PHENOTYPIC VARIATION IN THE RESPONSE TO THE HUMAN IMMUNODEFICIENCY VIRUS AMONG DERIVATIVES OF THE CEM T AND WIL-2 B CELL LINES

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Expression of the CD4 glycoprotein, the surface receptor for the human immunodeficiency virus (HIV) (1–4), and of specific transcription factors that promote the expression of HIV genes (5) are characteristics that target a subset of lymphocytes for the pathological consequences of HIV infection. Additional properties of $CD4^+$ lymphocytes and other HIV host cells could affect the efficiency of replication or the cytopathic effects of the virus, and thereby determine whether the outcome of infection is cell death; the establishment of a latent, nonproductive state; or the evolution of a chronic, virus-producing reservoir. The existence and potential importance of such host cell-specific factors is suggested by the variable response to HIV infection among cultured human lymphoid cell lines in vitro (6–8). The basis for this variability is poorly understood, and the spectrum of responses to HIV infection in vitro has not been well characterized.

The regulation of deoxyribonucleotide pool size and turnover in host cells could affect the reverse transcription of the HIV genome and subsequent replication of double-stranded circular viral DNA, and could strongly influence the therapeutic activity or cytotoxicity of antiretroviral nucleoside analogs. In view of these possibilities, we initiated studies of the ability of HIV to infect mutant lymphoblastoid cell lines with altered nucleoside metabolism. We chose the CEM human T cell line (9) and the WIL-2 human B cell line (10) because they have been shown to differ strikingly in their regulation of deoxynucleotide metabolism (11, 12), and because of the availability of mutants of each that are deficient in specific nucleoside kinase activities, which could be useful for studies of antiretroviral nucleoside analog metabolism. Here we report a systematic characterization of the responses of these mutants and other derivatives of CEM and WIL-2 to infection with the HTLV-IIIB strain of HIV.

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Materials and Methods

Cell Line Selection and Culture Methods. The strain of CEM used in this laboratory was obtained from Dr. Peter Creswell, Duke University. CEM4, a subclone of this strain, was used as wild type in the studies described in this report. CEM/3B, used as the HIV donor in cell fusion assays (see below), is a derivative of this strain of CEM that has been chronically infected with HTLV-IIIB (13). An independently maintained wild-type CEM strain and its deoxycytidine kinase-deficient (dCK^-)[1] derivative (14) were kindly made available by Dr. A. Fridland, St. Jude Children's Research Hospital. The nucleoside kinase-deficient mutants of CEM M1-1a (adenosine kinase-deficient, AK^-), ACO611a (dCK^-), and RC3b (doubly deficient in AK and dCK) have previously been described (11); in addition to their AK- and dCK-deficient phenotypes, each of these lines also lacks hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity.

The following mutants of the WIL-2 human splenic, EBV^+ B lymphoblastoid cell line were studied: 107 (AK^-) (15, 16); a dCK^- mutant (17) referred to as BdCK (obtained from B. Ullman, Oregon State University for the Health Sciences, OR); and an AK^-dCK^- double mutant derived from 107 (17), referred to as K2B. Additional independent AK^-dCK^- derivatives of WIL-2, not previously described, were isolated by stepwise selection in the presence of 10 μM 6-methylmercaptopurine ribonucleoside (6-MMPR; Sigma Chemical Co., St. Louis, MO) and 1 μM arabinosylcytosine (ara-C; Sigma Chemical Co.), cytotoxic substrate analogs of AK and dCK, respectively. The clones isolated by these selections had <1-3% of the AK and dCK activity found in WIL-2. Assay conditions for measurement of AK and dCK activities in cell extracts have been described (11). After initial screening for sensitivity to HTLV-IIIB (see below) one of these AK^-dCK^- mutants, termed AA, was subcloned and studied further.

All cell lines were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat inactivated FCS (Gibco Laboratories), nonessential amino acids, 2 mM glutamine, and 1 mM pyruvate, under 5% CO_2 in air at 37°C. Subclones of wild-type and mutant T and B cell lines were obtained by limiting dilution cloning in 96-well culture plates at 0.2 and 0.5 cells per well in this growth medium with the addition of 10% horse serum (Gibco Laboratories) and any selective drugs. The phenotypes of wild-type and mutant cell lines were routinely confirmed every 2-3 mo by evaluating resistance/sensitivity to 10 μM 6-MMPR (AK), 1 μM ara-C (dCK), and 10 μM 6-thioguanine (HPRT).

Cell Surface Antigen Expression. The percentage of cells expressing lymphocyte cell surface antigens was analyzed by indirect immunofluorescence and FACS using the following mAbs: Leu-3a + Leu-3b (Becton Dickinson Monoclonal Center, Mountain View, CA) and OKT4 and OKT4A (Ortho Diagnostic Systems, Raritan, NJ), which detect epitopes of the CD4 (T4) antigen; anti-human HLA-DR (Becton Dickinson & Co.), directed against a class II histocompatibility antigen; the 3A1 pan-T cell mAb, obtained from B. F. Haynes, Duke University; B1 and B4, markers of mature and immature B cells (Coulter Immunology, Hialeah, FL).

10^6 cells were washed twice with PBS containing 1% BSA and 0.1% sodium azide (PBS/BSA/NaN_3), and then were incubated with 1:40 to 1:200 dilutions of mAb for 30 min at 4°C. After three washes with PBS/BSA/NaN_3, cells were incubated for 30 min at 4°C with a 1:50 dilution of affinity-purified FITC-conjugated goat anti-mouse IgG (Tago Inc., Burlingame, CA). After three washes, cells were resuspended in PBS/BSA/NaN_3, fixed by the addition of 37% formaldehyde (10 μl/ml of cell suspension), and stored in the dark for later analysis. Background fluorescence was determined by incubating cells with P3 × 63/Ag/8 ascitic fluid (control murine myeloma protein, provided by B. F. Haynes). Membrane staining was assessed by FACS analysis of 10,000 cells, performed on a flow cytometer (EPICS 753; Coulter Electronics, Hialeah, FL).

Virus. Stocks of HIV strains HTLV-IIIB, MN, and RF (18, 19) prepared from medium of chronically infected H9 cells were kindly provided by Dr. A. Langlois, Duke University. Preparation of virus stocks and titration of virus have been described (20).

[1] *Abbreviations used in this paper:* AK^-, adenosine kinase-deficient; dCK^-, deoxycytidine kinase-deficient; HPRT, hypoxanthine-guanine phosphoribosyltransferase.

