

Table 3 Effect of sCD4 and anti-CD4 antibody on HIV-1 infection of different human cell types

Cell type	Input HIV (TCID)	Human anti-HIV-1		Leu3a anti-CD4		sCD4 180 µg ml ⁻¹		Control	
		SF	RF	SF	RT	SF	RT	SF	RT
T cell									
C8166	5 × 10 ³	-	-	-	-	-	-	+++	20,000
Myelomonocyte									
U937	5 × 10 ⁵	ND	ND	-	-	-	-	+++	27,000
	5 × 10 ³	-	-	-	-	-	-	+++	55,000
HL60	5 × 10 ⁵	ND	ND	-	-	-	-	++	49,000
	5 × 10 ³	-	-	-	-	-	-	++	9,000
Glioma									
U138	5 × 10 ⁵	-	-	++	21,000	++	23,000	++	23,000
U87	5 × 10 ⁵	-	-	-	-	-	-	-	-
Muscle									
RD/TE671	5 × 10 ⁵	-	-	++	18,000	++	26,000	++	19,000
	5 × 10 ³	-	-	++	9,000	++	20,000	++	18,000

HIV-1 (HTLV-IIIRF strain) was incubated with anti-HIV-1 antibody or with sCD4 for 1 h at 37 °C before plating on 10⁶ cells of the types indicated. Syncytium formation (SF) and reverse transcriptase (RT) activity in the medium was scored 5 days post-infection for the T-cell and myelomonocyte cultures. For the glial and myoblast cultures there were no detectable SF or RT activity as previously described²⁰⁻²³. After seven days and two trypsin-mediated passages of the glial and myoblast cells, C8166 cells were added to the cultures, and SF and RT activity was scored 3 days later. Virus production for cells exposed to HIV-1 (not treated with anti-CD4 or sCD4) was also detected after 3 weeks and 6 passages in U138 and RD/TE671 cells, but not U87 cells. The glial cells expressed glial fibrillary acidic protein, confirming their provenance. SF scores: +++, >50% giant syncytial cells in culture; ++, 10-50% syncytial cells; +, 1-9% syncytial cells; -, no discernable syncytia. RT scores in c.p.m. acid precipitable [³H]thymidine incorporation; -, <1,000 c.p.m. ND, not determined.

lymphocytes, HIV infection might occur by gp41-mediated membrane fusion in the absence of specific gp120-CD4 receptor interaction.

It is not clear whether HIV infection of astroglial cells has a role in HIV encephalopathy (as infection is mainly detectable in microglial and endothelial cells^{33,34}) or whether muscle infection is related to the weight loss and wasting seen in AIDS. Although our results may be restricted to cells *in vitro*, HIV infection of glial and muscle in the presence of excess sCD4 merits further investigation, given the interest in developing sCD4 as a therapeutic agent.

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Productive dual infection of human CD4⁺ T lymphocytes by HIV-1 and HHV-6

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Although infection by HIV-1 (refs 1 and 2) has been implicated as the primary cause of AIDS and related disorders^{3,4}, cofactorial mechanisms may be involved in the pathogenesis of the disease. For example, several viruses commonly detected in AIDS patients and capable of transactivating the long terminal repeat of HIV-1, such as herpesviruses⁵⁻⁷, papovaviruses⁷, adenoviruses⁸ and HTLV-I (ref. 9), have been suggested as potential cofactors. Another candidate is human herpesvirus-6 (HHV-6, originally designated human B-lymphotropic virus)¹⁰⁻¹², which has not only been identified in most patients with AIDS by virus isolation^{10,13,14}, DNA amplification techniques¹⁵ and serological analysis¹⁶, but is also predominantly tropic and cytopathic *in vitro* for CD4⁺ T lymphocytes^{17,18}. Here we demonstrate that HHV-6 and HIV-1 can productively co-infect individual human CD4⁺ T lymphocytes, resulting in accelerated HIV-1 expression and cellular death. We also present evidence that HHV-6 transactivates the HIV-1 long terminal repeat (LTR). These observations indicate that HHV-6 might contribute directly or indirectly to the depletion of CD4⁺ T cells in AIDS.

