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Growth of the HTLV-III Strain of Human Immunodeficiency Virus in Different Cell Types

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The major immunological abnormality in the acquired immunodeficiency syndrome (AIDS) appears to be a quantitative defect in the T4 antigen-positive helper/inducer T-cell subset. AIDS is etiologically linked to a retrovirus, designated human immunodeficiency virus (HIV), that has been shown to selectively infect T4 antigen-positive lymphoid cells in vitro [1]. Since virus replication in vitro is associated with a pronounced cytopathic effect, it has been suggested that

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Table 1. Growth of the HTLV-IIIB isolate in different cell types

Cell type[a]	T4[b] antigen positive cells (%)	Virus[c] dose (cpm × 10^3/ 10^6 cells)	No. of experiments	Weeks after infection					
				1			2		
				RT	IF[d] (%+)	CPE[e]	RT	IF (%+)	CPE
PBMC		32	15	26		+	72		+
Monocytes		180	3	68.4		−	31		−
		180	1	31		−	9.7		−
T-cell lines									
H9		75	10	8	10	+	35	64	+
		2.5	2	−		−	−		−
HUT-78		60	1	5.8		+	143		++
		12	1	2.8		+	65		++
Karpas45		70	2	4.8	7	+	293	83	+++
Molt-3		50	2	−	−	−	−	−	−
Monocytoid cell line and derived clones									
U937 parental	<10	150	1	−			−		−
U937 clone 4	<10	1800	1	6.5		−	3		−
U937 clone 1	50–60	350	1	1.3		−	1.7		+
U937 clone 16	>95	100	15	80	95	+++			
		25	2	15		++			+++
		2.5	2	0.4		−	5.8		++
Malignant glioma cell lines									
138	0	100	2	−	−	−	−	2	−
373	0	100	2	−	−	−	−	−	−
489	0	100	2	−	−	−	−	−	−

Footnote see p. 438.

immunodeficiency in vivo is a result of the virus killing the T4 cells. To gain further insight into the virus-cell interactions we studied the replication of the HTLV-IIIB strain of HIV in different cell types.

Peripheral blood mononuclear cells (PBMC) from blood donors were separated by Ficoll-Isopaque and treated with 2.5 mg/ml phytohemagglutinin (PHA-P, Difco) for 3 days prior to infection [2]. Monocytes were obtained by harvesting the surface-adherent cells 24 h after separation of PBMC and either exposing them to OKT3 antibodies and C′ (negative selection) or submitting them to FACS selection of M3 antigen-positive cells (positive selection). Both PBMC and monocyte cultures could readily be infected with HTLV-IIIB and yielded reverse transcriptase-positive culture fluids 1 week after infection (Table 1). PBMC cultures showed slight cytopathic changes. Virus production descreased by the 3rd week, probably due to depletion of virus-sensitive T4-positive cells. Monocyte cultures showed no cytopathic changes during the first 2 weeks after infection. From the 3rd week on, cell loss occurred due to gradual cell death.

All four T-cell leukemia lines could be infected by the HTLV-IIIB isolate. H9, HUT-78, and Karpas45 cultures produced small amounts of virus the first week after infection. Large amounts of virus were produced during the second week when the majority of cells became infected, as shown by immunofluorescence with monoclonal antibodies to viral core proteins p24 and p15. The particular Molt-3 line used began virus production with a 4-week delay. Virus spread was also slower in these cultures, since only 50% of cells were virus antigen positive 6 weeks after infection. Cultures of all T-cell lines showed cytopathic changes simultaneously with virus production. In the case of H9, HUT-78, and Molt-3 cells, cell death could be

3			4			9			Continuously producing line established	Virus detected by co-cultivation with U937–16
RT	IF (%+)	CPE	RT	IF (%+)	CPE	RT	IF (%+)	CPE		
2.5		–	0.5		–					
Gradual cell death										
3.0		–	Gradual cell death							
64	70	+	110	80	++				yes	
–		–	64		++					
135		+	161		–				yes	
134		+	49		–				yes	
104		+++							no	
–	–	–	72	5	+				yes	
–		–	–		–	79	90	–	yes	
12		–	19		–	170		–	yes	
35		++	65	70	–				yes	
									no	
		+++								
–	–	–	–	20	–	–	–	–	no	+
–	–	–	–	–	–	–	–	–	no	–
–	–	–	–	–	–	–	–	–	no	–

compensated by the addition of uninfected cells, and eventually a continuously virus-producing line, no longer showing cytopathic changes, could be established. In Karpas45 cultures, however, the cytopathic changes led to extensive cell death, and no producer line could be established.

