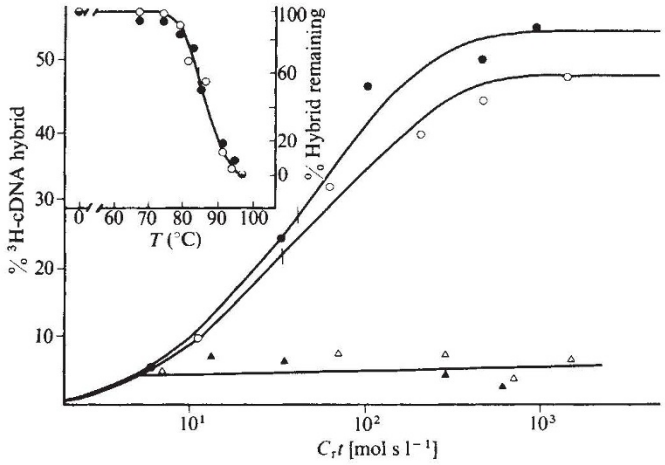

Fig. 5 Nucleic acid relatedness of $HTLV_{MB}$ to $HTLV_{CR}$. $HTLV_{CR}$ ^{3}H-cDNA was prepared as previously described[5], hybridized to 200 μg of the indicated cytoplasmic RNA in 50% formamide–3X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7) with 0.05% SDS and 1 mM diethylpyrocarbonate, and the amoun of hybrid assayed by S_1 nuclease digestion. The cDNA was hybridized to the indicated C_rt. Values were normalized to the maximum value with HTLV 70S RNA (actual value 90%). Inset: cDNA was hybridized to a C_rt of 500, diluted 20-fold with 2X SSC, and incubated at the indicated temperature for 5 min. Hybridization is normalized to 100% for each hybrid (actual values 48% and 44%, respectively). ●, HUT102 cytoplasmic RNA; ○, IUdR-treated CTCL-2 cytoplasmic RNA; △, human embryonic cell line cytoplsmic RNA; ▲, PHA-stimulated normal human blood T lymphoblasts obtained as previously described[23].

infected by and producing HTLV. These findings are consistent with the presence of HTLV proviral sequences in DNA of a fraction of the primary tumour cells and/or the presence of only fractions of the provirus in most tumour cells.

Thus, the present data indicate that an isolate of a type C retrovirus ($HTLV_{MB}$) from the cultured leukaemic cells of a patient with Sézary syndrome is closely related to $HTLV_{CR}$ from a patient with T-cell lymphoma, by virtue of their structural protein and nucleic acid sequence homology. Further studies are required to identify any differences between the two isolates. The observations that the HTLV isolates are not ubiquitous genetically transmitted viruses of man[5], that HTLV-related nucleic acid sequences and proteins are found in fresh leukaemic blood cells of patient M.B. and that specific antibodies to HTLV occur in some human sera[16,17] indicate that HTLV has infected some humans. The results also suggest that its provirus was integrated into the human genome of at least some of the leukaemic cells. Consistent with these findings, recent electron microscopic studies of van der Loo *et al.* show apparent retrovirus-like particles in primary (uncultured) tumour cells of a patient with cutaneous T-cell lymphoma[18].

Many animal retroviruses are known to cause leukaemias and lymphomas of T cells in their host[19]. Particularly interestingly, a cutaneous lymphoma can occur in sheep after inoculation with BLV[20,21]. HTLV has so far been found only in the neoplastic tissue of three patients with T-cell malignancies. Seroepidemiological studies and surveys of cell nucleic acids for HTLV sequences are in progress to obtain further evidence for a possible relationship of HTLV particles to these diseases.

