Analysis of human immunodeficiency virus type 1 (HIV-1) variants and levels of infection in dendritic and T cells from symptomatic HIV-1-infected patients

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Dendritic cells (DC) are required to initiate primary cellular immune responses. Human immunodeficiency virus type 1 (HIV-1) infection of DC may be central to transmission and persistence of virus and in the pathogenesis of AIDS. In symptomatic HIV-1-infected patients the proportion of DC in the mononuclear cell population was reduced. Provirus load in the T cells was 3–100 times higher than in DC and there was no correlation between the levels of infection in the two cell types. Phylogenetic analysis of amino acids in the V3 loop and flanking regions indicated intermingling of sequences and thus provides the first evidence for transfer of virus between DC and T cells in vivo. In one of three patients analysed there were significant differences in amino acid residues in the V3 region. This may reflect reduced interactions between DC and T cells in infected individuals and for the existence of variants with a stronger tropism for DC, which could play a role in transmission by initiating infection in mucosal DC.

Introduction

Dendritic cells (DC) are required for the initiation of primary immune responses. In tissues they acquire and process antigens to short peptides that complex with major histocompatibility complex (MHC) class II molecules to be presented on the cell surface. Migrating to T dependent areas of the draining lymph node they may lose the ability to process antigen but cluster and stimulate T helper cells. DC are also effective at stimulating both primary and secondary cytotoxic T cell responses. Macrophages, which also express MHC class II, can present antigens to memory T cells but are significantly less potent than DC and are unable to stimulate naive T cells. The unique stimulatory properties of DC are thought to reflect high level expression of MHC, adhesion and co-stimulatory molecules including B7.1, B7.2 and CD40 in addition to their unique ability to cluster naive T cells (Knight & Stagg, 1993).

The presence of DC at sites of human immunodeficiency type 1 (HIV-1) infection (skin, blood and genital mucosa), together with their pivotal role in initiating immune responses, has led investigators to study their involvement in the pathogenesis of AIDS. There is general agreement that DC are important in HIV-1 mediated disease processes, and a number of different mechanisms have been proposed. DC are targets for infection and, as a consequence, antigen presentation may be impaired leading to failure to recruit naive T cells. The evidence comes from observations that DC express CD4 (Patterson et al., 1991a, 1995; O’Doherty et al., 1993; Ferbas et al., 1994) and chemokine receptors (Granelli-Piperno et al., 1996) and are susceptible to infection in vitro (Patterson & Knight, 1987; Patterson et al., 1991b; Langhoff et al., 1991; Chehimi et al., 1993; Ludewig et al., 1995). Furthermore, studies on cells from patients have documented infection and loss of dendritic skin Langerhans’ cells (Belsito et al., 1984) and blood DC (Macatonia et al., 1990). Blood DC infected in vitro or blood DC and skin Langerhans’ cells from HIV-1-infected individuals show impaired capacity to stimulate T cell proliferation in a mixed leukocyte reaction (Blauvelt et al., 1995; Macatonia et al., 1989, 1990, 1992). In contrast to studies showing infection and dysfunction of DC, cells exposed for a shorter period in vitro may appear resistant to HIV-1 infection but can bind virus, stimulate T cells and transmit
infection to the T cells as they become activated (Cameron et al., 1992a, b, 1994). Thus, high levels of virus production result when virus pulsed DC are incubated with T cells. In contrast, only low or negligible levels of virus replication were observed when pure populations of either DC or T cells were infected (Pope et al., 1994). Some authors have reported little evidence of virus infection of DC in vivo or in vitro (Cameron et al., 1992a, b, 1994), although more recently this group has described low levels of infection in DC exposed to HIV-1 in vitro (Pope et al., 1995). To clarify the picture, fluorescence-activated cell sorting (FACS) analysis was used to estimate DC numbers in blood mononuclear cells, and virus load in highly purified preparations of DC and T cells was measured.

