value obtained in the presence of an equivalent volume of lysis buffer.

Radioimmune Precipitation Assay. Disrupted $HTLV_{CR}$ (20 μg) was extensively dialyzed against PBS and reacted with 1 mCi of monoiodinated Bolton-Hunter reagent (New England Nuclear, Boston, Mass.) for 1 h at 4°C. The reaction was terminated by the addition of 0.2 M glycine and 0.1 M sodium borate, pH 8.5; and the mixture was passed through an 8-ml Bio-Gel p10 column (Bio-Rad Laboratories) previously equilibrated with a buffer of 0.05 M sodium phosphate, pH 7.5, containing 0.25% gelatin. Fractions were eluted with PBS and collected into an equal volume of PBS containing 1% BSA. Titration curves were constructed by incubating ~150,000 cpm of iodinated $HTLV_{CR}$ proteins (7,500 cpm/ng protein) in 50 μl of 0.1% gelatin; 300 mM NaCl; 0.2% Triton X-100; 0.01% Tween 80; 10 mM sodium phosphate, pH 7.5; 0.02% sodium azide (radioimmunoprecipitation [RIP] buffer) with serial twofold dilutions of serum in RIP buffer. The final volume of the mixture was adjusted to 500 μl with RIP buffer. After incubation for 16 h at 4°C, the antigen-antibody complexes were precipitated at 4°C for 24 h by adding sufficient staphylococcal protein A Sepharose (previously swollen in RIP buffer) in 250 μl RIP buffer to give a hydrated bead volume of 10 times the serum volume added. Precipitates were washed three times with 1-ml portions of RIP buffer and counted in the gamma counter.

Competition RIP Assay. Sera were diluted in RIP buffer to give ~50% precipitation of iodinated HTLV proteins. Mixtures containing serum, ^{125}I-HTLV proteins, and competing proteins (either disrupted viruses or cell lysates) were incubated for 16 h at 4°C. Precipitation of immune complexes and subsequent processing of the precipitates were carried out as for the radioimmune precipitation assay.

Polyacrylamide Gel Electrophoresis. Samples were electrophoresed on 12% polyacrylamide gels containing sodium dodecyl sulfate according to the method of Laemmli (34). 1-mm slices of each gel were counted in the gamma counter.

Molecular weights were determined by comparing relative mobilities of $HTLV_{CR}$ proteins to those of standard proteins, including phosphorylase A, BSA, ovalbumin, chymotrypsinogen, and ribonuclease.

Results

Reactivity to $HTLV_{CR}$ in CTCL Patient Sera. The coded sera of 17 patients with CTCL and 55 normal donors were screened for the presence of antibodies to $HTLV_{CR}$ and other retroviral proteins by a solid-phase radioimmunoassay (Table I). Only the data for the first 20 normal sera assayed are listed individually; results for the entire 55 are summarized at the bottom of Table I. The purpose of this limited survey was to determine whether antiviral reactivity could be found in the sera of patients with CTCL, especially in sera previously collected from the patient CR, and to determine whether the antibody binding was specific for $HTLV_{CR}$ proteins. Unfortunately, no serum was available from patient MB, the source of $HTLV_{MB}$. No attempt was made to use this series to define the interface between a "positive" and "negative" value.

Two sera, one from the patient CR and a second from patient CTCL-4 (CTCL-17 serum is a second sample from patient CTCL-4), bound strongly to $HTLV_{CR}$; patient CR serum also reacted strongly with $HTLV_{MB}$ (Table I). The amount of $HTLV_{MB}$ available was limited, so CTCL-4 serum was not tested with it. None of the control sera bound to $HTLV_{CR}$ as significantly as these two sera. The binding of patient CR and CTCL-4 sera to $HTLV_{CR}$ compared with the mean binding of control sera was 20- and 12-fold greater, respectively. Whereas 75-fold dilutions of sera were used in this survey, significant binding of both patient CR and CTCL-4 sera to $HTLV_{CR}$ could easily be detected at dilutions of 1:10,000. CTCL-4 serum was obtained from a patient with Sezary syndrome, a T cell leukemia. A sufficient amount of this serum was available for demonstrating the copurification of the antiviral reactivity with the IgG fraction after elution from a DEAE cellulose column (35; data not shown).