Virus Infection: Standard Protocol. Exponentially growing cells were pelleted and resuspended in fresh medium at 10^5 cells/ml. Duplicate 10-ml aliquots (10^6 cells) were distributed in culture flasks, one of which was infected with 1 ml of a 1:10 dilution of the virus stock (estimated at $\sim 2 \times 10^4$ infectious units/ml, undiluted). 24 h after virus addition, the cells were centrifuged and resuspended in 10 ml of fresh medium. Centrifugation and replacement of medium was repeated every 24 h for the next 10–12 d, and every 2–3 d thereafter for the course of the experiment. Cultures were diluted to 10^5 cells/ml in fresh medium every 3–5 days to permit continuation of exponential growth by still viable cultures. Depending upon the rate of infection, viable cell count (trypan blue exclusion) and virus production were assessed every 24–72 h by measuring virion-associated reverse transcriptase (RT) activity (19, 20) or amount of p24 protein in culture medium (p24 was determined either by RIA [NEK-040; E. I. DuPont de Nemours & Co., Wilmington, DE] or by ELISA [NEK-041; E. I. DuPont de Nemours & Co.]. Cultures were monitored until the HIV-exposed cells exhibited a cytopathic effect or until no cytopathic effect had been observed for 12–15 d. A cell line was considered to be permissive if it supported the production of RT at more than five times the background for the assay. Sensitivity to virus cytopathic effect was determined by inspection and by comparing viable cell count of infected cultures to a parallel culture of the uninfected cell line. A similar protocol was followed for studies of infection by the RF and MN isolates of HIV.

In some experiments susceptibility to infection was assessed at various times after cells were exposed to serial dilutions of a standard virus stock, or after infection was carried out in the presence of specific mAbs. Details of these experiments are described in the text and figure legends.

Syncytium Formation. As previously described (13, 21) 75×10^3 uninfected target cells were combined in medium containing or lacking 5 μg/ml OKT4A mAb, with 5×10^3 CEM/3B cells (CEM cells chronically infected with HTLV-IIIB) in wells of a 96-well one-half area microtiter culture dish. After incubation for 24 h entire wells were inspected by inverted phase microscopy and foci of giant cells were counted. Wells were scored according to the number of giant cell foci according to the following scale: 0 = none; 1+ = 10 or less; 2+ = 10–20; 3+ = 20–30; 4+ = 30–40.

Binding of ^{125}I gp120. Purified gp120 from HTLV-IIIB was prepared as described (22). Binding of gp120 to cells will be described elsewhere (Skinner, M., and T. J. Matthews, manuscript in preparation). Briefly, duplicate aliquots of 10^6 cells in 0.1 ml of medium (containing or lacking 10 μg/ml of OKT4A mAb) were incubated for 30 min at room temperature with $\sim 10^5$ cpm of ^{125}I-gp120. The cell suspension was then layered over 0.3 ml of phthalate oil in a 0.4 ml microcentrifuge tube and the cells were separated from unbound gp120 by centrifugation for 30 s at 13,000 *g*. After careful aspiration of the overlying medium and oil, radioactivity in the cell pellet was determined in a gamma counter.

Results

Sensitivity to Infection by Free Virus

After screening of a larger number of cell lines and clones derived from the CEM T and WIL-2 B cell lines, a selected group of wild-type and nucleoside kinase–deficient lines (Table I) were evaluated in more detail for ability to support productive infection by the HIV strain HTLV-IIIB and their susceptibility to the cytopathic effect of the virus (Table II, Figs. 1 and 2). In these experiments, cell growth and viability and production of RT were followed for at least 15 d on at least one occasion and on other occasions for 10–15 d, depending on the characteristics of the particular cell line.

CEM T Cells. The growth and viability of our wild-type strain of CEM declined markedly by 7–10 d after infection; RT production by infected cells peaked after ~7–8 d. Similar results were observed with 10 of 11 randomly selected wild-type subclones and with M1-1a, an AK^- derivatives of this CEM strain. In contrast to

TABLE I
Cell Line Characteristics

Cell line	Nucleoside kinase phenotype
CEM strains	
CEM	Wild type
CEM 4	Wild type
CEM (St. Jude)	Wild type
dCK^- (St. Jude)	dCK^-
ACO611a	dCK^-
M1-1a	AK^-
WIL-2 strains	
WIL-2	Wild type
BdCK	dCK^-
107	AK^-
K2B	AK^--dCK^-
AA clones (1-5)	AK^--dCK^-

wild-type CEM and M1-1a, strain ACO611a, which lacks dCK, was nonpermissive, showing no change in growth rate or decrease in cell viability, and almost no production of RT during 15 d after exposure to HTLV-IIIB (Table II, Fig. 1). RC3b, an AK^--dCK^- double mutant derived from ACO611a (11), was also nonpermissive (data not shown).

M1-1a and ACO611a were selected in different laboratories from independently maintained strains of CEM. The sensitivity to HIV infection of the CEM parent of ACO611a is unknown. Thus, the HIV-resistant phenotype of ACO611a could have been a property of its dCK-expressing parent, rather than a consequence of the loss of dCK activity. The former possibility is underscored by the finding that another independently maintained CEM wild-type strain and a dCK^- derivative selected from it were both nonpermissive for HTLV-IIIB (St. Jude strains, Table II). Since dCK^- cell lines derived from WIL-2 can be infected by HIV (see below), expression of dCK cannot be an absolute determinant of HIV sensitivity.

WIL-2 B Cells. Similar studies with nucleoside kinase-deficient variants of the WIL-2 B cell line (Table II, Fig. 2) can be summarized as follows: (*a*) wild-type WIL-2 cells and some cloned AK^--dCK^- derivatives of WIL-2 supported essentially no replication of HTLV-IIIB (data for the latter not presented). (*b*) The cell lines 107 (AK^-), BdCK (dCK^-) and some AK^--dCK^- clones supported HTLV-IIIB replication, with maximal RT production ocurring 6-8 d after infection. The level of RT (also p24, see below) produced by 107 and BdCK approached that observed with CEM in some experiments, but in others was 10-20% of the CEM values. In contrast to permissive strains of CEM, infection by HTLV-IIIB had minimal effect on growth and viability of these derivatives of WIL-2. (*c*) Five subclones (AA1-AA5) of the AA (AK^-dCK^-) cell line were strikingly permissive for HTLV-IIIB and sensitive to its cytopathic effects (representative results for the AA2 subclone are reported below). K2B, another AK^--dCK^- double mutant, was also permissive, but somewhat less sensitive to virus cytopathic effect.

Viable AA2 cells decreased to <10% of an uninfected AA2 control culture by 3-5 d after exposure to HTLV-IIIB; RT production was maximal by day 3-5 and the

TABLE II
Sensitivity to HIV Infection

Cell lines	Cytopathic effect* Onset	Reverse transcriptase Maximum cpm	Syncitiom formation
CEM derived			
CEM	day 8	353,320 (day 9)	4+
CEM4	day 8	565,810 (day 8)	3+
M1-1a	day 9	553,567 (day 11)	4+
ACO611a	None	3,500	0
CEM (St. Jude)	None	3,890	4+
dCK^- (St. Jude)	None	3,550	4+
WIL-2 derived			
WIL-2	None	1,500	0
BdCK	None	59,000 (day 9)	0
107	None	103,790 (day 6)	0
K2B	day 5-7	231,270 (day 6)	1+
AA2	day 4-5	354,860 (day 4)	2+

Viable cell counts and RT activity were monitored daily for 12-15 d after infection with $HTLV\text{-}III_B$ using the standard protocol (see Materials and Methods).

