Herpesvirus saimiri-immortalized human T-cells support long term, high titred replication of human immunodeficiency virus types 1 and 2

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Herpesvirus saimiri strain C488 transforms human CD4⁺ T-lymphocytes to continuous interleukin-2-dependent growth. Unlike human T-cell lines derived from tumours or those transformed by human T-lymphotropic virus 1, herpesvirus saimiri-immortalized T-cells (HVS T-cells) retain many functions of primary activated T-lymphocytes. We have characterized the course of human immunodeficiency virus types 1 and 2 (HIV-1/-2) infection in three HVS T-cell lines. Our results confirm that HVS T-cells are highly permissive to both HIV-1/-2 prototype viruses and to poorly replicating HIV-2 strains of restricted cell tropism. However, the infection was persistently productive for up to 5 months. The down-regulation of surface CD4 molecules was delayed and virus yields significantly exceeded those obtained in T-cell lines.

Two culture systems for the isolation, propagation and biological characterization of human immunodeficiency viruses types 1 and 2 (HIV-1/-2) are in routine use. These employ cytokine-independent conventional human cell lines of lymphoid (T-cell) or monocytic (monocyte/macrophage) origin and cytokine-dependent primary peripheral blood mononuclear cells (PBMC). Conventional cell lines have a relatively stable phenotype, are technically simple to maintain and support the replication of tissue culture-adapted strains of HIV. However, they are permissive for only one-third of primary isolates (Fenyö et al., 1988; Kong et al., 1988; Schwartz et al., 1989) and viruses from asymptomatic patients are rarely able to grow in them. Primary isolates grow better in cytokine-activated PBMC (Levy & Shimabukuro, 1985; Asjo et al., 1988) but isolation rates from asymptomatic patients remain poor. PBMC are technically difficult to maintain, have a limited life span and pools derived from different donors vary in composition (cell phenotype), growth characteristics and ability to support virus replication (Williams & Cloyd, 1991; Spira & Ho, 1995).

CD4-bearing T-lymphocytes from primary PBMC can be transformed to continuous growth by a γ₂ herpesvirus, herpesvirus saimiri (HVS) strain C488. Unlike conventional T-cell lines, HVS-immortalized T-cells (HVS T-cells) retain the phenotypic cell-surface markers and cytokine profile of activated mature T-lymphocytes (Biesinger et al., 1992; Mittrucker et al., 1992). Saha et al. (1996) have described the generation of stable HVS T-cell clones from HIV-infected individuals. Nick et al. (1993) reported that HVS T-cells were permissive to the replication of both prototype HIV-1/-2 viruses and primary HIV-1 isolates. This suggested that HVS T-cells may provide an alternative tissue culture system for HIV but clearly, a full characterization of the infection was required to assess this potential further. We have achieved this using well-characterized prototype HIV-1/-2 viruses, for which good reagents are available, and HIV-2 CBL-24, an isolate from an asymptomatic individual reported to replicate poorly in both PBMC and one T-cell line (Molt-4 cl.8) (Schulz et al., 1990).

For this study, HIV-1/-2 virus pools were prepared in conventional T-cell lines grown in RPMI 1640 (Gibco) containing 10% heat inactivated foetal calf serum (FCS), 2 mM L-glutamine and 2000/1000 IU/ml penicillin/streptomycin. HIV-1 MN [MN, ARP (AIDS Reagent Program) 102.1] and HIV-1 GB8 (GB8, ARP131.1) were grown in C8166 (ARP013), HIV-2 ROD (ROD, ARP121.1) and HIV-2 CAM-2 (CAM-2, kindly provided by A. Karpas, MRC Centre, Cambridge, UK) were grown in CEM-SS (ARP048) as previously described (Vella et al., 1995). HIV-2 CBL-24 (Schulz et al., 1990) was passaged three times in Molt-4 cl.8 (#175 NIH AIDS Research and Reference Program) and harvested at 18 days post-infection (p.i.) when showing extensive syncytia. The TCID₅₀ was determined by syncytium assay in the cell line used for growth as previously described (Vella et al., 1995) and the core-antigen titre determined by ELISA (Coulter p24 and p27).

