CD34 cells and their derivatives contain mRNA for CD4 and human immunodeficiency virus (HIV) co-receptors and are susceptible to infection with M- and T-tropic HIV

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Highly purified (> 98%) CD34 cells directly after isolation (D0) or 2 weeks in culture (D14) were CD4 and contained mRNA for the T-tropic HIV co-receptor, CXCR-4, and minor co-receptor, CCR-2B. D14 but not D0 cells were RT–PCR positive for mRNA for the major M-tropic human immunodeficiency virus (HIV) co-receptor, CCR-5, and potential co-receptor, CCR-1. D14 and D0 cells were susceptible to T- (HXB2) and M-tropic HIV (Bal), showing greater virus production with Bal than HXB2, and with higher virus production levels in D14 compared to D0 cells. Seven days post-infection of D0 cells Bal DNA was present in CD14bright and CD14 fractions, suggesting D0 infection of diverse progenitor types. HXB2 DNA was detected in CD14bright cells alone indicating D0 infection of monocyte progenitors only. It is concluded that CD34 cells and cultured derivatives are susceptible to M- and T-tropic HIV and this correlates in part with co-receptor expression at the mRNA level.

Human immunodeficiency virus (HIV)-positive patients often suffer haematological dysfunctions that have been partly attributed to HIV-induced effects on haematopoietic progenitor cells. CD34+ cells are a heterogeneous population of multipotent haematopoietic progenitors residing in the adult bone marrow. Reports on whether CD34+ cells from HIV-positive patients are infected in vivo have been controversial (Davis et al., 1991; De Luca et al., 1993; Molina et al., 1990; Stanley et al., 1992; von Laer et al., 1990). Several studies have shown successful in vitro infection of the CD34+ population (Folks et al., 1988; Kitano et al., 1991), although studies in this area have focused on the haematological consequences of HIV infection and its effects on progenitor cells (Chelucci et al., 1995; Kaushal et al., 1996; Steinberg et al., 1991).

In this study we have analysed HIV infection of haematopoietic progenitor cells in relation to virus production and initial cell phenotype. We have defined and compared the in vitro susceptibility of freshly isolated and cultured CD34+ cells to prototype laboratory M- and T-tropic strains of HIV. Recently, major tropism determinants have been identified that act through distinct co-receptor usage for entry by different strains of HIV. Two molecules, fusin or CXCR-4 (Feng et al., 1996) and CCR-5 (Deng et al., 1996; Dragic et al., 1996) have been identified as the major T- and M-tropic HIV co-receptors, respectively. Other β-chemokine receptors, such as CCR-2B (Doranz et al., 1996) and CCR-3 (Choe et al., 1996; Doranz et al., 1996), function as ‘minor’ co-receptors for some HIV strains while CCR-1 (Dragic et al., 1996) potentially may also allow HIV entry. We report here the detection of mRNA for CD4 and HIV co-receptors in CD34+ cells and their derivatives, and dual susceptibility of these cells to M- and T-tropic HIV. Such information is essential for understanding the in vivo consequences of HIV infection of progenitor cells.

Bone marrow was aspirated from the posterior iliac crest and sternum of healthy volunteers after informed consent and used with the approval of the Human Ethics Committee of the Royal Adelaide Hospital. Bone marrow mononuclear cells (BMMNC) were collected after centrifugation (400 g) over Ficoll (Lymphoprep, 1-077 g/ml; Nycomed Pharma) and washed twice with HHF (Hanks’ balanced salt solution [Gibco BRL], 20 mM HEPES pH 7.35, 5% (v/v) foetal calf serum [FCS]) at 4 °C. BMMNC were incubated in HHF + 2% (v/v) human serum for 30 min on ice before immunolabelling with the anti-CD34 antibody, HPCA-2-FITC (Haylock et al., 1992). Cell sorting was performed using a FACStar Plus cell sorter (Becton Dickinson Immunocytometry Systems). The threshold for selection of CD34+ cells was based on staining with an isotype-matched control antibody. Separated cells were routinely greater than 98% CD34+. Cells were used immediately (D0) or cultured (D14) in Delta medium [Iscove’s modified Dulbeco’s media: 30% (v/v) FCS, 1% (w/v) bovine serum albumin (BSA), 2-7 mM L-glutamine, 0-05 mM β-mercaptoethanol] + 4 haemopoietic growth factors [4HGF: 10 ng/ml interleukin (IL)-3, 20 ng/ml IL-6, 100 ng/ml granu-
loctocyte-colony stimulating factor (G-CSF), 100 ng/ml stem cell factor] (Delta + 4HGF), as described by Haylock et al. (1992). At D₀ cells are quiescent and non-adherent. Culture of cells with 4HGF stimulates proliferation and after 14 days in culture the cell number had expanded and some cells had started to differentiate into cells of the myeloid lineage (Haylock et al., 1992).