* Onset of cytopathic effect was the day when increase in viable cell count ceased. Syncytium formation (see Materials and Methods) was monitored in a separate experiment (identical results were obtained on two separate occasions).

level of RT was comparable to that of infected, permissive CEM cells (Table II, Fig. 2). AA2 was in fact more sensitive than CEM to HTLV-IIIB. This was evident when virus production was determined as a function of multiplicity of infection. For example, in the experiment presented in Fig. 3, p24 production was determined 5, 7, and 10 d after exposure of AA2 and CEM4 cells to serial fourfold dilutions of a standard HTLV-IIIB stock (1:10 to 1:164,000). With AA2, maximal production of p24 was evident at every virus dilution on day 5. In contrast, with CEM4 submaximal p24 production was detected only at the 1:10 dilution of virus on day 5; by day 10, p24 was detectable only through the first five dilutions (to 1:2,560). A more quantitative comparison of p24 production by AA2 and CEM4 is presented below (Fig. 5). In addition to HTLV-IIIB, AA2 supported replication of HIV clinical isolates, including MN and RF (23 and data not shown).

Syncytium Formation

As another index of virus cytopathic effect, we evaluated the formation of multinucleate syncytia within 24 h after uninfected cell lines were added in 15-fold excess to a culture of CEM/3B, a chronically infected CEM line that continuously produces low levels of HTLV-IIIB (Table II). HIV-sensitive CEM wild-type clones and M1-1a were strongly positive in the fusion assay. No syncytia were observed with the nonpermissive strain ACO611a, but the HIV-resistant St. Jude wild-type and dCK^- strains of CEM formed syncytia as effectively as the permissive CEM strains. Among WIL-2 strains, syncytium formation after exposure to CEM/3B was only observed with the sensitive cell lines K2B and AA2, and fusion was less efficient than with CEM strains that formed giant cells.

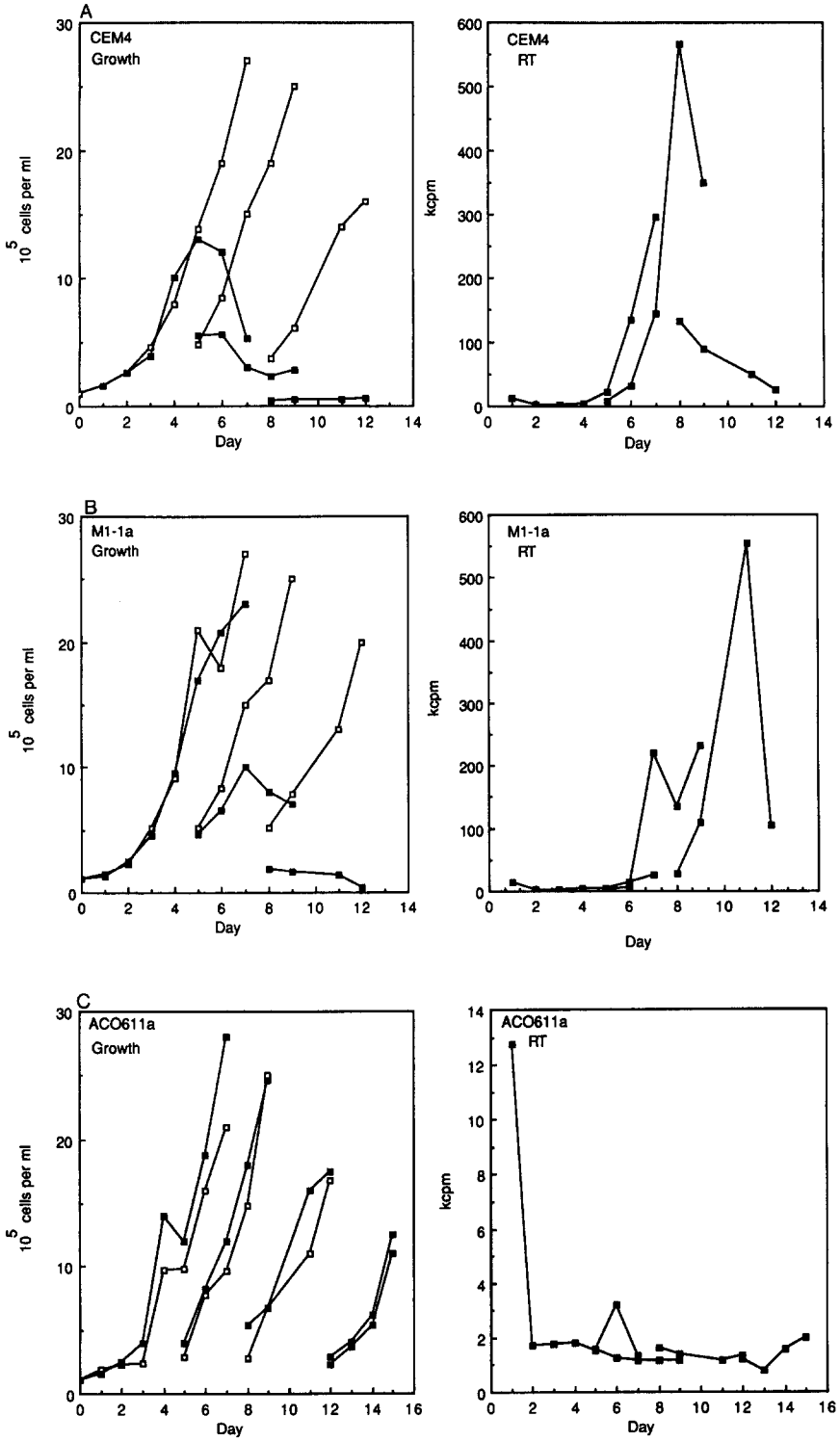

FIGURE 1. Figure continued on facing page.

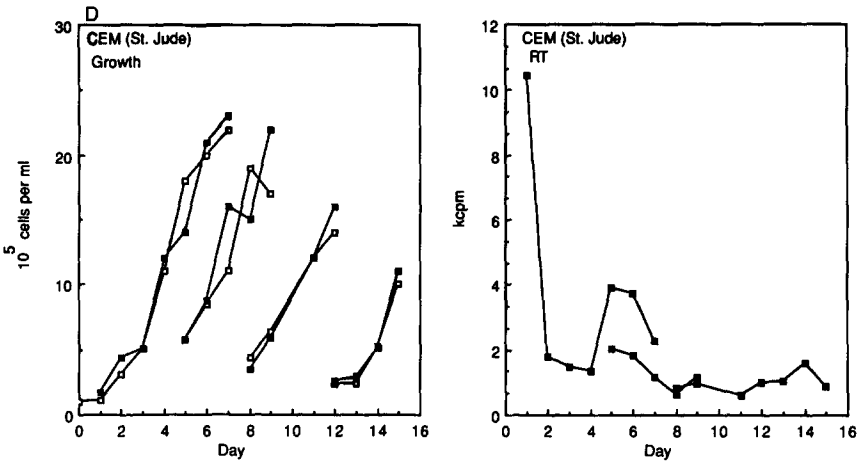

FIGURE 1. (*A–D*) Response of CEM-derived cell lines to infection with HTLV-IIIB. The experimental procedure is as described in the Materials and Methods seciton. (*left hand panels*) Growth (viable cell count) of the indicated uninfected (*open symbols*) and HTLV-IIIB-infected (*solid symbols*) cell lines. (*Right hand panels*) Virion associated reverse transcriptase activity (RT) in culture medium in the same cultures shown on left. (*A*)CEM4, (*B*) M1-1A, (*C*) ACO611a, (*D*) CEM wild type (St. Jude).