A wide variety of other antiviral reactivities was also seen in patient and control sera (Table I). We show below that the apparent cross-reactivities to animal type C retroviruses, including BaEV, SSV, GaLV, RLV, and FeLV proteins are not viral specific and are therefore probably artifacts. Recent reports have shown that the bulk of and perhaps all human serum antibody reactivities to animal RNA tumor viruses can be traced to either a fetal calf antigen that copurifies with the virus or to antigenic components that are appended to the virus by the cell in which the virus is grown (15–17). In this initial report, we chose to examine the broad spectrum of antiviral rectivities and to determine whether natural antibody specific for $HTLV_{CR}$ existed by focusing only on the two serum samples (patient CR and CTCL-4) that reacted strongly with $HTLV_{CR}$.

Specificity of Serologic Reactivity to $HTLV_{CR}$. The specificity of the serologic reactivities observed was first investigated using a solid-phase immunocompetition assay that allowed us to test reactivity against several viral preparations at an equivalent level of sensitivity after iodination of a single stable probe. This assay avoided exposure of virus to strong oxidants, such a chloramine T, which may destroy antigenic sites. In addition, short incubation times minimized protease digestion.

The reactivities of patient CR serum and CTCL-4 serum were remarkably specific

TABLE I

Serologic Reactivity to HTLV$_{CR}$ and Other RNA Tumor Viruses in Patients with Cutaneous T Cell Lymphomas and Leukemias and in Normal Individuals

Serum	HTLV$_{CR}$	BoLV	FeLV	M7	SSV	GALV	RLV	(AMV)	BSA*	FCS*
Patient sera										
Patient CR	8,217 5,788‡	282	795	4,930	190	194	558	(560)	385	268
CTCL-2	0	0	0	0	0	0	0	(643)	265	229
CTCL-3	127	0	73	0	215	106	224	(563)	289	320
CTCL-4	5,295	503	1,685	1,726	1,659	2,841	1,952	(611)	280	306
CTCL-5	211	607	0	36	134	235	343	(535)	328	265
CTCL-6	1,023	385	700	406	1,034	974	836	(590)	281	351
CTCL-7	0	130	0	0	0	0	0	(437)	299	216
CTCL-8	898	392	1,232	188	1,284	1,731	1,431	(632)	272	234
CTCL-9	315	5	169	0	145	159	51	(563)	289	204
CTCL-10	462	123	464	46	216	252	688	(502)	291	230
CTCL-11	1,607	868	1,430	653	2,512	2,913	1,613	(559)	451	406
CTCL-12	984	1,526	2,560	1,702	3,972	3,930	2,565	(510)	376	373
CTCL-13	442	270	175	73	237	156	575	(323)	256	265
CTCL-14	198	63	54	0	265	163	203	(507)	356	363
CTCL-15	24	0	189	0	64	0	822	(839)	296	414
CTCL-16	291	55	370	7	385	246	782	(440)	330	224
CTCL-17	5,403	716	1,402	1,196	1,434	1,655	1,635	(494)	223	351
CTCL-18	405	0	386	0	242	353	548	(531)	226	240
Normal sera										
1	2	0	0	0	135	36	78	(590)	286	196
2	0	361	671	142	1,000	851	845	(559)	329	203
3	0	0	0	0	221	207	0	(544)	246	224
4	54	64	79	0	148	108	225	(441)	318	233
5	151	378	57	12	269	318	243	(317)	326	186
6	363	233	82	0	241	309	0	(494)	208	215
7	394	326	1,319	0	693	1,600	1,161	(1,444)	387	359
8	165	62	410	0	149	868	493	(1,119)	359	394
9	259	39	330	101	484	377	681	(471)	209	185
10	0	0	410	171	533	360	626	(799)	368	281
11	127	102	304	0	404	211	624	(631)	195	185
12	602	319	982	248	1,363	1,338	1,464	(517)	260	310
13	652	292	734	0	880	964	1,234	(1,226)	367	460
14	477	459	531	37	652	914	1,385	(1,206)	513	539
15	726	395	250	0	499	738	43	(635)	199	196
16	0	6	0	0	0	0	191	(814)	214	237
17	741	463	558	388	935	1,297	779	(551)	211	190
18	375	271	570	43	1,213	822	1,190	(472)	188	155
19	0	0	0	0	24	0	134	(817)	170	222
20	59	53	24	163	109	378	108	(614)	194	172
Mean§	380 (±413)	153 (±201)	563 (±611)	136 (±246)	ND‖	664 (±714)	789 (±719)	876 (±489)	510 (±716)	ND

* AMV background values not subtracted.