Different HIV-1 isolates have diverse biological properties. Virus from asymptomatic individuals is usually slow growing and non-syncytium inducing (NSI) and displays a tropism for macrophages but does not replicate in T cell lines. In contrast, symptomatic patients often harbour fast-growing syncytium inducing (SI) virus with a tropism for T cell lines but not for primary macrophages (Asjo et al., 1986; Cheng-Mayer et al., 1988; Fenyo et al., 1988; Tersmette et al., 1989; Chesebro et al., 1992; Fouchier et al., 1992, 1995). Infection in chronically infected individuals is characterized by the presence of many virus variants or quasi species (Meyerhans et al., 1989), but virus in acutely infected individuals is relatively homogeneous and usually of the NSI type (Roos et al., 1992; Zhang et al., 1993; Zhu et al., 1993). Selective pressures may therefore, act on the infecting inoculum during transmission or during the initial expansion of virus. The first cells to encounter the virus may be DC in the mucosal tissues, and selection could act at this level. This possibility is supported since DC are more susceptible to in vitro infection with NSI macrophage-tropic strains of virus than with SI lymphotropic strains (Chehimi et al., 1993). Additionally, clade E HIV-1, prevalent in South-East Asia and sub-Saharan Africa and associated with heterosexual transmission, infected Langerhans’ cells more efficiently than clade B, the predominant virus in Europe and the United States (Soto-Ramirez et al., 1996). This suggests that Langerhans’ cells in the urogenital tract may be the initial targets of infection. Amino acid sequences within the V3 loop of the HIV-1 envelope glycoprotein may determine cell tropism (O’Brien et al., 1990; Hwang et al., 1991; Shioda et al., 1991; Westervelt et al., 1991) and syncytium inducing properties of the virus (Freed et al., 1991; DeJong et al., 1992). These sequences were compared in proviral DNA obtained from DC and T cells from patients.

Methods

**Patients.** A single 50 ml blood sample was taken from 17 patients at CDC stage B or C who were due to start antiviral therapy.

**Cell preparation.** Mononuclear cells from patients and controls were separated from heparinized blood by centrifugation over Ficoll, washed and resuspended in RPMI 1640 medium. Dutch modification, containing 100 IU penicillin, 100 µg/ml streptomycin and 10% heat-inactivated foetal calf serum. After overnight culture the non-adherent cells were removed and used for flow cytometry and DC purification. The first step in the purification of DC was to centrifuge the mononuclear cells for 10 min at 600 × g over a 13.7% metrizamide (Nygaard) gradient. Low density blood mononuclear cells (LDC) harvested from the metrizamide–medium interface contained 8–15% DC, as assessed by flow cytometry, and were further purified by panning. The majority of lymphocytes collected at the bottom of the gradient. The LDC were labelled with a cocktail of cell lineage-specific antibodies that included anti-CD3, CD14, CD16, CD19 and CD56 (Becton-Dickinson). After washing, labelled cells were depleted by incubating on flasks coated with goat anti-mouse immunoglobulin (Applied Immuno-Sciences). The non-adsorbed cells were then used for virus load estimation or after positive selection for MHC class II using immunomagnetic beads (Dynal). T cells were separated by labelling mononuclear cells with antibody against CD3 and then the labelled cells were isolated using immunomagnetic beads (MiniMacs, Miltenyi).

**Flow cytometry.** In order to detect DC, mononuclear cells were labelled with a fluorescein isothiocyanate (FITC) conjugated anti-MHC class II DR antibody and a cocktail of phycoerythrin (PE)-conjugated antibodies specific for T cells (CD3), monocytes (CD14), B cells (CD19) and NK cells (CD56). Cells were also labelled in parallel with appropriately conjugated isotype control antibodies. After gating out dead cells, identified by the forward scatter/side scatter profile, 100 000 cells were analysed. DC are located in a population of large cells which, on forward scatter/side scatter analysis, are distinct from the small lymphocyte population. DC were identified in this large cell population by the absence of labelling with the cocktail of PE-conjugated antibodies and by FITC labelling for MHC class II DR (see Fig. 2). The percentage of CD4+ cells in the T cell population was estimated by labelling with a mixture of PE-conjugated anti-CD3 antibody and FITC-conjugated anti-CD4. All antibody reagents were purchased from Becton-Dickinson except a PE-conjugated IgG2b isotype control antibody which was supplied by Pharmingen.