Analysis of Cell Surface Markers

Over the 14–20-mo period during which their responses to HIV were studied, we examined expression by the above cell lines of the CD4 and DR class II histocompatibility antigens on as many as six occasions; expression of other antigens was evaluated less frequently (Table III). All of the CEM-derived cell lines expressed a panel of markers consistent with a T cell phenotype. DR was found on <12% of cells, and the pan-T cell marker 3A1 (CD7) on 47–87%. 60–99% of cells reacted with the Leu-3 and OKT4 mAbs to CD4, and 43–99% reacted with OKT4A. Small fluctuations in the levels of expression of each antigen were observed for each cell line.

WIL-2 and its derivatives expressed cell surface markers consistent with a B cell phenotype. DR was present on 75–95% of cells, and <12% expressed the 3A1 T cell marker. Except for AA2, expression of CD4 epitopes by these lines was uniformly low: 0–19% of cells reacted with the Leu-3 and OKT4, and 0–7% with the OKT4A mAb. Again, small fluctuations in the levels of expression were noted. Expression of immunologically detectable CD4 epitopes by the HIV-sensitive cell line AA2 (and other AA subclones) was considerably more variable, ranging from 1 to 6% on three occasions to 60 to 89% of cells on three others. The kinetics of HIV infection and susceptibility to cytopathic effect were essentially constant during this 20-mo period, regardless of the level of CD4 expression (Table III, Table IV).

OKT4A Inhibition of gp120 Binding and HTLV-IIIB Infection

The CD4 protein acts as a receptor for HIV by virtue of the specific interaction between the OKT4A epitope of CD4 and the HIV envelope protein gp120 (1, 2, 4, 24–28). The low frequency of CD4 expression by HIV-susceptible WIL-2-derived cell lines, and conversely, the HIV insensitivity of some CEM derivatives that ex-

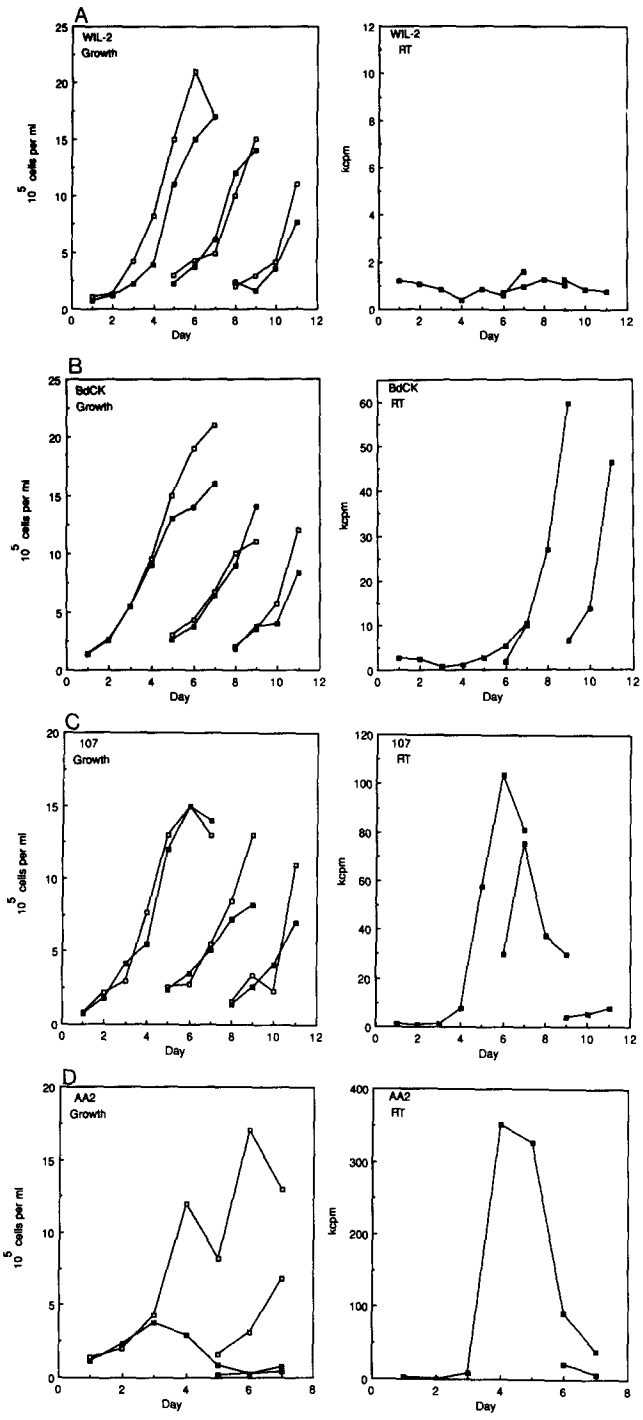

FIGURE 2. Response of WIL-2-derived cell lines to infection with HTLV-IIIB. Details as described in legend to Fig. 1. (*A*) WIL-2, (*B*) BdCK, (*C*) 107, (*D*) AA2.

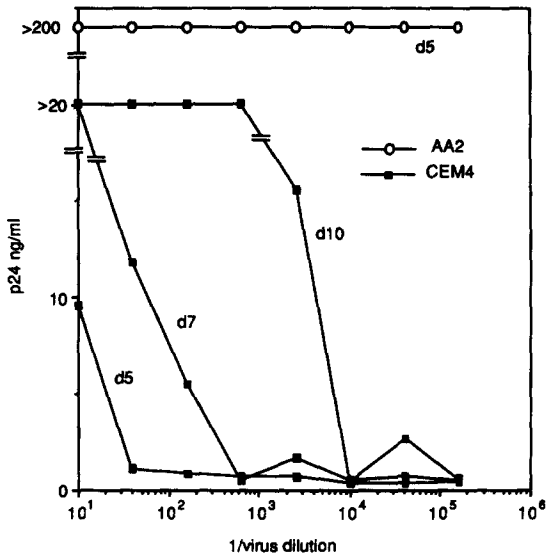

FIGURE 3. Production of p24 antigen by CEM4 and AA2 cell lines infected with dilutions of HTLV-IIIB stock. Serial 1:4 dilutions were prepared from a 1:10 starting dilution of a standard stock of HTLV-IIIB. 1-ml aliquots of cultures of CEM4 and AA2 (10^5 cells/ml) were inoculated with 0.1 ml of virus-free diluent (control) or with a dilution of virus (1:10 to 1:164,000). On days 3 and 5, 2 ml of fresh medium was added to cultures, and on day 7, 3 ml was added. On days 5, 7, and 10, 0.4-ml samples of the culture medium were frozen for RIA analysis of p24. For the p24 assay of day 5 AA2 samples, the culture medium was diluted 1:10, while for CEM4 (all days), the undiluted medium samples were assayed for p24.