‡ Serologic reactivity of patient CR serum to HTLV$_{MB}$.

§ Mean value of 55 normal sera including the 20 shown here, ± SD.

‖ Not done on all 55 normal sera.

for HTLV$_{CR}$, and in the one case tested, patient CR, also specific for HTLV$_{MB}$. These reactivities could be competed only by HTLV$_{CR}$ or HTLV$_{MB}$, and not by a variety of other solubilized viral preparations, including the primate type C viruses BaEV, GALV, and SSV (Fig. 1a and b). The specificity was further verified using protein extracts of cells in similar competition assays. Lysates of HUT102 cells, one of the cell lines that produces HTLV$_{CR}$, competed the reactivity of patient CR serum to HTLV$_{CR}$. There was no significant competiton with other cell lysates, including those from normal human T cells; from established normal or transformed human B cell lines; from established transformed immature T blast cell lines; or from HUT78 cells,

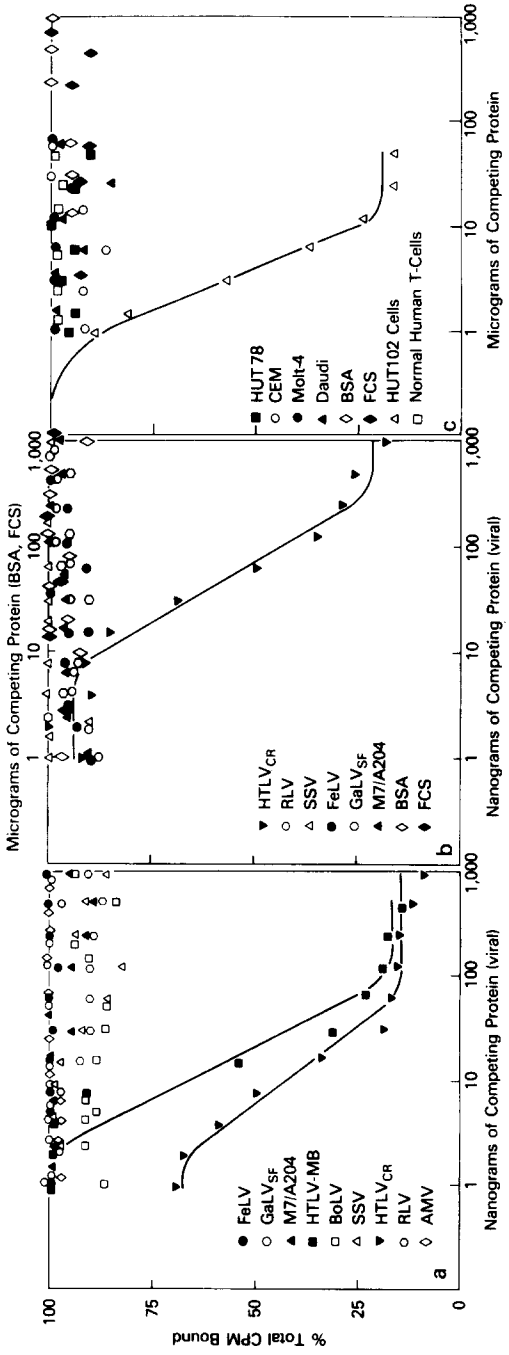

FIG. 1. Solid-phase immunocompetition assay of patient sera reactivities for HTLV$_{CR}$. (a) Reactivity of serum from patient CR to proteins of HTLV$_{CR}$ competed by solubilized viruses. (b) Reactivity of serum from patient CTCL-4 to proteins of HTLV$_{CR}$ competed by solubilized viruses. (c) Reactivity of serum from patient CR to proteins of HTLV$_{CR}$ competed by cell lysates.

a transformed cell line established from cells of another patient with cutaneous T cell lymphoma but not producing HTLV (Fig. 1c). In addition, competition with up to 1 mg of BSA or FCS failed to diminish binding of either CTCL-4 serum or patient CR serum to $HTLV_{CR}$ (Fig. 1b and c).