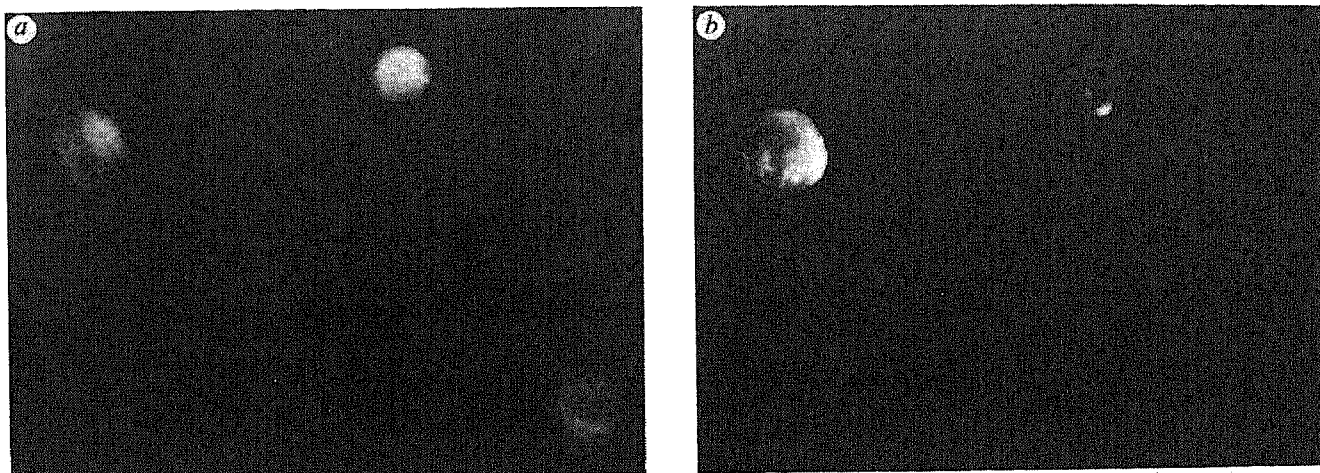
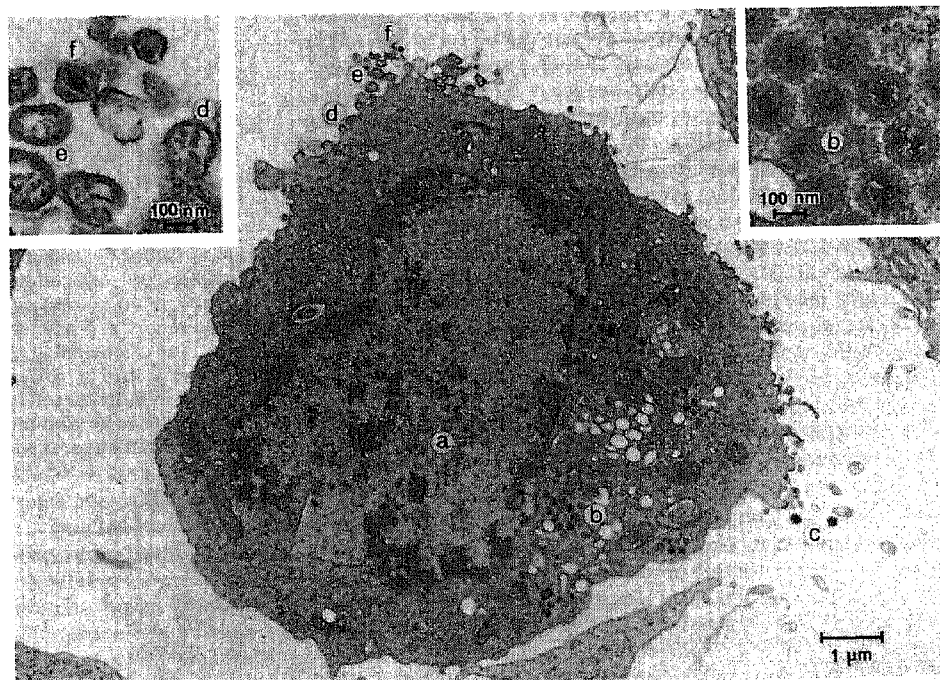


Fig. 1 Simultaneous expression of specific antigens of HHV-6 (a) and HIV-1 (b) in doubly infected cell cultures, as assessed by two-colour indirect immunofluorescence.