pressed CD4 abundantly, prompted us to examine the OKT4A-inhibitable binding of purified, ^{125}I-labeled gp120 by these cell lines (Fig. 4). In the absence of antibody, binding of ^{125}I-gp120 correlated with the relative reactivity of the cell lines with antibodies to the CD4 complex. Binding to CEM derivatives was inhibited 53–86% by OKT4A. There was no detectable OKT4A-inhibitable gp120 binding to WIL-2, or to its HIV-permissive derivatives 107 and BdCK, which were resistant to HIV-induced fusion and expressed nearly undetectable levels of CD4. The amount of gp120 bound by these cells was approximately equal to the level bound by CEM

TABLE III

Surface Antigen Expression

	Monoclonal antibodies						
	Leu-3*	OKT4	OKT4A	3A1	B1	B4	DR
Cell line	Range of percent of cells positive						
CEM strains							
CEM	78–96	60–92	88–95	71	4	4	0–12
CEM4	84–99	86–99	89–99	47	2	1	0–5
ACO611a	60–91	61–67	43–61	86	ND	ND	0–10
M1-1a	81–84	85	81–85	87	ND	ND	0–1
CEM St. Jude	71	66	69	ND	ND	ND	0
dCK$^-$ St. Jude	81	76	83	ND	ND	ND	1
WIL-2 strains							
WIL-2	0–14	0–15	0–3	0–3	57	29	83–90
BdCK	0–19	17	7	0	ND	ND	83–93
107	0–2	0–9	0–2	4–11	65	46	84–95
K2B	6–19	3–8	0–1	1	ND	ND	76–94
AA2	6–82	1–68	1–63	1–2	80	4	75–95

* Leu-3a + Leu-3b

TABLE IV

CD4 Expression and Susceptibility to Infection by HTLV-IIIB of the AA2 Cell Line during the Period of Study

	Reverse transcriptase	Antibody reactivity			
		Leu3	OKT4	OKT4A	DR
Month	Max cpm	Percent cells positive			
1	206,900	–	–	8	95
3	–	89	–	–	95
6	251,800	–	–	–	–
8	350,800	12	8	6	75
12	339,500	–	68	63	87
13	350,500	57	45	35	79
19	345,400	6	1.4	0.5	75

derivatives in the presence of OKT4A. AA2, which does undergo fusion and shows variable expression of CD4, bound about 1.5-fold more gp120 than the other WI-L2 lines; and this binding was inhibited ~20% by OKT4A.

Despite the failure to detect either CD4 expression or OKT4A-inhibitable gp120 binding by the 107 and BdCK B cell lines, productive infection of these lines, and of CEM4, by HTLV-IIIB was completely blocked by OKT4A; their infection was delayed by the OKT4 antibody (Fig. 5). Infection of AA2 was only delayed by OKT4A and was not detectably affected by OKT4. The remarkable efficiency of HTLV-IIIB replication in AA2 is indicated in this experiment by both the more rapid kinetics of infection and the production of 100-fold more p24 protein by AA2 than CEM.

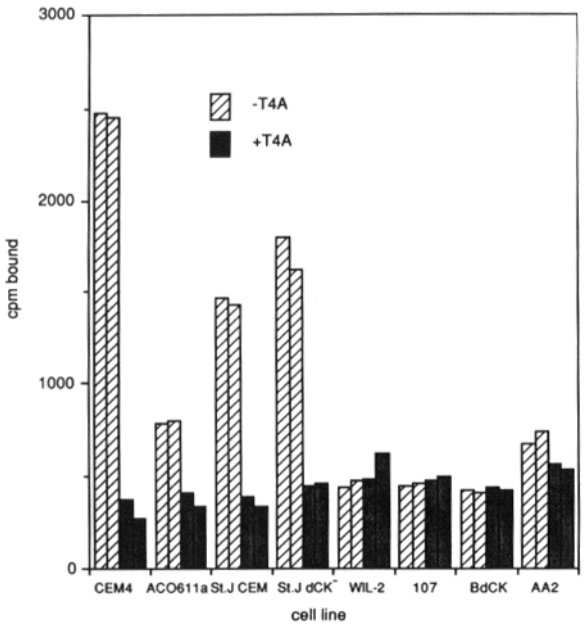

FIGURE 4. OKT4A inhibition of the binding of ^{125}I-labeled gp120 to cell lines.

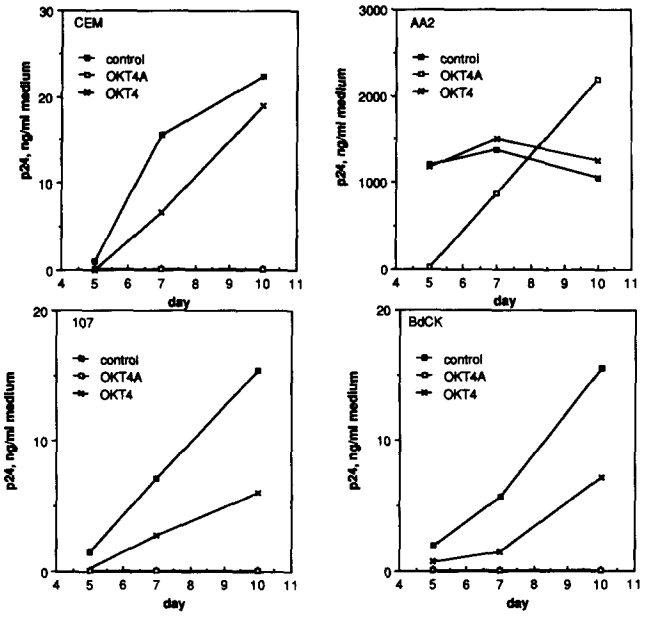

FIGURE 5. Effect of OKT4 and OKT4A mAbs on p24 production of HTLV-IIIB-infected cell lines. Aliquots of cultures of the indicated cell lines containing 10^5 cells were centrifuged and resuspended in growth medium containing no added antibody (control) or containing 1 μg/ml of OKT4 or OKT4A mAbs. After 2 h, each culture was infected with 0.1 ml of a standard stock of HTLV-IIIB. After another 24 h cells were centrifuged, washed and resuspended in fresh medium without virus and lacking (control) or containing the appropriate antibody (1 μg/ml). On the fourth day after infection all cultures were fed with 1 ml of fresh medium lacking antibody. On days 5 and 7 after infection, aliquots of 0.4 ml were removed from each culture for assay of p24 by ELISA, and the cultures were fed with the same volume of medium lacking antibody. A final 0.4 ml sample for p24 assay was removed on day 10.