To partly characterize the phenotype of these cells, lineage-specific markers were analysed by two colour flow cytometry using the following antibodies: CD45-FITC/CD14-PE (Simul-stain Leukogate, Becton Dickinson), CD34-PE (HCPA2, Becton Dickinson), CD4-PE (Dako). Isotype-matched controls, monocyte-derived macrophages and total peripheral blood mononuclear cells (PBMC) were co-analysed as controls. Results indicate D₀ cells to be a highly pure leukocyte population of 97–100% CD34⁺, 97–100% CD45⁺ with low levels of CD14 and CD4 (3–5%) and negligible levels of CD3⁻ (< 2%). Louache et al. (1994) reported low levels of CD4 within the CD34⁺ population, while CD34⁺/CD14⁺ cells also exist (Tjonnfjord et al., 1996). Thus, the low levels of CD4 and CD14 at D₀ do not indicate contaminating mature cells. After 14 days in culture the cells were still 97–100% CD45⁺, lost expression of CD34 (< 2%), gained expression of CD4 (10–20%) and CD14 (30–60%) and remained CD3⁻ (< 2%), indicating myeloid characteristics and a lack of mature T-cells.

Total RNA was extracted from 10⁶–10⁷ uninfected CD34⁺ cells from two donors at D₀ and D₁₄ and subjected to RT–PCR to investigate the presence of mRNA for HIV receptor and co-receptors. RNA was extracted by the method of Chomczynski & Sacchi (1987), DNase treated and the RNA integrity and concentration determined. DNA-free RNA was reverse transcribed with M-MuLV RT (New England Biolabs) and random hexamers (Bresatec). Reverse transcriptase minus (RT⁻) control reactions were performed under identical conditions but contained no M-MuLV RT. PCR was performed with primer pairs as follows: CD4, cggaattcattgggctaggcatc, gtcaagcttcatctggtccgcagg; CXCR-4, ggaattcctctccaaaggaaag, cacag-gatcctgcctagac; CCR-1, tgcacctggttaactggctc, gcccagaattcctcagaagc; CCR-2B, aactgcagggagacagtggatg, gcattggttaacatagtctg; CCR-5, tgcgcatgctgttctattttccagcaa, catgtcgagaactctgactgg; β-actin, gcttcg-cgggcgacgatgccccccgggcc, gacgtagcacagcttctccttaatgtcacgca. Reactions were amplified with TAQ DNA polymerase (Biotech International) for 30 cycles annealing at 58 °C except for CCR-5 and CCR-2B, which were annealed at 50 °C. RT⁻ reactions were universally negative (data not shown). Fig. 1 shows representative reactions.

Results indicate that, under these PCR conditions, D₀ cells
are RT–PCR positive for CD4, CXCR-4 and CCR-2B but negative for CCR-5 and CCR-1. In contrast, under identical conditions, D14 cells are RT–PCR positive for CD4, CXCR-4, CCR-1, CCR-2B and CCR-5. The mRNA for CCR-3 could not be reliably demonstrated.