Methods. PBMC from healthy volunteers were isolated by Ficoll-Hypaque gradient centrifugation and cultured at 37 °C in a humidified atmosphere of 5% CO₂ at a density of 1×10^6 ml⁻¹ in RPMI medium supplemented with 15% fetal bovine serum, $1 \mu\text{g ml}^{-1}$ phytohaemagglutinin-P and $5 \mu\text{g ml}^{-1}$ polybrene. Cells were washed with RPMI after 24 h, counted and exposed for one hour at 37 °C to HIV-1 alone (isolate HTLV-III_B), HHV-6 alone or to HIV-1 plus HHV-6; each virus was at an infectious multiplicity of ~0.3. Infected cells were then cultured at 10^6 per ml in RPMI supplemented with 10% fetal bovine serum and 20% of their original culture medium collected before infection. The absolute numbers of viable cells, CD4⁺ cells and giant multinucleated cells (syncytia) were recorded at 2-day intervals. After removal of dead elements by Ficoll-Hypaque gradient centrifugation, viral antigen expression was evaluated in parallel by indirect immunofluorescence on cytocentrifuge preparations fixed for 5 min in cold acetone¹⁸. A murine monoclonal antibody against the gag p24 protein of HIV-1 (M26)²⁰ was used at $2 \mu\text{g ml}^{-1}$; an anti-HHV-6 human serum (N828), extensively adsorbed with uninfected phytohaemagglutinin-activated PBMC, was used at a final dilution of 1:15. Phycoerythrin-conjugated (red) goat anti-mouse IgG and fluorescein-isothiocyanate-conjugated (green) goat anti-human IgG polyclonal antisera (both specific for γ -chain; Kirkegaard and Perry Inc., Maryland), were used as second layer antibodies. Samples were visualized with a Leitz Ortholux II fluorescence microscope equipped with a $\times 40$ dry objective. Two-colour immunofluorescence also demonstrated that cells infected with HHV-6 and HIV-1 displayed a CD4⁺ T-cell phenotype.

Fig. 2 Electron micrograph of a cell with the morphological features of an activated lymphocyte, eight days after simultaneous infection of PBMC cultures with HIV-1 and HHV-6. a, Unenveloped intranuclear HHV-6; b, unenveloped intracytoplasmic HHV-6; c, mature extracellular HHV-6; d, budding HIV-1 particles; e, immature extracellular HIV-1 (rarefied core); f, mature HIV-1 (dense core). Magnification is $\times 15,000$ (detailed inserts, $\times 75,000$). Cells were extensively washed with cold PBS, centrifuged at 1,500 r.p.m. and fixed with 1.25% glutaraldehyde, 0.1 M sodium cacodylate, 0.5 \times PBS for electron microscopy.



Freshly isolated normal human peripheral blood mononuclear cells (PBMC) were activated *in vitro* with purified phytohaemagglutinin and subsequently co-infected with HIV-1 and HHV-6. Two-colour indirect immunofluorescence was used to detect specific viral antigens and demonstrated that individual cells could serve as a simultaneous target for both viruses (Fig. 1). Electron microscopy illustrated the productive nature of this dual infection, showing the complete pathway of HHV-6 and HIV-1 maturation within individual lymphocytes. HHV-6 was visible as both immature (namely, nuclear or cytoplasmic un-

veloped nucleocapsids) and mature extracellular particles (Fig. 2a-c). Budding, extracellular immature and mature HIV-1 virions could be recognized if associated with the same cell (Fig. 2d-f).

The expression of viral messenger RNA, as analysed by northern blotting, was qualitatively unaltered in dually infected cultures and the viruses released were found to be infectious for normal human PBMC (data not shown). As summarized in Table 1, however, HIV-1 antigen expression was consistently accelerated in the course of co-infection with HHV-6. It must