Discussion

The diversity of in vitro responses to HIV among clonal derivatives of the CEM T and WIL-2 B cell lines is striking. Several fairly distinct patterns could be distinguished, based on the ability to support replication of HIV and sensitivity to its cytopathic effects (Table V, discussed below). These phenotypes were stable and where it was evaluated, the phenotypes of subclones of the same strain were similar. The set of phenotypes expressed by CEM and WIL-2 derivatives overlapped, but were not identical. Taken together, the various phenotypic combinations expressed by CEM- and WIL-2-derived cell lines clearly indicate a dissociation of HIV replication and the two characteristic cytopathic effects of the virus. Moreover, our results emphasize that while expression of the CD4 protein is necessary for HIV infection, other host cell factors can play a dominant role in determining the outcome of infection.

All strains of CEM that were permissive (P^+) for HTLV-IIIB (i.e., supported production of high levels of virion-associated RT activity and p24 core protein), were sensitive to viral cytopathic effect (C^+), as measured by loss of viability after exposure to free virus. All permissive (P^+) strains of CEM underwent rapid fusion (F^+) to form multinucleate syncytia when placed in contact with HIV-infected cells, another CD4-dependent manifestation of HIV cytopathic effect (29–33). The converse correlation of C and F phenotypes did not hold among nonpermissive (P^-) CEM strains: ACO611a was P^-C^- and F^-, but two other P^-C^- strains of CEM were F^+. In contrast to the $P^+C^+F^+$ phenotype of permissive CEM strains, two permissive derivatives of WIL-2, 107, and BdCK were insensitive to both cytopathic effects: they were not killed after infection by free HTLV-IIIB and they did not un-

TABLE V

Host Cell-HIV Phenotypes Observed among Strains of CEM T and WIL-2 B lymphoblastoid Cell Lines

Cell lines	Phenotype*
CEM-derived	
CEM	$P^+C^+F^+CD4^+$
CEM4	$P^+C^+F^+CD4^+$
M1-1a	$P^+C^+F^+CD4^+$
ACO611a	$P^-C^-F^-CD4^+$
CEM St. Jude	$P^-C^-F^+CD4^+$
dCK^- St. Jude	$P^-C^-F^+CD4^+$
WIL-2 derived	
WIL-2	$P^-C^-F^-CD4^\pm$
BdCK	$P^+C^-F^-CD4^\pm$
107	$P^+C^-F^-CD4^\pm$
K2B	$P^+C^\pm F^\pm CD4^\pm$
AA2	$P^+C^+F^+CD4^{\pm,+}$

* P, permissive: P^+, >5 × background in RT assay, P^-, <5 × background. C^-, cytopathic effect: C^+, >75% of cells killed; C^- <15% decrease in viability compared with uninfected control. F, fusion: F^+, scores ≥3 + in standard syncytium assay (see Materials and Methods); F^-, no fusion; $F^\pm$, scores ≤2 + . CD4, reactivity with T4A monoclonal antibody: $CD4^+$, >35% of cells react; $CD4^-$, near level of detectability by indirect immunofluorescence FACS analysis (≤10% of cells react).

dergo fusion ($P^+C^-F^-$ phenotype). On the other hand, AA2, another HIV-permissive WIL-2-derived cell line, was rapidly killed by the virus and did undergo fusion ($P^+C^+F^+$ phenotype).

How does this complexity relate to current understanding of the role of CD4 expression as a determinant of HIV sensitivity? The CD4 antigen, through interaction with the glycosylated (13, 32) gp120 envelope protein of HIV, acts as a receptor for free HIV on a subset of T cells, and on cells induced to express CD4 by transfer of the CD4 cDNA (1, 2, 4, 24–28). CD4 expression is also necessary for HIV-induced cell fusion (29–33). Although HIV preferentially infects $CD4^+$ T cells, it also infects cells of different lineages, including monocytes and macrophages (6, 34–39), B lymphocytes (6, 8, 39, 40), promyelocytes (6), and cells of neural origin (41–43). CD4 can be expressed by these cell lines (4, 6, 35, 44, 45), but whether HIV only infects cells of these lineages that express CD4, or whether other receptors, or other modes of cell entry, are involved is unclear. Moreover, the relationship between the level of CD4 expression and susceptibility to infection by HIV is complex. For example, Levy et al. (6) found that the level of Leu-3 expression did not always correlate with production of HIV in a study of unrelated cell lines. Independently isolated clones of the Molt-4 T cell line varied in their susceptibility to infection by HIV while expressing similar levels of the CD4 antigen (7). Salahuddin et al. (8) observed that mAbs to the CD4 antigen incompletely blocked infection of B cell lines by HIV.

In the present studies we found that some WIL-2-derived strains were permissive for HTLV-IIIB, even though they expressed nearly undetectable levels of CD4 by

indirect immunofluorescence FACS analysis, and exhibited no OKT4A-inhibitable gp120 binding. Nevertheless, the fact that OKT4A completely blocked their infection by HTLV-IIIB indicates that CD4 is essential for infection. It is clear that the level of CD4 necessary for infection of these permissive WIL-2 strains (and perhaps other non-T cells) can be very low, perhaps on the order of a few hundred or a few thousand molecules per cell. Conversely, the finding that some nonpermissive (P^-) CEM strains expressed similar high levels of CD4 and bound similar amounts of gp120 as permissive strains of CEM, is evidence that CD4 expression is not sufficient for productive HIV infection.

Some P^- CEM strains were able to undergo fusion with HIV-infected cells, suggesting a block in infection at a stage after virus entry. On the other hand, the inability of the P^- CEM strain ACO611a to undergo fusion, despite its ability to express CD4 and bind gp120, suggests that some host cell membrane component, other than CD4, is involved in virus entry and the fusion process. It is intriguing that this host cell phenotype can be mimicked by specific mutations in HIV *env* gene sequences encoding the hydrophobic NH_2 terminus of gp41, the transmembrane virus envelope protein (28). Expression of these mutant *env* genes yielded gp120 that was indistinguishable from wild type and bound to $CD4^+$ lymphocytes normally, but was unable to induce cell fusion (28). It is possible that ACO611a is altered in a host cell membrane component that interacts with gp41 to mediate a step in virus entry and cell fusion that follows gp120-CD4 binding.

The level of CD4 expression by AA2 cells ranged from <5% to >80% of cells during a 20-mo period of observation. The basis for this variable expression of CD4 is not known, but neither the susceptibility of AA2 to HIV infection, nor the time course of infection, changed over this period. The level of expression of CD4 does not seem to be a sufficient explanation for the sensitivity of this cell line to infection by HTLV-IIIB. AA2 can produce up to two orders of magnitude more virus than CEM and exhibits a marked cytopathic effect within 3–5 d, compared with 8–10 d for CEM. This remarkable sensitivity suggests the operation of host cell factors that can powerfully potentiate HIV replication, or alternatively a defect in, or suppression of, a host or viral gene product that normally would inhibit viral gene expression or viral replication.