Identification of Involved Proteins and Confirmation of Anti-HTLV Specificity. To identify the proteins of $HTLV_{CR}$ responsible for the reactivities observed with the patient CR and CTCL-4 sera, we developed an RIP assay against the proteins of the solubilized virus. We have performed these experiments with both CTCL-4 and patient CR sera with the same results, but only results with the latter are shown. The patient CR serum recognized $HTLV_{CR}$ proteins with molecular weights of 24,000 and 19,000 (Fig. 2a and b). A control serum from a normal donor failed to precipitate any $HTLV_{CR}$ proteins. These two proteins, p24 and p19, have previously been identified as components of $HTLV_{CR}$ and appear to be components of the viral core (27). The evidence for this is that (*a*) they copurify with the $HTLV_{CR}$ particles and cores; (*b*) they can be identified when preparations of the solubilized $HTLV_{CR}$ proteins and not the intact virus are radiolabeled; (*c*) in the case of p24, the protein elutes from phosphocellulose in a manner similar to known viral p30 core proteins; (*d*) the p24 migrates with reverse transcriptase activity with a density of intact virions and shifts to a density of viral cores when the virus is treated with detergent; and (*e*) these proteins are readily detected in virus-producing transformed T cells but not in normal growing human cells, including T cells, until the cells are infected in the laboratory.

Because the patient CR serum precipitated p24 and p19 of $HTLV_{CR}$, we developed a competition RIP assay against $HTLV_{CR}$ to confirm the results of the solid-phase

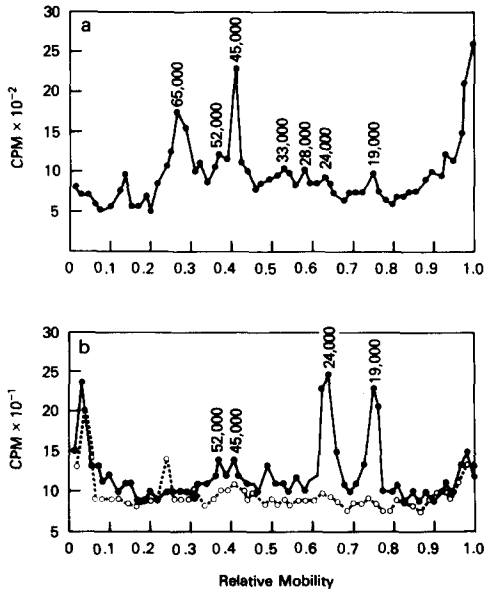

FIG. 2. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of iodinated proteins of $HTLV_{CR}$ (a) and immune precipitates obtained using serum from patient CR or normal control serum and iodinated proteins of $HTLV_{CR}$ (b). The immune precipitates in (b) contained nearly equivalent counts of ^{125}I and were obtained using either patient CR serum (●) or normal serum (○).

immunocompetition assay. We were able to use greater amounts of competing viral protein in the expanded volumes of the RIP assay to confirm the specificity of the natural antibody response to $HTLV_{CR}$. The results of the RIP competition failed to demonstrate significant homology between the $HTLV_{CR}$ p19 or p24 and the proteins of any other virus tested (Fig. 3a). Only $HTLV_{CR}$ effectively inhibited $HTLV_{CR}$ precipitation over the range of protein concentrations tested. Similarly, when cell lysates were used in the compeition assays, only the $HTLV_{CR}$-producing HUT102 cells were able to compete the precipitation of $HTLV_{CR}$ proteins (Fig. 3b).

Lack of Specificity of Serologic Reactivities to Animal RNA Tumor Viruses. We next turned our attention to the apparent cross-reactivities of patient CR and CTCL-4 sera with BaEV and of CTCL-4 with both the woolly monkey-gibbon ape retroviruses and FeLV (Table I). Solid-phase immunocompetition assays were carried out to determine the specificity of these reactivities (Fig. 4).