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Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus

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Although retroviruses (RNA tumour viruses) have been implicated in the causation of naturally occurring leukaemias and lymphomas of animals[1], it is not yet clear whether they are involved in the human versions of these malignant diseases, particularly because of the difficulty in isolating viruses truly ascribable to human origin[2,3]. However, a novel type C retrovirus (called $HTLV_{CR}$) has been isolated in our laboratory from T cells (fresh and in culture) from a lymph node biopsy of a patient (C.R.) with cutaneous T-cell lymphoma (mycosis fungoides)[4] and a very similar virus ($HTLV_{MB}$) from the peripheral blood T cells of another patient (M.B.) with cutaneous T-cell leukaemia (Sézary syndrome, see accompanying report[5]). The nucleic acid sequence[6], the reverse transcriptase[7] and the major internal structural protein (p24)[8] of $HTLV_{CR}$ are not significantly related to any of the known retroviruses; nucleic acid sequences and p24 protein of $HTLV_{MB}$ have been recognized in fresh and cultured cells[5]. We now describe the results of a limited survey of the occurrence, in patients with T-cell malignancies (and among normal people), of antibodies against $HTLV_{CR}$ proteins. We find that antibodies against p24 are present in human sera (including those of patient C.R. and his wife), and that these are specifically directed at $HTLV_{CR}$ proteins and not at cell-specific determinants—in other words, the immunological reactions are not those reported in human sera[9–11] against animal virus glycoproteins which, lacking virus specificity, are directed against the carbohydrate residues of the glycoprotein[12,13]. The antibodies against HTLV are thus the first evidence for a specific immune response in humans against a retrovirus.

A limited survey was designed to determine whether natural antibodies reactive against the human retrovirus isolate, HTLV, can be detected in human sera. The assay used in this preliminary survey was a radioimmune precipitation of ^{125}I-labelled $HTLV_{CR}$ p24 by human sera using a double antibody system[8]. The p24 of $HTLV_{CR}$ was purified to homogeneity from density-

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Table 1 Specificity of precipitation of $HTLV_{CR}$ p24 by natural antibodies in human sera

^{125}I-labelled antigen used in RIA	Serum	Diagnosis	Per cent competition by							
			$HTLV_{CR}$	R-MuLV	FeLV	SSV	BaEV	MPMV	SMRV	BLV
$HTLV_{CR}$ p24	Patient C.R.	Cutaneous T-cell lymphoma	98	3	6	4	3	5	0	2
	Patient M.J.	Cutaneous T-cell leukaemia	93	2	0	0	1	0	3	0
	Wife of patient C.R.	Normal	90	0	1	4	9	5	9	2
R-MuLV gp70	M.R.G.	Normal	100	100	100	100	97	100	100	100
	Patient S-924	Chronic myelogenous leukaemia	72	88	83	80	68	78	79	84

Competition radioimmunoassay (RIA) was performed as described in Fig. 2 legend using the competing viruses at 10 μg protein. Buffer 1 was used in the precipitations of ^{125}I-labelled $HTLV_{CR}$ p24 and buffer B in the precipitations of ^{125}I-labelled R-MuLV gp70. FeLV, feline leukaemia virus, SSV, simian sarcoma virus, BaEV, baboon endogenous virus; MPMV, Mason–Pfizer monkey virus; SMRV, squirrel monkey retrovirus; BLV, bovine leukaemia virus.

banded virus and radiolabelled with ^{125}I as described elsewhere[8]. The serum from patient C.R. precipitated >90% of the labelled p24, and the serum of another patient (M.J.) with Sézary syndrome showed a similar high reactivity towards $HTLV_{CR}$ p24. Twenty-one other serum samples from various T-cell malignancies and 50 sera from random normal donors showed no significant precipitating activity. Sera were also obtained from 11 close family members of patients with T-cell malignancies, with previous evidence of HTLV infection. Out of these, one serum (no. 81-5, from the wife of patient C.R.) reacted strongly in the immunoprecipitation.

The reactivity of the positive sera was characterized in detail, and Fig. 1 shows the pattern of precipitation obtained. The radiolabelled probe used was 95% immunoprecipitable with a hyperimmune rabbit serum against disrupted $HTLV_{CR}$, whereas sera 38-7 and 24-1 from patients C.R. and M.J. respectively precipitated >80% and the apparently normal serum 81-5 precipitated >50% of the ^{125}I-labelled p24 at the highest serum concentration tested. Thus, the two highly positive human sera had antibody titres very similar to that of the hyperimmune rabbit serum. The profile of normal serum M.R.G. in Fig. 1 is typical of the results obtained with all the negative sera tested. These results concur with data obtained in an independent study using solid phase radioimmunoassay which showed specific reactivities in these sera towards $HTLV_{CR}$ (ref. 14).