The AA2 cell line appears to be significantly more susceptible to HIV infection than many T and B cell lines that have been reported as being particularly sensitive to strains of HIV. For example, X50-7, an EBV-transformed B cell line that expresses CD4 on 60–70% of cells, exhibits a cytopathic effect within 15 d (46). A3.01, a HAT-sensitive derivative of CEM, showed maximal RT activity at 10 d after infection, coincident with cell death (47), which is similar to the time course we observe with our CEM4 clone. The ATH8 T cell line exhibits a cytopathic effect within 4 d of infection by HTLVIIB, but it requires IL-2 for growth and is HTLVI transformed (48). The ease of culturing AA2 cells and its sensitivity to HIV infection make AA2 potentially useful for producing high titer stocks of HIV and for isolation of HIV from clinical samples. A subclone of AA (AA5) with comparable characteristics to AA2 has been used as an indicator strain in a rapid, colorimetric assay for HIV-neutralizing antibody that depends on killing of infected cells (23). In the case of AA2, "grow through" survivors of HTLV-IIIB infection do not actively produce virus (data not presented). We are investigating the ability of other derivatives of CEM

and WIL-2 to serve as chronic virus producers in the manner of the H9 T cell line (19) or the FR8 EBV-transformed B cell line (39).

Clearly, further work will be required to determine the basis for the host cell–virus interactions displayed by the cells we have studied (Table V), but this report may provide some direction for future investigation. It would, for example, be of interest to use these cell lines to examine HIV replication intermediates and gene expression, and the expression of HIV constructs bearing defined mutations in virus-encoded regulatory elements. The identification of nucleoside kinase–deficient mutants capable of supporting HIV replication provides a panel of cells that will be useful for studying the mechanism of activation and metabolism of nucleoside analogs with antiretroviral activity. We would encourage the use of this panel of cell lines for studies aimed at identifying and isolating host factors and genes that play important roles in determining the outcome of HIV infection.

Summary

Derivatives of the CEM T and WIL-2 B cell lines showed striking diversity in their responses to the HTLV-IIIB strain of the human immunodeficiency virus (HIV). Several stable phenotypic patterns could be defined, based on whether cells were permissive (P^+, P^-) for virus production, were sensitive or insensitive to cytopathic effects after infection by free virus (C^+, C^-), and whether they underwent fusion on contact with virus-infected cells (F^+, F^-). Although expression of CD4 was essential for infection by HTLV-IIIB, very low levels were sufficient for productive infection of WIL-2 derivatives. Conversely, some CEM T cell lines that expressed ample CD4, and which were able to bind virus gp120 and undergo fusion, did not support productive infection by free virus. One nonpermissive, $CD4^+$ derivative of CEM could bind gp120 but failed to undergo fusion, suggesting an alteration in some membrane protein other than CD4 that is essential for virus entry and HIV-induced cell fusion. The AA2 derivative of the WIL-2 cell line is also described, which is remarkably permissive for HIV replication and exquisitely sensitive to virus cytopathic effect. The panel of related cell lines with different host-virus phenotypes could be useful for more precisely defining steps in the infectious cycle of HIV, and for identifying host cell genes and gene products that determine the outcome of HIV infection.

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References

1. Dalgleish, A. G., P. C. L. Beverly, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss. 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature (Lond.).* 312:763.
2. Klatzmann, D., E. Champagne, S. Chameret, J. Gruest, D. Guetard, T. Hercend, J.-C. Gluckman, and L. Montagnier. 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature (Lond.).* 312:767.
3. Campbell, J. A., D. Hicks, and J. Sligh. 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. 1. Role of T cell activation and expression of the T4 antigen. *J. Immunol.* 135:3151.

4. Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell.* 47:333.
5. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (Lond.).* 326:711.
6. Levy, J. A., J. Shimabukuro, T. McHugh, C. Casavant, D. Stites, and L. Oshiro. 1985. AIDS-associated retroviruses (ARV) can productively infect other cells besides human T helper cells. *Virology.* 147:441.
7. Kikukawa, R., Y. Koyanagi, S. Harada, N. Kobayashi, M. Hatanaka, and N. Yamamoto. 1986. Differential susceptibility to the acquired immunodeficiency syndrome retrovirus in cloned cells of human leukemic T-cell line Molt-4. *J. Virol.* 57:1159.
8. Salahuddin, S. Z., D. V. Ablashi, E. A. Hunter, M. A. Gonda, S. Sturzenegger, P. D. Markham, and R. C. Gallo. 1987. HTLV-III infection of EBV-genome-positive B-lymphoid cells with or without detectable T4 antigens. *Int. J. Cancer.* 39:198.
9. Foley, G. E., H. Lazarus, S. Faber, B. G. Uzman, B. A. Boone, and R. E. McCarthy. 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer.* 18:522.
10. Levy, J. A., M. Virolainen, and V. Defendi. 1968. Human lymphoblastoid lines from lymph node and spleen. *Cancer.* 22:517.
11. Hershfield, M. S., J. E. Fetter, W. C. Small, A. S. Bagnara, S. R. Williams, B. Ullman, D. W. Martin, D. B. Wasson, and D. A. Carson. 1982. Effects of mutational loss of adenosine kinase and deoxycytidine kinase on deoxyATP accumulation and deoxyadenosine toxicity in cultured CEM human T-lymphoblastoid cells. *J. Biol. Chem.* 257:6380.
12. Kurtzberg, J., and M. S. Hershfield. 1985. Determinants of deoxyadenosine toxicity in hybrids between human T and B lymphoblasts as a model for the development of drug resistance in T-cell acute lymphoblastic leukemia. *Cancer Res.* 45:1579.
13. Matthews, T. J., K. J. Weinhold, H. K. Lyerly, A. J. Langlois, H. Wigzell, and D. Bolognesi. 1987. Interaction between the human T-cell lymphotropic virus type IIIB envelope glycoprotein gp120 and the surface antigen CD4: role of carbohydrate in binding and cell fusion. *Proc. Natl. Acad. Sci. USA.* 84:5424.
14. Dow, L. W., D. E. Bell, L. Poulakos, and A. Fridland. 1980. Differences in metabolism and cytotoxicity between 9-B-D-arabinofuranosyladenine and 9-B-D-arabinofuranosyl-2-fluoroadenine in human leukemic lymphoblasts. *Cancer Res.* 40:1405.
15. Hershfield, M. S., and J. E. Seegmiller. 1976. Regulation of de novo purine biosynthesis in human lymphoblasts. *J. Biol. Chem.* 251:7348.
16. Hershfield, M. S., F. F. Snyder, and J. E. Seegmiller. 1977. Adenine and adenosine are toxic to human lymphoblast mutants defective in purine salvage. *Science (Wash. DC).* 197:1284.
17. Ullman, B., B. B. Levinson, M. S. Hershfield, and D. W. Martin. 1981. A biochemical genetic study of the role of specific nucleoside kinases in deoxyadenosine phosphorylation by cultured human cells. *J. Biol. Chem.* 256:848.
18. Wong-Staal, F., G. M. Shaw, B. H. Hahn, S. Z. Salahuddin, M. Popovic, P. Markham, R. Redfield, and R. C. Gallo. 1985. Genomic diversity of human T-lymphotropic virus type III (HTLV-III). *Science (Wash. DC).* 229:759.
19. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science (Wash. DC).* 224:497.
20. Matthews, T. J., A. J. Langlois, W. G. Robey, N. T. Chang, R. C. Gallo, P. J. Fischinger, and D. Bolognesi. 1986. Restricted neutralization of divergent human T-cell lymphotropic virus type III isolates by antibodies to the major envelope glycoprotein. *Proc. Natl. Acad. Sci. USA.* 83:9709.