The binding of patient CR serum to BaEV could be reduced by preincubation with BaEV, strain M7, grown in A204 cells (M7/A204), with RD114, and at high concentrations with $HTLV_{CR}$ (Fig. 4A). Similar data were obtained using CTCL-4 serum (not shown). The competition by $HTLV_{CR}$ was unidirectional, i.e., $HTLV_{CR}$ could reduce the binding of patient CR and CTCL-4 sera to M7/A204, but preabsorbing either serum sample with M7/A204 could not reduce binding to $HTLV_{CR}$ (Fig. 4A and B). This result cannot be explained by a single cross-reactive viral determinant present in greater quantity in $HTLV_{CR}$ than in M7/A204. If this were the case, $HTLV_{CR}$ should reduce the serum reactivity to M7/A204 at a significantly lower concentration than M7/A204 itself, exactly opposite to what is found (Fig. 4). In addition, the reactivity of patient CR serum (and CTCL-4 serum) to M7/A204 could not be competed by BaEV grown in canine thymus cells (M7/A7573), suggesting that the reactive antigen from M7/A204 is cellular and not viral in origin. Barbacid et al. (15) have suggested that 1% BSA will preabsorb human natural antibodies directed toward animal RNA tumor viral proteins. The fact that our buffers did not include BSA does not affect our results because the cross-reactivity

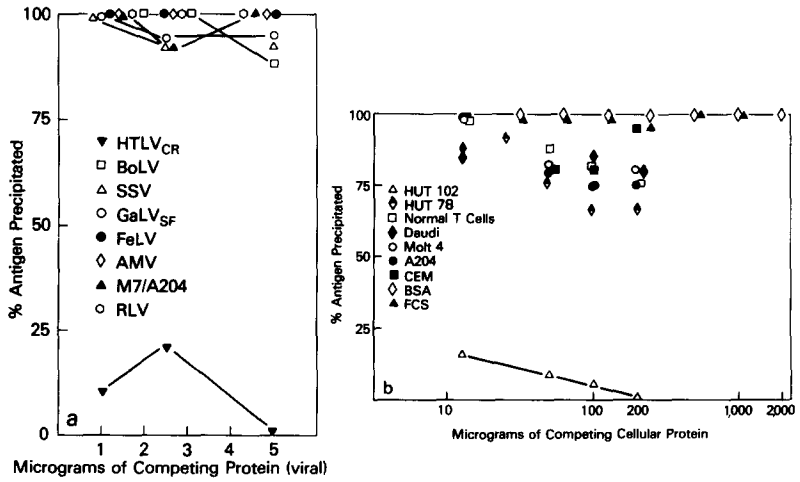

FIG. 3. Competition radioimmunoprecipitation assay of patient CR serum against solubilized viral preparations (a) and cell lysates (b).