The U937 monocytoid cell line was originally derived from a histiocytic lymphoma [3]. It has retained a basic phenotype corresponding to that of an immature monocyte [4]. The cell line is inducible by various agents to phenotypic alterations similar to those of normal monoblasts undergoing differentiation. It has recently been cloned, and several clonal lines with different properties have been derived. Susceptibility to infection with HTLV-IIIB and sensitivity to cytopathic changes following infection correlated with the expression of T4 antigen on the cell surface [5]. The parental U937 line and one of its subclones, clone 4, had less than 10% T4-positive cells; hence, productive infection could be established only after a long latency (parental line) or with a high virus inoculum (clone-4 line). These lines showed no or only marginal cytopathic effects.

The clone-1 line contained 50%–60% T4-positive cells and showed moderate susceptibility to infection. Cytopathic changes, even if pronounced, could be overcome in the infected cultures by the addition of uninfected cells, and in each case a producer line could be established. Similarly, the U937 parental and clone-4 lines, once infected, gave rise to continuously virus-producing lines. The clone-16 line contained more than 95% T4-positive cells and was the line most sensitive to infection and to cytopathic changes. Cell death was so extensive following infection that no continuously virus-producing line could be established. The clone-16 line thus resembled monocytes in its prompt virus production but was similar to the Karpas45 T-cell line in its sensitivity to cytopathic changes. The results suggest that the T4 molecule may also play a role in the effector mechanisms leading to cytopathic changes as recorded by cell death.

None of the malignant glioma cell lines used [6, 7] in these experiments appeared to be T4 antigen positive. In spite of this, one of the lines, MG138, could be infected with the HTLV-IIIB isolate [8]. Infection appears to be latent rather than productive, since only a minority of cells in the MG138 culture show transient expression of viral antigens. No virus production could be detected in these cultures. However, the presence of virus could be demonstrated through cocultivation with sensitive target cells, U937 clone 16 in the present experiments. Through contact with the monocytoid cells the virus present in the glioma cells can be transmitted and can give rise to a fully productive infection. The fact that the T4 antigen-negative glioma cells do not show any cytopathic effect provides further support for the notion that the T4 antigen is necessary for the cytopathic effect after virus infection.

In conclusion, T-lymphoid, monocytoid, and glioma cell lines can be infected with HIV. Whereas infection is productive in T-lymphoid and monocytoid cells, glioma cells appear to be latently infected. Cells with low expression or apparent lack of T4 antigen do not show cytopathic changes after virus infection. This suggests that the T4 antigen is necessary for the cytopathic effect after virus infection.

Footnote of Table 1, p. 436:

[a] Peripheral blood mononuclear cells were grown in RPMI medium supplemented with 10% fetal calf serum (FCS), 10% T-cell growth factor (Cellular Products), 45 IU of sheep anti-human α-interferon serum, and 2 μg/ml polybrene (PB). Monocytes, T-cell lines, and monocytoid cell lines were grown in RPMI medium with 10% FCS and 2 μg/ml PB. Malignant gliomas were grown in Eagles MEM with 10% FCS and PB.

[b] Indirect membrane immuniofluorescence with the *Fab* fragment of an anti-T4 monoclonal antibody (received from Dr. Ellis Reinherz) and fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark).

[c] Estimated by reverse transcriptase activity (RT) [5].

[d] Immunofluorescence on methanol-fixed cells with monoclonal antibodies to HTLV-III p24 and p15.

[e] Cytopathic effect: syncytia formation and cell death; +, <5% of cells, ++, 5%–50%, +++, >50% of cells show CPE.

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