In view of the past controversy[15] concerning the presence of natural antibodies in human sera reactive with antigens from animal retroviruses, the reactivities shown in Fig. 1 were analysed for their specificity. Various retroviruses and cell

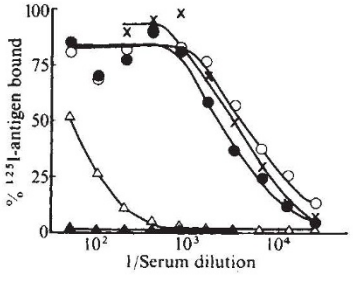

Fig. 1 Immunoprecipitation of ^{125}I-labelled $HTLV_{CR}$ p24 by different human sera. $HTLV_{CR}$ p24 was purified and labelled with ^{125}I as described elsewhere[8]. The labelled p24 (8,000–10,000 c.p.m.) was mixed with two-fold serial dilutions of human sera or a hyperimmune rabbit serum raised against HTLV in a volume of 20 μl of buffer 1 (200 mM Tris-HCl, *p*H 7.5, 200 mM NaCl, 1 mM EDTA, 0.3% Triton X-100, 0.1 mM phenylmethylsulphonyl fluoride and 2 mg ml^{-1} bovine serum albumin). The reaction mixture was incubated for 2 h at 37 °C and overnight at 4 °C. A 20-fold excess of goat anti-human IgG (or goat anti-rabbit IgG when rabbit IgG was the primary antibody) was then added and the volume made up to 500 μl with buffer 1. The samples were incubated for 1 h at 37 °C and an additional 2 h at 4 °C and then centrifuged at 2,500 r.p.m. for 15 min in a Beckman centrifuge. The supernatants were aspirated and the radioactivity in the pellets was counted in an LKB Ultrogamma counter. ●, Human serum (patient C.R.); ○, human serum (patient M.J.); △, human serum (wife of patient C.R.); ▲, normal human serum, M.R.G.; ×, rabbit antiserum to $HTLV_{CR}$.

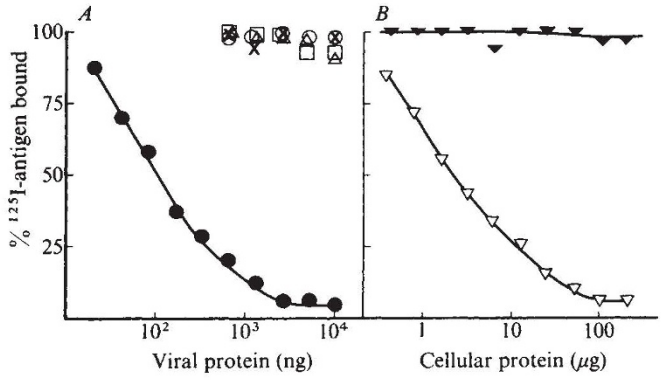

Fig. 2 Specificity of the precipitation of $HTLV_{CR}$ p24 by serum of patient M.J. Competition radioimmunoassays were set up using ^{125}I-labelled $HTLV_{CR}$ p24 and a limiting dilution (1 : 7,500) of the human serum (patient M.J.). Serial dilutions of the unlabelled antigens were preincubated with the serum for 1 h at 37 °C. Labelled p24 (8,000–10,000 c.p.m.) was then added and the reaction mixture incubated and processed as described in Fig. 1 legend. *a*, Competition by retroviral extracts: ●, $HTLV_{CR}$; ○, SSV; ×, BaEV; △, SMRV; □—□, BLV. *b*, Competition by cell extracts: ▽, HUT102 cells producing $HTLV_{CR}$; ▼, HUT78 cells.