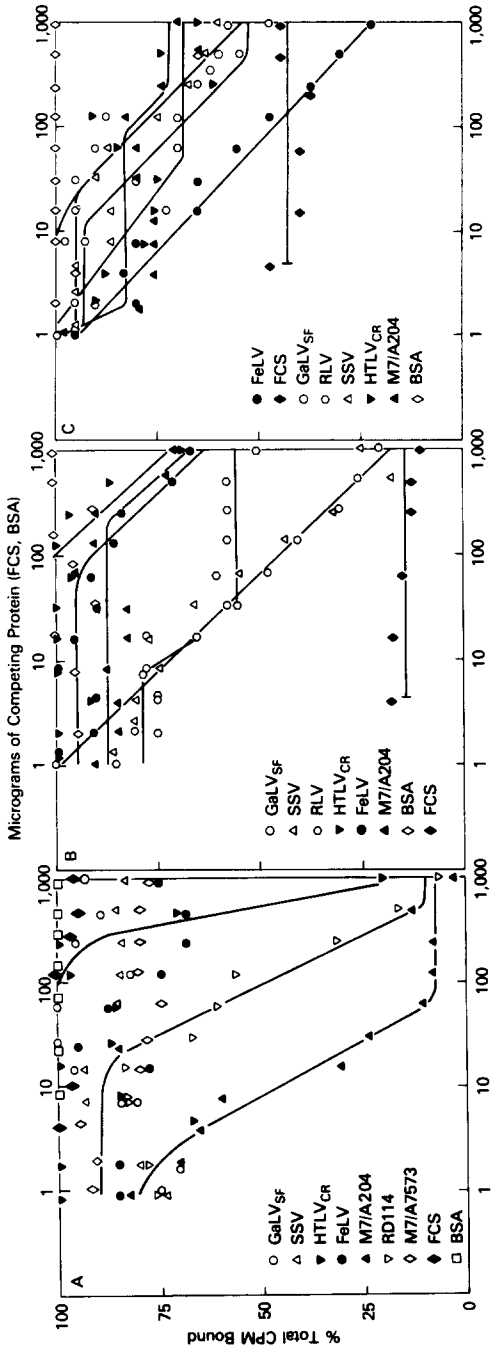

FIG. 4. Solid-phase immunocompetition assay of patient CR and CTCL-4 sera reactivities for various RNA tumor viruses. (A) Patient CR serum reactivities for BaEV, M7 strain, grown in A204 cells (M7/A204) competed by FCS, BSA, or viral proteins. The latter included BaEV, M7 strain, grown in dog thymus cells (M7/A7573). (B) CTCL-4 serum reactivities for GALV$_{SF}$ competed by FCS, BSA, or viral proteins. (C) CTCL-4 serum reactivities for FeLV competed by FCS, BSA, or viral proteins.

described is only found when the virus of interest is grown in nonhuman cells (15). This is not the case with $HTLV_{CR}$, $HTLV_{MB}$, or M7/A204, which are all grown in human cells. However, to erase any doubts as to the presence of BSA or FCS shared determinants, immunocompetition assays were carried out using BSA or FCS as competitors. Neither one competed the observed binding to M7/A204 (Fig. 4A).

These data suggest no homology between $HTLV_{CR}$ and BaEV. This conclusion was verified by molecular hybridization studies (26), by competition radioimmunoassays using various viral core proteins (p30) and $HTLV_{CR}$ p24 (27), and by comparisons of the cross-reactivity of the reverse transcriptase of HTLV and that of other retroviruses (28). All tests failed to show any relationship between HTLV and BaEV. The results indicate therefore that whereas patient CR serum reactivity to BaEV is probably directed against a cellular antigen, its reactivity to HTLV is clearly viral specific.

The CTCL-4 serum was the only one identified that reacted with the primate viruses of the woolly monkey-gibbon ape group, with RLV and FeLV, as well as with $HTLV_{CR}$ and BaEV (Table I). Solid-phase immunocompetition studies revealed that binding to $GALV_{SF}$ could be completely inhibited by preabsorption with high concentrations of FCS (Fig. 4B). No inhibition of binding occurred with purified BSA. This parallels the data of Snyder et al. (16, 36) who found that the apparent specificity of human antibodies for the gp70 of SSV was in fact cross-reactive with a carbohydrate antigen present in FCS.

The binding of CTCL-4 serum to FeLV (Table I) was also nonspecific. The reactivity could be reduced to 50% after incubation with FCS, whereas preincubation with the same FeLV preparation present on the solid phase could reduce binding to <25% of maximum (Fig. 4). This indicates that an antigen associated with the FeLV preparation cross-reactive with a fetal calf antigen is partially responsible for the reduction in serum binding and that a second distinct antigen is also present. We do not know if the latter antigen is viral or cellular in origin.

The identification of the reactive antigen in FeLV or $GALV_{SF}$ is not the purpose of this publication. These relationships were explored with the CTCL-4 serum to show that they were distinct from the viral-specific cross-reactivity of both patient CR and CTCL-4 sera for $HTLV_{CR}$. By inference, however, one may conclude that the wide variety of reactivities present in patient and control sera (Table I) directed toward FeLV, GALV, and the related viruses SSV and RLV, share the same lack of specificity.