HVS T-cells CB23 and Kesting were described by Nick et al.
p27 production in HIV-infected herpesvirus saimiri-immortalized T-cell cultures. (a) p27 produced by Kesting (□) and CB23 (□) cultures infected with HIV-2 CBL-24, together with the result of infection in the T-cell line Molt-4 cl.8 with cell-free virus (− indicates syncytia not formed, + indicates syncytia formed). (b) p27 produced by CB23 (□) and AC (□) cultures infected with HIV-2 ROD (all supernatants produced syncytia on the T-cell line CEM-SS). (c) p24 produced by Kesting (□) and CB23 (□) cultures infected with HIV-1 MN (all supernatants produced syncytia on the T-cell line C8166). * Not done.

Fig. 1. p24/p27 production in HIV-infected herpesvirus saimiri-immortalized T-cell cultures. (a) p27 produced by Kesting (□) and CB23 (□) cultures infected with HIV-2 CBL-24, together with the result of infection in the T-cell line Molt-4 cl.8 with cell-free virus (− indicates syncytia not formed, + indicates syncytia formed). (b) p27 produced by CB23 (□) and AC (□) cultures infected with HIV-2 ROD (all supernatants produced syncytia on the T-cell line CEM-SS). (c) p24 produced by Kesting (□) and CB23 (□) cultures infected with HIV-1 MN (all supernatants produced syncytia on the T-cell line C8166). * Not done.
p24 (Fig. 2). MN-infected C8166 T-cells gave a significantly lower p24 yield (peak 700 ng/ml). Results were comparable in two independent experiments.

Viral antigen expression was analysed by flow cytometry in a FACscan Vantage (Becton Dickinson) set to accumulate 10,000 events per test. Surface staining was investigated with phycoerythrin-conjugated Leu 3a, 5 µl per test (IgG1, anti-CD4, Becton Dickinson) and FITC-conjugated anti-gp120, 1:100 (IgG1, Agmed). HIV-2 gp105 was detected using human HIV-2 seropositive serum (ARP501, A and C) at 1:100 after extensive cross-adsorption on HVS T-cells, followed by FITC-conjugated goat anti-human IgG (Sigma) at 1:150.

Cytoplasmic staining for p24 in permeabilized cells was detected with FITC-conjugated KC57, 5 µl per test (IgG1, Coulter). FITC-conjugated isotype controls and uninfected stained cells were included as negative controls. Again, results were reproducible.

Antigen expression in MN-infected HVS T-cell cultures (Fig. 2) showed that following an input of 200 ng of p24 small numbers of cells became infected, but by 21 days p.i. the number of productively infected cells was greater than 80% in terms of cytoplasmic p24 staining. HIV-2-infected cultures were not analysed for p27 by flow cytometry because all products, Nef, Vpu and Env (see review by Bour et al., 1996) are not present in HIV-2. Loss of the viral receptor prevents superinfection toxicity and may facilitate the release of viral progeny (Benson et al., 1993). In MN- and ROD-infected conventional T-cells there is well-documented evidence to show that as gp120 expression increases there is rapid down-regulation of CD4 at the surface (Dalgleish et al., 1984; Kanner & Haffar, 1995; Cucchiarini et al., 1995). We found that with ROD- and MN-infected C8166 T-cells, CD4 was down-regulated by more than 90% at 4 days p.i., whereas in CBL-24-infected Molt-4 c.l8 T-cells, CD4 was down-regulated by 50% at 7 days p.i. and 96% at 12 days p.i. (not shown). Taking 30% as a clear downward shift (Lusso et al., 1995) CD4 modulation was delayed in most HIV-infected HVS T-cell cultures, despite high gp120/105 expression (Figs 2 and 3). Double labelling confirmed that 50% gp120+ cells co-expressed CD4 (Fig. 3). Notably, extensive syncytium...
formation in CBL-24-infected Kesting and CB23 cultures resulted in rapid loss of CD4, after which CD4 expression recovered. Other workers (Evans et al., 1988; Lusso et al., 1995) have also reported minimal down-regulation of CD4 with particular cell lines or virus isolates and the process is less efficient in PBMC in vivo (Schnittman et al., 1989) and in vitro (Chen et al., 1996).

Although HVS T-cells were previously reported to support prototype viruses and primary isolates, little was known of the course of infection. Our results show that HVS T-cell cultures undergo a persistent, highly productive infection with HIV-1 and HIV-2, which is characterized by a delay in cell-surface CD4 down-regulation. We report p24\textsuperscript{27} titres that are significantly higher than those obtained in conventional T-cell lines. Our results with HIV-2 CBL-24, an isolate that replicates poorly in PMBC and in the T-cell line Molt-4 cl.8, are particularly encouraging for further studies focusing on samples from asymptomatic and non-progressing individuals from whom primary isolates are difficult to obtain.

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References


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