extracts were tested for their capacity to compete with the precipitation of ^{125}I-labelled p24 induced by the positive human sera. Figure 2 shows results obtained with the serum of patient M.J. Simian sarcoma virus, baboon endogenous virus, Mason–Pfizer monkey virus and bovine leukaemia virus failed to neutralize the reactivity of the serum for p24. Only $HTLV_{CR}$ effectively competed in the precipitation (Fig. 2*a*). Similarly, extracts from cells which produce $HTLV_{CR}$ totally competed in the precipitation, whereas extracts from a neoplastic human T-cell line (HUT78) which is negative for $HTLV_{CR}$ (ref. 8) failed to compete (Fig. 2*b*). This specificity is very similar to that observed in the precipitation of $HTLV_{CR}$ p24 by a hyperimmune serum against disrupted $HTLV_{CR}$ (ref. 8). The same strict specificity was observed with all the human sera which showed an immune reactivity with $HTLV_{CR}$ p24 (Table 1). Similarly, identical results were obtained whether the immune complex was precipitated with goat anti-human IgG or by the addition of inactivated *Staphylococcus aureus* cells, indicating that the reactive species in the human sera are intact immunoglobulin molecules.

The extent of immune precipitation of ^{125}I-labelled p24 by the human sera was not affected by the assay conditions used, unlike the situation encountered with human natural antibodies reported to be reactive to envelope glycoproteins of animal retroviruses (refs 12, 13 and data below). The extent and pattern of precipitation of $HTLV_{CR}$ p24 by human sera were identical, irrespective of whether the immune precipitation medium was supplemented with ovalbumin or bovine serum albumin as a protein carrier (Fig. 3*a*,*b*). In contrast, many human sera exhibit strong precipitating activity towards Rauscher murine leu-

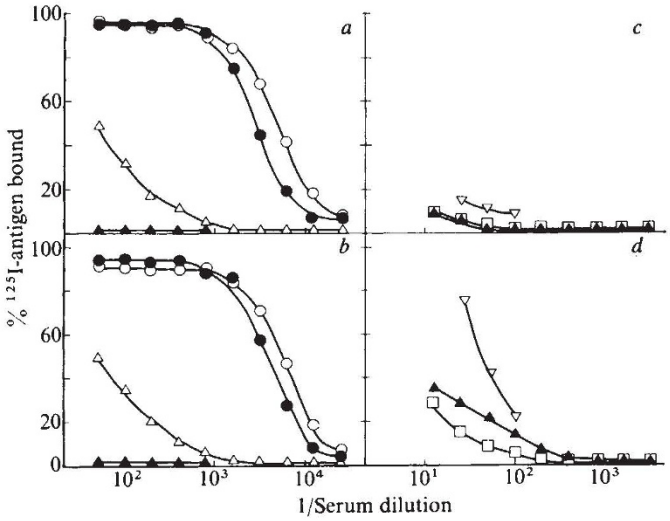

Fig. 3 Effect of buffer composition on the precipitation of ^{125}I-labelled $HTLV_{CR}$ p24 and ^{125}I-labelled R-MuLV gp70 by human sera. Immunoprecipitation of ^{125}I-labelled $HTLV_{CR}$ p24 and ^{125}I-labelled R-MuLV gp70 by the human sera was performed in buffer A (buffer 1 containing 5 mg ml^{-1} bovine serum albumin and 0.5% Triton X-100) or in buffer B (buffer A containing 5 mg ml^{-1} ovalbumin instead of bovine serum albumin). Labelled antigen (8,000–10,000 c.p.m.) was incubated with twofold serial dilutions of the human sera in 200 μl of the appropriate buffer and the extent of immune precipitation measured as described in Fig. 1 legend. *a*, *b* Represent precipitation of $HTLV_{CR}$ p24 in buffer A and buffer B respectively. ●, Serum of patient C.R.; ○, serum of patient M.J.; △, serum of the wife of patient C.R.; ▲, normal serum. *c*, *d* Represent precipitation of R-MuLV gp70 in buffer A and buffer B, respectively. ▽, serum of chronic myelogenous leukaemia patient, 7-117; □, serum of patient with chronic myelogenous leukaemia, S-964; ▲—▲, normal human serum, M.R.G.

