HBLV (or HHV-6) in human cell lines

SIR—Last year we described the isolation from patients with lymphoproliferative disorders of a novel human herpesvirus, human B lymphotropic virus (HBLV), which infects freshly isolated human mononuclear cells from cord blood, adult peripheral blood and spleen but not from a number of established human and animal cell lines[1,2]. The requirements for repeated infection of fresh lymphocytes as the target population presented a hinderance to biological, immunological and molecular studies. Now that we have found several cells lines that can be productively infected with the virus we would like to describe them for the sake of those who might find them of value for research.

Five such cell lines are listed in the table. All of them can be infected by HBLV with either cell-free virus or by cocultivation with previously infected cord blood cells. HSB-2, a T-cell line, was highly susceptible to infection by HBLV. By nine days after infection > 90% of the cells are fatally infected. Morphologically, the cells resemble HBLV-infected cord blood mononuclear cells[1] and diffuse staining of the entire cell is obtained in indirect immunofluorescence assay (IFA). The amount of HBLV released by day 9 into supernatant fluids of infected HSB-2 cells is 10^3–10^4 $TCID_{50}ml^{-1}$ assayed on fresh cord blood mononuclear cells and HSB-2 cells.

The megakaryocyte cell line, HEL, showed a peak of IFA-positive cells (~30 %) by 9-10 days after infection, with predominantly nuclear fluorescence. HEL cells did not domonstrate a clear morphologic change following infection. Furthermore, only a low per cent of HEL cells continued to express HBLV proteins and release virus. By day 45, the number of IFA-positive cells was less than 5 %. It is possible that these residual of HEL cells, like some EBV cell lines, are persistently but nonproductively infected.

The other three cell lines shown in the Table are substantially less susceptible to HBLV infection. In two of them, the infection persists at a low level for several months, with a continuing release of low levels of infectious HBLV but virus becomes undetectable in HTB-14 cells within 14 days, suggesting lytic infection.

Cell clones were established from the WIL-2 cell line by limiting dilution and tested for susceptibility to HBLV infection. In a selected cloned cell population (ET62), > 90 % of cells were infected and released high levels of infectious virus.

High molecular weight DNA extracted from the HBLV-infected HSB-2, HEL, ET62 and HBT-14 cells yielded characteristic restriction endonuclease banding patterns by Southern blot analysis, whereas uninfected cells were negative.

Of the five cells lines, HSB-2 and HEL should be the most useful. They are free of other herpesviruses and are therefore more suitable for immunovirologic and molecular analysis and for developing HBLV-specific reagents. Moreover, HSB-2 should be an excellent source for large-scale production of HBLV. Such reagents may lead to a linkage of HBLV with one or more human diseases. Our virus-producing cell lines will be made available to investigators interested in research on HBLV. We feel that the virus should be classified HHV-6 (human herpes virus-6) in accord with the published provisional classification of herpesviruses[7].

D.V. ABLASHI
S.Z. SALAHUDDIN
S.F. JOSEPHS
F. IMAM
P. LUSSO
R.C. GALLO

National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20892, USA

C. HUNG
J. LEMP

Electro-Nucleonics, Inc.,
Silver Spring, Maryland 20904, USA

P.D. MARKHAM

Bionetics Research, Inc.,
Kensington, Maryland 20850, USA

1. Salahuddin, S.Z. *et al. Science* **234**, 596-601 (1986).
2. Josephs, S.F. *et al. Science* **234**, 601-603 (1986).
3. Ponten *et al. Acta path. Microbiol. Scand.* **74**, 465-486 (1960).
4. Foley *et al., M.D. Anderson Hospital and Tumor Inst. Monograph* **21**, (1967).
5. Levy, J.A. *et al. Cancer* **22**, 517-524 (1968).
6. Buell, D.N. *Ann. N. Y. Acad. Sci.* **190**, 221-234 (1972).
7. Roizman, B. IRA *IRAC Monographs* **19** (1979).

Table 1 Infection of continuous human cell lines with HBLV

Source	Cell line identification	HBLV infectivity* titre ($TCID_{50}$/ml)	Average number of HBLV infected cells (IFA)(%)	Endogenom herpesviruses
1	HSB-2	10^3–10^4	90 (day 9)	None
2	HEL	10^1	30 (day 10) 5 (persistent)	None
3	HTB-14	None	3 (day 7)	None
4	WIL-2	~10^{1-2}	2–5 (persistent)	EBV
5	IM-9	~10^{1-2}	2–5 (persistent)	EBV

*Infectivity titres were performed on human cord blood mononuclear cells according to the procedure described previously[1]. Source: 1, T-lymphoblastoid cell line[4] (ATCC CCRF-HSB-2); 2, megakaryocytic cell line from normal human bone marrow (gift from Doris Morgan, Hahnemann University, Philadelphia); 3, glioblastoma cells[3] (ATCC); 4,5 HTB-14 B-lymphoblastoid cell line[6] (ATCC).

Comments on the sombre view of AIDS

SIR—We want to comment on the method Rees[1] used to estimate the mean incubation period of transfusion-associated AIDS (acquired immune deficiency syndrome).

First, Rees did not consider the left censoring of tranfusion-associated AIDS data. Because the association between AIDS and blood transfusions was not recognized until the middle of 1982[2,3], cases of AIDS with onsets before 1982 may have been missed. Consequently, Rees might have overestimated the true mean. (Rees's Table 1 contains cases with diagnosis dates between 1981 and 1982, because he transformed the crude data reported by Peterman[4].)

Second, Rees considered only the densities in the family of normal distributions that have a ratio of mean to standard deviation equal to 3. If this restriction on the ratio is removed, then other normal distributions could be found that have only a minor increase of standard deviation, means much shorter than 15 years, and total absolute differences smaller than 14.67 (the smallest total absolute difference presented in Rees's Table 3).

Third, Rees focused only on the fit of the assumed model to the marginal observed frequencies, and there was no interval estimate or standard error of the estimate. Furthermore, he stated no statistical optimal properties for the method.

Finally, another approach is to use the maximum likelihood estimator based on the truncated Weibull model[2]. This method can take into account the left and right censoring. Using this approach, we calculated an estimate of 4.5 years for the mean incubation period with a 90% confidence interval of 2.6 to 14.2 years. A recent calculation based on current data suggests that the mean incubation period may be even longer than 4.5 years. However, with a limited observation time and a very long mean incubation period for transfusion-associated AIDS, it is very difficult to pinpoint the mean at present.

KUNG-JONG-LUI
Division of Injury Epidemiology and Control,
Center for Environmental Health,
THOMAS A. PETERMAN
AIDS Program,
DALE N. LAWRENCE
Division of Host Factors,
Center for Infectious Diseases,
Centers for Disease Control,
Public Health Services,
US Department of Health and Human Services,
Atlanta, Georgia 30333, USA

1. Rees, M. *Nature* **326**, 343–345 (1987).
2. Lui, K.J. *et al. Proc. natn. Acad. Sci.* **83**, 3051–3055 (1986).
3. Curran, J.W. *et al. New Engl. J. Med.* **310**, 69–75 (1984).
4. Peterman, T.A. *et al. J. Am. med. Ass.* **254**, 2913–2917 (1985).