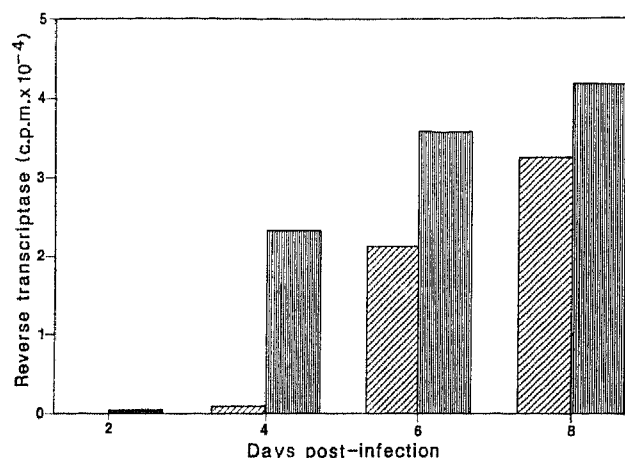


Fig. 3 Reverse transcriptase activity in cultures of PBMC infected with HIV-1 alone (cross-hatched) or with HIV-1 and HHV-6 together (shaded). Details of infection and culture procedures are given in the legend to Fig. 1. Polyethyleneglycol-precipitated cell culture supernatant fluids were assayed for reverse transcriptase activity as previously described².

be emphasized that the majority of HIV-1-positive cells were simultaneously expressing HHV-6 antigens. Moreover, these immunofluorescence determinations probably underestimated the actual magnitude of the infection, as dead (presumably infected) cells were removed before testing. An accelerated expression of HIV-1 in dually infected cultures was further indicated by the earlier detection of reverse transcriptase activity (Fig. 3).

Consistent with the acceleration of HIV-1 expression, we also observed a more rapid induction of cytopathicity in the course of dual infection. The loss of CD4⁺ T lymphocytes was significantly accelerated in dually infected PBMC cultures (Fig. 4a) and mathematical analysis of the data suggested a synergistic cytotoxic action of HIV-1 and HHV-6 (Fig. 4b). A parallel decline of total cell viability was also observed in these cultures. For example, eight days after infection, the absolute numbers of viable cells were $4.3 \pm 0.5 \times 10^5$ with HIV-1 and $2.7 \pm 0.5 \times 10^5$ with HHV-6, as compared to $0.6 \pm 0.2 \times 10^5$ with both viruses together ($P < 0.01$). These observations indicate that, despite the documented down-regulation of CD4 molecules by HIV-1 gp120/160 glycoproteins¹⁹, the chief mechanism for CD4⁺ cell depletion in our cultures was a virus-induced cytopathic effect. Moreover, HIV-1 induced syncytia were identified earlier in doubly infected cultures (Table 1). Dual infection of CD4⁺ T cells was also observed using established human CD4⁺ T cell lines (not shown).

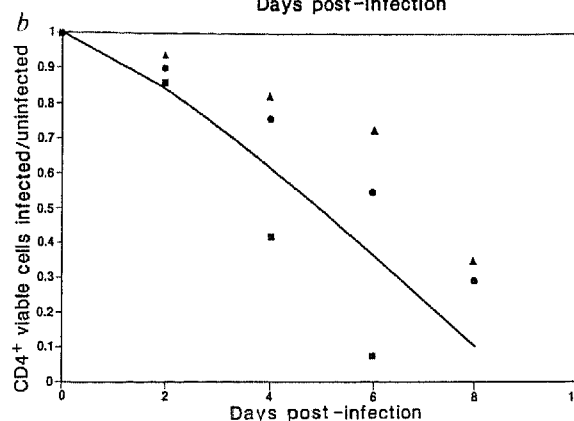
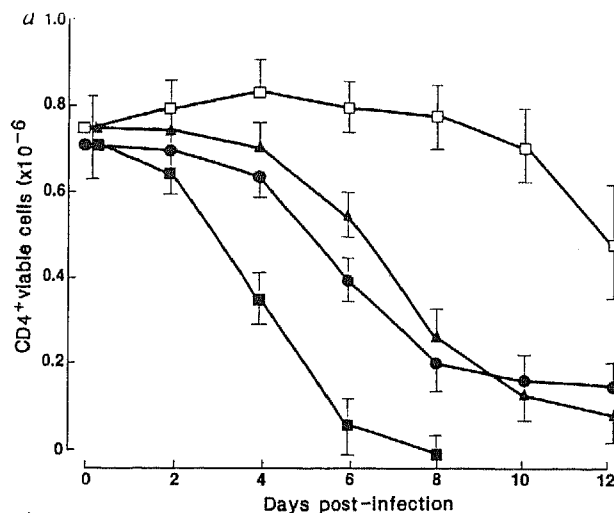