kaemia virus (R-MuLV) gp70 when the reaction is done in the presence of ovalbumin as the carrier (Fig. 3*d*). This activity is nearly eliminated if bovine serum albumin is used instead of ovalbumin (Fig. 3*c*). Such reactivity is also observed with gp70s of other retroviruses and is thought to be directed, not against the polypeptide portions of the gp70 that are virus coded, but against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific[12,13]. This lack of viral specificity is clearly shown by the finding that, unlike the activity towards $HTLV_{CR}$ p24, all the viruses tested competed totally in the precipitation of R-MuLV gp70 (Table 1).

We have described here a specific immune reactivity in some human sera towards the internal structural antigen p24 of $HTLV_{CR}$, a type C retrovirus, isolated from the T cells of two different patients with T-cell malignancies. Of 84 sera tested, 3 have shown significant reactivity towards this viral antigen. It is possible that other sera contain anti-$HTLV_{CR}$ antibodies but were missed because the assay detects only antibodies to the $HTLV_{CR}$ core protein, p24. In this regard, use of an envelope antigen of the virus as the probe may show a more representative frequency of natural antibodies in humans towards the virus. These and independent tests using solid phase radioimmunoassay (ref. 14) are the first evidence of a specific immune response to a type C retrovirus in humans. As animal retroviruses can cause T-cell leukaemias and lymphomas, the finding of these antibodies only in sera of people with T-cell neoplasias and in the positive normal case (the wife of the patient from whom HTLV was first isolated), combined with other recent evidence for the presence of this virus in some of these patients, warrants careful consideration of a possible role for HTLV in the origin of these kinds of human leukaemias and lymphomas.

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The *v-sis* transforming gene of simian sarcoma virus is a new *onc* gene of primate origin

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The defective transforming simian sarcoma virus (SSV) and its nondefective helper virus (SSAV) are retroviruses isolated from a fibrosarcoma of a pet woolly monkey. Together with the gibbon ape leukaemia viruses, they are the only group of retroviruses known to cause spontaneous and experimentally induced neoplasia in primates (see refs 1 and 2 for review). Molecular cloning has shown that SSV contains a 1.2-kilobase (kb) transformation-specific viral *onc* gene (*v-sis*)[3,4] which, like other viral *onc* genes, is derived from a set of conserved cellular DNA sequences[5]. A human DNA fragment containing sequences homologous to the entire *v-sis* gene has also been cloned and analysed[6]. We present here experiments carried out on the cloned SSV genome which show that: (1) *v-sis* is distinct from other viral transforming (*onc*) genes; and (2) *v-sis* is derived from a woolly monkey naturally infected once with gibbon ape leukaemia virus (GaLV) and is to date the only *onc* gene of primate origin.

Different viral *onc* genes have been known to share a common cellular progenitor. There are many examples of viruses isolated from the same species acquiring the same *onc* gene, and at least one example of a cellular *onc* gene represented in viruses from two different species (chicken and cat)[7]. To test possible homology between *v-sis* and other viral *onc* genes, we digested DNA from a recombinant phage clone (C60) of SSV with the restriction endonucleases *Bgl*II or a combination of *Sal*I and *Pvu*II to localize the *v-sis* sequences on a gel, which we blot-hybridized[8] to ^{32}P-labelled plasmid clones containing *onc* sequences from different acutely transforming retroviruses. Table 1 summarizes the origin and properties of the probes used. The detailed restriction map of C60 has been published elsewhere[3]. This clone has two copies of the long terminal repeat (LTR) and an inversion involving one LTR and 0.1 kb of adjacent viral sequences. In each case *Bgl*II or *Sal*I and *Pvu*II generated a 0.65-kb fragment that was highly enriched in *v-sis* sequences, as demonstrated by the poor detection of this

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