21. Putney, S. D., T. J. Matthews, W. G. Robey, D. L. Lynn, M. Robert-Guroff, W. T. Mueller, A. J. Langlois, J. Ghrayeb, S. R. Petteway Jr., K. J. Weinhold, P. J. Fischinger, F. Wong-Staal, R. C. Gallo, and D. Bolognesi. 1986. HTLV-III/LAV-neutralizing antibodies to an *E. coli*-produced fragment of the virus envelope. *Science (Wash. DC).* 234:1392.
22. Robey, W. G., L. O. Arthur, T. J. Matthews, A. J. Langlois, T. D. Copeland, N. W. Lerche, S. Oroszlan, D. P. Bolognesi, R. V. Gilden, and P. J. Fischinger. 1986. Prospect for prevention of human immunodeficiency virus infection: purified 120-kDa envelope glycoprotein induces neutralizing antibody. *Proc. Natl. Acad. Sci. USA.* 83:7023.
23. Langlois, A. J., T. J. Matthews, K. J. Weinhold, S. Chaffee, J. M. Leeds, M. S. Herschfield, and D. P. Bolognesi. 1988. Detection of HIV-1 neutralizing antibodies by a simple, rapid, and colorimetric assay. *AIDS Human Retrovirus Res.* 4:63.
24. McDougal, J. S., A. Mawle, S. P. Cort, J. K. A. Nicholson, G. D. Cross, J. A. Sheppler-Campbell, D. Hicks, and J. Sligh. 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. 1. Role of T cell activation and expression of the T4 antigen. *J. Immunol.* 135:3151.
25. McDougal, J., M. Kennedy, J. Sligh, S. Cort, A. Mawle, and J. Nicholson. 1986. Binding of the HTLV-III/LAV to $T4^+$ T cells by a complex of the 110K viral protein and the T4 molecule. *Science (Wash. DC).* 231:382.
26. McDougal, J., J. Nicholson, G. Cross, S. Cort, M. Kennedy, and A. Mawle. 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition and potential for idiotypic mimicry. *J. Immunol.* 137:2937.
27. Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverly. 1986. Epitopes of the CD4 antigen and HIV infection. *Science (Wash. DC).* 234:1120.
28. Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski. 1987. Functional Regions of the envelope glycoprotein of human immunodeficiency virus type I. *Science (Wash. DC).* 237:1351.
29. Lifson, J. D., G. R. Reyes, M. S. McGrath, B. S. Stein, and E. G. Engleman. 1986. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. *Science (Wash. DC).* 232:1123.
30. Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine. 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature (Lond.).* 322:470.
31. Lifson, J. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steimer, E. G. Engleman. 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature (Lond.).* 323:725.
32. Lifson, J., S. Coutre, E. Huang, and E. Engelman. 1986. Role of envelope glycoprotein carbohydrate in human immunodeficiency virus (HIV) infectivity and virus-induced cell fusion. *J. Exp. Med.* 164:2101.
33. Yoffe, B., D. E. Lewis, B. L. Petrie, C. A. Noonan, J. L. Melnick, and F. B. Hollinger. 1987. Fusion as a mediator of cytolysis in mixtures of uninfected $CD4^-$ lymphocytes and cells infected by human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA.* 84:1429.
34. Ho, D. D., T. R. Rota, and M. S. Hirsch. 1986. Infection of monocyte/macrophages by human T lymphotropic virus type III. *J. Clin. Invest.* 77:1712.
35. Nicholson, J. K. A., G. D. Gross, C. S. Callaway, and J. S. McDougal. 1986. In vitro infection of human monocytes with human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). *J. Immunol.* 137:323.
36. Salahuddin, S. Z., R. M. Rose, J. E. Groopman, P. D. Markham, and R. C. Gallo. 1986. Human T lymphotropic virus type III infection of human alveolar macrophages. *Blood.* 68:281.
37. Gartner, S., P. Markovits, D. M. Markovitz, N. H. Kaplan, R. C. Gallo, and M. Po-

povic. 1986. The role of mononuclear phagocytes in HTLV/LAV infection. *Science (Wash. DC).* 233:215.

38. Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science (Wash. DC).* 233:1089.
39. Montagnier, L., J. Gruest, S. Chamaret, C. Dauguet, C. Axler, C. Guetard, M. T. Nugeyre, F. Barre-Sinoussi, J.-C. Chermann, J. B. Brunt, D. Klatzman, and J.-C. Gluckman. 1984. Adaption of lymphadenopathy-associated virus (LAV) to replication in EBV-transformed B lymphoblastoid cell lines. *Science (Wash. DC).* 225:63.
40. Tersmette, M., F. M. Miedema, H. G. Huisman, J. Goudsmit, and C. J. Melief. 1985. Productive HTLV-III infection of human B cell lines. *Lancet.* i:815.
41. Chiodi, F., S. Fuerstenberg, M. Gidlund, B. Asjo, and E. M. Fenyo. 1987. Infection of brain-derived cells with human immunodeficiency virus. *J. Virol.* 61:1244.
42. Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen. 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropism. *Science (Wash. DC).* 236:819.
43. Cheng-Mayer, C., J. T. Rutka, M. L. Rosenblum, T. McHugh, D. P. Stites, and J. A. Levy. 1987. Human immunodeficiency virus can productively infect cultured human glial cells. *Proc. Natl. Acad. Sci. USA.* 84:3526.
44. Talle, M. A., P. E. Rao, E. Westberg, N. Allegar, M. Makowski, R. S. Mittler, and G. Goldstein. 1983. Patterns of antigenic expression on human monocytes as defined by monoclonal antibodies. *Cell Immunol.* 78:83.
45. Funke, I., A. Hahn, E. P. Rieber, E. Weiss, and G. Reithmuller. 1987. The cellular receptor (CD4) of the human immunodeficiency virus is expressed on neurons and glial cells in human brain. *J. Exp. Med.* 165:1230.
46. Dahl, K., K. Martin, and G. Miller. 1987. Differences among human immunodeficiency virus strains in their capacities to induce cytolysis or persistent infection of a lymphoblastoid cell line immortalized by Epstein-Barr virus. *J. Virol.* 61:1602.
47. Folks, T., S. Benn, A. Rabson, T. Theodore, M. D. Hoggan, M. Martin, M. Lightfoote, and K. Sell. 1985. Characterization of a continuous T-cell line susceptible to the cytopathic effects of the acquired immunodeficiency syndrome (AIDS)-associated retrovirus. *Proc. Natl. Acad. Sci. USA.* 82:4539.
48. Mitsuya, H., and S. Broder. 1986. Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2',3'-dideoxynucleosides. *Proc. Natl. Acad. Sci. USA.* 83:1911.