PCR products for CD4, CXCR-4 and CCR-5 were cloned and sequenced to confirm their identity and to establish a more sensitive and semi-quantitative PCR. RT–PCR of CD4, CXCR-4 and CCR-5 or PCR of the corresponding known copy number DNA clones were performed, as above, for 27 cycles and PCR products detected by Southern hybridization (Fig. 1C). Assuming equivalent RT efficiencies, CD4 and CXCR-4 mRNA were present at similar levels in both D0 and D14 samples. However, even using this more sensitive detection method (at least 10 copies of DNA) CCR-5 mRNA was again detected only in RNA extracted from D14 but not D0 cells.

CXCR-4 is present in many tissues and is the receptor for the chemokine, stromal-derived factor 1 (SDF-1) (Bleul et al., 1997; Oberlin et al., 1996). SDF-1 is produced by bone marrow stroma and has been recently shown to have chemoattractant properties for CD34+ cells (Aiuti et al., 1997). Consistent with this, CD34+ cells, residing in the bone marrow, contained mRNA for CXCR-4. Deichmann et al. (1997) have also recently described CXCR-4 mRNA in CD34+ cells. CCR-1 and CCR-2B are β-chemokine receptors, which are undescribed in the bone marrow. CCR-5 is also a β-chemokine receptor which Deichmann et al. (1997) have recently reported to be present in only 24% of CD34+ samples examined. This is, in part, consistent with our results. If CCR-5 mRNA is present at low levels in only a few individuals as indicated by Deichmann and co-workers, the larger number of separate CD34+ samples analysed by these investigators may account for the differences in our observations. Alternatively, Deichmann et al. (1997) used CD34+ cells isolated from patients receiving chemotherapy and G-CSF, which may have affected CCR-5 mRNA levels. The detection of CXCR-4, CCR-1, CCR-2B and CCR-5 on D14 cells is similar to that reported for primary monocytes (Deng et al., 1996; Loetscher et al., 1994), which is consistent with the acquisition of monocyte-like characteristics on CD34+ cells under the described culture conditions.

CD34+ cells at D0 or D14 (1–2 × 10^5 cells) were mock-infected or infected with M-tropic (Bal) or T-tropic (HXB2) HIV at 0–1 TCID50 per cell and markers of virus infection followed for 21 days post-infection (p.i.). The M-tropic nature of Bal was maintained by passage in monocyte-derived macrophages in between amplifications in PBMC. Cultured D14 cells were infected by centrifugal enhancement (Pietroboni et al., 1989) or a 2 h incubation at 37 °C. D0 cells were infected in the latter manner. After infection, cells were washed and resuspended in Delta + HGF. On day 1 p.i., cells were harvested and resuspended in fresh medium. Cultures were assessed every 3–4 days for cell number and seeded at 1 × 10^6/ml in Delta + HGF.

Virus production was assessed by supernatant P24 ELISA (Dupont) (Fig. 2A). Infection of D0 or D14 cells with either strain of HIV produced significant amounts of P24, which were maintained for at least 21 days p.i. In experiments from different donors the amount of P24 produced varied although there was always more P24 released from cells infected at D14 than D0. Bal-infected cultures produced more P24 than HXB2-infected cultures in all experiments.

At day 21 p.i. total DNA was extracted and subjected to PCR for HIV Gag to confirm the presence and persistence of HIV-positive cells. Cells were washed, resuspended at 1 ×
10^5/ml in 50 mM KCl, 10 mM Tris pH 8.3, 1.5 mM MgCl₂, 0.5% (v/v) Triton X-100 + 0.2 mg/ml proteinase K and incubated at 56 °C for 60 min. The DNA extract was heated at 95 °C for 10 min then used directly in PCR for HIV Gag using primers gagaaatggcacaatgg and cgctgagaagtggtgcctc and for β-globin using caactctctccacctcggt and gccgcagcagcagcttgc. PCR reactions were annealed at 58 °C and amplified for 28 cycles. Viral DNA was present in all infected but not uninfected cultures (Fig. 2B) with highest levels (relative to β-globin) in cells infected with Bal at D₁₄.