Fig. 4 Cytopathic effect in the course of co-infection of normal human PBMC by HIV-1 and HHV-6. Open square, uninfected; filled triangle, HIV-1 alone; filled circle, HHV-6 alone; filled square, HIV-1 plus HHV-6. *a*, Kinetics of CD4⁺ cell viability in singly and doubly infected cultures. Data represent the averaged results (\pm standard deviation) from 3 separate experiments. *b*, Synergistic effect of HIV-1 and HHV-6 in inducing cytopathicity on normal PBMC cultures. All values are normalized with respect to uninfected control cultures (representing the ratio between viable CD4⁺ cell counts in infected cultures and viable CD4⁺ cell counts in uninfected cultures for each time point). The solid line represents the predicted curve in the course of an additive effect and was calculated according to the following formula: $N_t = p_t q_t$ (where p_t and q_t correspond to normalized CD4⁺ cell counts at time t in cultures infected with HIV-1 and HHV-6 respectively). By day 4 or 6 post-infection, the observed mortality in dually infected cell cultures was 2–3 times greater than the values predicted for a simple additive effect.

Table 1 Viral antigen expression and syncytia formation in normal PBMC cultures infected with HIV-1, HHV-6 or both viruses simultaneously

Day post-infection	Virus used for infection*							
	HIV-1		HHV-6		HIV-1 plus HHV-6			S‡
	HIV-1† %	S‡	HHV-6† %	S‡	HIV-1† %	HHV-6† %	HIV-1+HHV-6† %	
2	—	—	—	—	0.7 (0.6)	—	—	—
4	1.7 (1.6)	—	6.7 (5.8)	—	10.7 (7.5)	14.7 (10.3)	9.3 (6.5)	—
6	9.3 (7.2)	—	30.7 (14.4)	—	38.3 (12.6)	45.7 (15.1)	24.0 (7.9)	+
8	22.7 (9.7)	+	68.3 (18.4)	—	61.0 (3.7)	82.7 (5.0)	50.3 (3.0)	+
10	30.3 (11.2)	+	80.0 (18.4)	—	NA	NA	NA	+

* Phytohaemagglutinin-activated PBMC were infected with HIV-1, HHV-6 or both viruses simultaneously. Details of the infection procedure and indirect immunofluorescence assay are given in the legend to Fig. 1.

† Per cent immunofluorescent-positive viable cells and in parenthesis absolute numbers of positive viable cells $\times 10^{-4}$ (averaged results from three separate experiments) for the indicated virus, determined as described in the legend to Fig. 1.

‡ Presence (+) or absence (–) of syncytia (S) as evaluated on Wright-Giemsa stained cytocentrifuge preparations, each containing 2×10^5 cells.

|| NA, not applicable. In these cultures no viable cells were detected by day 10 post-infection.

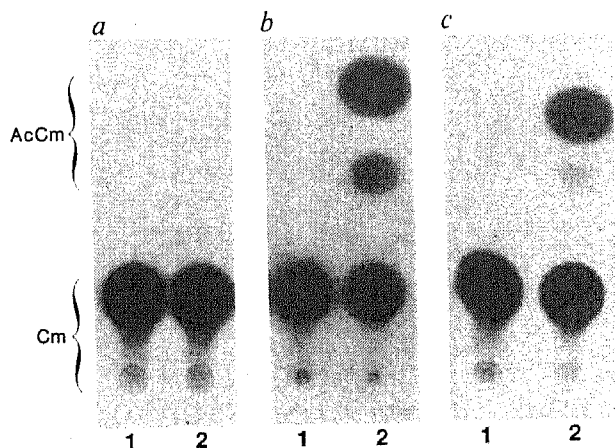


Fig. 5 Transactivation of the chloramphenicol acetyltransferase (CAT) gene linked to the HIV-1 LTR when transfected into MOLT-3 cells which were uninfected (a), infected with HIV-1 (b) or infected with HHV-6 (c). Plasmids were pC15CAT (lanes 1; ref. 21) containing the HIV-1 LTR linked to the bacterial CAT reporter gene, and the control pSVOCAT (lanes 2; ref. 22) that lacked promoter-enhancer sequences.