Discussion

The HTLV are new retroviruses not yet detectably related to known animal retroviruses. These viruses were isolated from cells of different patients, one with a cutaneous T cell lymphoma, the other with a cutaneous T cell leukemia. There were strong arguments that the HTLV isolates were not contaminants: (*a*) the repeated detection of viral particles in different clinical specimens of patient CR; (*b*) the detection of viral particles within the first few days of putting the primary tumor cells in culture; and (*c*) the lack of a close relationship to other known animal retroviruses, as with the bovine leukemia virus. However, it was still important to determine whether HTLV could be detected in vivo. This has now been accomplished by two different methodologies, including the demonstration of HTLV protein and nucleic

acids in primary (uncultured) neoplastic T cells of a few patients,[2] and the finding of antibodies to HTLV in human sera as shown in this report and elsewhere.[3] We are aware that previously reported antibody responses toward purported animal viral proteins subsequently have been shown to be nonspecific cross-reactions. Therefore, detection of a reactivity against a viral protein may not verify an in vivo infection. Others have shown (15–17), and we were able to confirm with respect to primate and feline retroviruses that such reactivities may be devoid of viral specificity. However, the data shown here regarding HTLV, isolated from the lymph node-derived cell line of patient CR, are the first to unequivocally demonstrate antibodies from human sera with a high degree of specificity for retroviral antigens. We used a plate competition assay that demonstrated that patient CR serum was specific for $HTLV_{CR}$ and $HTLV_{MB}$ proteins and that the antigens recognized were not cross-reactive with those of any commonly encountered retroviruses. The results of the solid-phase assay agreed completely with the RIP competition studies performed against disrupted $HTLV_{CR}$, substantiating the specificity of the natural antibodies and the uniqueness of the $HTLV_{CR}$ determinants. The antibodies present recognized core proteins that would be expected if the virus were truly replicating in vivo. Data reported elsewhere show that the same patient CR and CTCL-4 sera possess antibodies to homogeneously purified HTLV p24, the major core protein of the virus.[3]

Antibodies to HTLV have not been detected in the majority of patients with cutaneous T cell leukemias and lymphomas studied to data. However, from experience gained from animal models (37, 38), it is clear that the absence of antibodies in a given patient does not necessarily mean that HTLV never infected these people. Cellular transformation may be more common than virus production, and antibodies will not be produced unless viral proteins are expressed. It is also possible that $HTLV_{CR}$ expression may be associated only with pathologic subsets of CTCL, such as those with peripheral blood or visceral involvement. A much more extensive study using sera from patients with CTCL and other T cell neoplasias, and normal donors is clearly needed now to gain information on the natural virus reservoir and its mode of transmission.

Summary

Sera from patients with cutaneous T cell lymphoma and leukemia were screened for the presence of natural antibody to the human T cell lymphoma (leukemia) virus, $HTLV_{CR}$, using a solid-phase radioimmunoassay. Sera from two patients, including patient CR, from whose cultured T lymphoblastic cell line (HUT102) the retrovirus $HTLV_{CR}$ was isolated, reacted specifically with proteins of $HTLV_{CR}$. Serum from patient CR also reacted specifically with proteins of $HTLV_{MB}$, an independent but highly related retroviral isolate from a patient with Sezary T cell leukemia. The specificity for $HTLV_{CR}$ proteins was demonstrated by solid-phase immunocompetition assays and competition radioimmunoprecipitation assays. Analysis of radioimmunoprecipitates indicated that the natural antibodies were directed against $HTLV_{CR}$ core proteins with molecular weights of 24,000 and 19,000 (p24 and p19). Whereas the serum reactivities for $HTLV_{CR}$ proteins were shown to be highly specific,

[3] Kalyanaraman, V. S., M. G. Sarngadharan, P. A. Bunn, J. D. Minna, and R. C. Gallo. Antibodies in human sera reactive against an internal structural protein (p24) of human T cell lymphoma virus (HTLV). Manuscript submitted for publication.

additional reactivities seen against proteins of animal retroviruses including GaLV, SSV, FeLV, and BaEV were clearly shown not to be viral specific but rather were due to reactivity with cellular antigens contaminating the viral preparations or with related antigens present in fetal calf serum. These results demonstrating natural antibodies to $HTLV_{CR}$ provide the first evidence for a specific antibody response to a retrovirus in humans.

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