The detection of viral protein (P24) and DNA indicated that both strains of HIV can establish productive infection in freshly isolated CD34+ cells and CD34+ cells cultured for 14 days in 4HGF. This is consistent, for D₁₄ cells, with the presence of mRNA for CD4 and M- and T-tropic HIV co-receptors. D₀ cells were susceptible to both strains of HIV and contained mRNA for CD4, CXCR-4 and CCR-2B but not the major M-tropic HIV co-receptor, CCR-5. Since some strains of HIV may use other β-chemokine receptors for entry (Choe et al., 1996; Doranz et al., 1996; Dragic et al., 1996), Bal may have entered D₀ cells using alternative receptors or D₀ cells may contain CCR-5 at levels below the sensitivity of RT-PCR but still high enough to allow virus entry. Confirmation of the co-receptor usage by Bal should be further analysed using chemokine blocking experiments.

The observed susceptibility of isolated progenitor cells to both Bal and HXB2 is in agreement with previous studies using either M-tropic (Kaushal et al., 1996; Kitano et al., 1991) or T-tropic HIV (Chelucci et al., 1995; Folks et al., 1988; Steinberg et al., 1991) and our results are comparable to the previous literature. In contrast, Kitano et al. (1991) reported a lack of susceptibility of CD34+CD4+ cells to T-tropic HIV, which may relate to the different cell population used. In our study, D₀ and D₁₄ cells produced higher virus yields with Bal infection than with HXB2 infection, consistent with the observed monocyte/macrophage-like phenotype of the cells.

The yields of virus released and viral DNA levels at day 21 p.i. were greater with D₁₄ than with D₀ cells, suggesting differences in virus entry and/or replication. D₁₄ cells are rapidly proliferating, while D₀ CD34+ cells are initially quiescent, which may make intracellular conditions for replication less permissive while the lack of detectable CCR-5 and lower levels of CD4 at D₀ compared to D₁₄ may restrict virus entry.

To assess the proportion of infected cells, P24 immunofluorescence was performed at day 21 p.i. using a P24 antibody (Dako) and an anti-mouse IgG Fc-specific-FITC conjugate (Sigma). In cells infected with Bal at D₀ or D₁₄, P24 was detectable in approximately 40–90% of cells compared to 1–5% of the population with HXB2 (data not shown). The low percentage of HXB2-infected cells was suggestive of a subpopulation of susceptible cells. To assess the cell-type infected with HIV, CD34+ cells were infected at D₀ and on day 7 p.i. cells were harvested, immunolabelled with CD45-FITC/CD14-PE and sorted for CD14. CD14bright and CD14− populations were collected, total DNA extracted and subjected to PCR for HIV Gag and β-globin (Fig. 3). HIV DNA was detected in the CD14bright fraction with both HXB2 and Bal infection, indicating D₀ infection of monocyte progenitors and demonstrating that HXB2 susceptibility was not due to infection of contaminating T-cells. Infection with Bal also led to detectable HIV DNA in the CD14− fraction. Taken together these results suggest Bal infects a broad range of progenitors whereas HXB2 infects myeloid progenitors.

Infection with either strain of HIV was non-cytopathic and cells were visually similar to uninfected control cells. However, in some experiments there was a reduction in cell proliferation with Bal but not HXB2 infection (data not shown) without an increase in cell death (by trypan blue assay), syncytium formation or obvious increase in the number of differentiated adherent cells. However, these morphological effects were not extensively assessed and remain to be investigated more rigorously.

Infection of CD34+ cells by HIV may have important consequences in vivo, as has been suggested previously (Folks et al., 1988). Our results suggest that CD34+ progenitors are susceptible to different strains of HIV, particularly as they begin to differentiate. This may contribute to a chronically infected pool of functionally altered cells containing viruses of different tropism across different cell lineages. This heterogeneous reservoir of virus may form a significant pool of diversity from which HIV can evolve against host selection pressures.

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References


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