Methods. 10^7 cells were transfected with 10 μ g plasmid DNA by the DEAE-dextran technique²³, collected after 40 h and processed to obtain 100 μ l cellular extract; aliquots of 20 μ l were subsequently used for CAT assays in a 3-h incubation²⁴. Different percentages of cell viability were found in the various cultures at the time of collection: a1, 79%; a2, 89%; b1, 51%; b2, 49%; c1, 26% and c2, 29%. The acetylation of chloramphenicol (%) as estimated from liquid scintillation counting was a1, 0.19; a2, 0.24; b1, 2.5; b2, 30; c1, 0.27 and c2, 19.7. Cm, chloramphenicol; AcCm, acetylated chloramphenicol.

The accelerated virus expression and cytopathicity in dually infected cultures suggested that a direct interaction occurs between HIV-1 and HHV-6. We therefore studied the transactivation of the HIV-1 LTR by HHV-6 using the human CD4⁺ T-cell line MOLT-3. As illustrated in Fig. 5, there was a consistent transactivation in HHV-6-infected cells which was not found in uninfected cells. We obtained similar results using the CD4⁺ T-cell line CEM (data not shown).

The part played by cofactorial mechanisms in the pathogenesis of AIDS and in particular the relevance of other potentially immunosuppressive agents are still to be clarified. We have previously demonstrated that HHV-6 exerts a direct lytic effect on human CD4⁺ T lymphocytes¹⁸. Here we have shown that HHV-6 can co-infect individual T cells with HIV-1 and can transactivate the expression of HIV-1. The simultaneous presence of both viruses in the same cell emphasizes the potential importance of this transactivation. Replication of HHV-6 might in turn be enhanced by co-infection with HIV-1, thus generating a vicious cycle. Our observations, although they do not prove the aetiological involvement of HHV-6, are consistent with a possible cofactorial role of this virus in one of the critical pathological features of AIDS, namely the CD4⁺ T-cell depletion. Prospective long-term seroepidemiological and *in vivo* investigations, using animal models or two-probe hybridization to detect dual infection in individual patient cells, will help evaluate the contribution of potential cofactors to the course of HIV-1 infection.

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Genetically haploid spermatids are phenotypically diploid

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Because chromosomal homologues segregate from one another during meiosis, spermatids are genetically different. Post-meiotic gene expression could lead to gametic differences, some of which might lead to preferential transmission of certain alleles over others. In both insects and mammals, however, all the cells derived from a single spermatogonial cell develop within a common syncytium formed as a result of incomplete cytokinesis at each of the mitotic and meiotic cell divisions¹⁻³. It has been proposed that the intercellular bridges connecting the cells, which are about 1 μ m in diameter⁴, permit the sharing of cytoplasmic constituents, thus ensuring the synchronous development of a clone of cells and gametic equivalence between haploid spermatids^{2,5,6}. By analysing the product of a transgene which is expressed exclusively in post-meiotic germ cells in hemizygous transgenic mice, we have shown that genetically distinct spermatids share the product of the transgene and hence can be phenotypically equivalent.

The experiments described here were designed to determine whether either RNA or protein can pass through the intercellular bridges which connect spermatids. To address these questions we have generated transgenic mice that carry a chimaeric gene consisting of the mouse protamine 1 (*mPI*) transcriptional regulatory sequences, fused to the human growth hormone (*hGH*) structural gene. The advantage of using transgenic mice for such an analysis is that mice can be generated which are hemizygous for the transgene, that is, the introduced DNA is present on only one of the two chromosomal homologues, so that after meiosis only half of the spermatids carry the transgene (Fig. 1a). The *mPI* gene is transcribed exclusively during spermiogenesis, the haploid phase of spermatogenesis⁷. Previous studies have shown that 4.8 kilobase (kb) of 5' sequence is sufficient for the proper tissue-specific and temporal regulation of *mPI* in transgenic mice⁸. The construct used in this study contains 4.1 kb of *mPI* 5' sequence fused to the *hGH* gene (Fig. 1b).

Two independent lines of transgenic mice harbouring the *mPI-hGH* gene were established (Fig. 1b). To determine the transmission frequency of the transgene, tail DNA from

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