**PCR.** Purified T cells and DC were pelleted and then digested for 2 h at 50 °C with protease K (100 µg/ml) in 20 mM Tris pH 7.6 containing 0.5% SDS and 5 mM EDTA. After extraction with phenol and chloroform, DNA was precipitated with ethanol in the presence of 0.3 M sodium acetate. The provirus load was estimated by a semi-quantitative nested PCR technique on limiting dilutions of DNA employing primers based on the polymerase gene (Simmonds et al., 1990). Preliminary titrations were on 10-fold dilutions of DNA and subsequently were performed in quadruplicate on doubling dilutions. The lowest DNA dilution at which two or more of the four PCR reactions were positive was selected and the number of replicates at that dilution increased to 20.

The number of provirus gene copies was then estimated from the Poisson distribution where 63% positive reactions indicates an average of one copy per replicate. The external primers were 5′ CATGGTAC-CCAGCACAAGG 3′ (4149–4169) and 5′ TCTACTGGTCCATGC-ATGGCTTC 3′ (4370–4392) and the internal primers were 5′ GGAGGAAAATGAAACGATGAATAATTAGTCA 3′ (4174–4206) and 5′ TCTACTGGCCATTGCTCCATT 3′ (4280–4302). Nucleotide numbers are taken from the HXB2 sequence. The yield of purified DC was usually too low for accurate cell counts to be made with a counting chamber, and cell number was therefore estimated by nested PCR on limiting dilutions of DNA using primers for the single haploid copy gene β-globin. The cell number was calculated from the Poisson distribution on 20 replicate PCRs as described above. The external primers were 5′ GGTTGGCCAAATCTACTCCAGG 3′ and 5′ GTCACCTCAGTG-
Nucleotide sequencing. Single molecules of HIV-1 provirus were isolated by limiting dilution, and a region of the envelope gene that included the V3 loop was amplified by nested PCR (Donaldson et al., 1994). The external primers were 5’ CAGTCAATGATACATG-GAAT 3’ (6954–6975) and 5’ GATCCCATATGCTTCTGCTGCT 3’ (7795–7818). The internal primers were 5’ ATGCGAGTCTAGCAGAAAGA 3’ (7007–7027) and 5’ CTCTCAATTGCTCCCTACATA 3’ (7645–7665). The thermostable polymerase enzyme, Pfu polymerase (Stratagene), which exhibits proofreading properties, was used in the first-round PCR reactions to reduce the chances of PCR generated errors. Amplified DNA was purified by electrophoresis in a 1% agarose gel and extracted from the agarose using glassmilk (Geneclean; Bio 101). The nucleotide sequences were determined by the dideoxynucleotide chain termination technique using Sequenase version 2 (United States Biochemical), forward and reverse primers and [α-35S]S-ATP following the protocol for sequencing PCR products advocated by Winship (1989).

Results

A total of 17 patients was studied. Number of DC was estimated by flow cytometry on 13 of these patients and virus load was assessed in 14. For 10 of the patients estimates were made of both DC number and virus load. HIV-1 provirus was detected by PCR at a level of one or more copies per 10000 cells in purified DC in 13 of 14 patients studied. The highest virus load in DC was 320 provirus copies per 10000 cells (Table 1). DC were purified from LDC by panning with a cocktail of antibodies against T, B, NK and mononuclear cells to remove non-DC. This procedure removes about 98% of these contaminating cells (Patterson et al., 1995). It may be argued that provirus detected in the purified DC preparation reflects a high level of infection in the few lineage positive contaminating cells that were not removed by panning. It seems reasonable to expect that the level of infection in these contaminating cells would be similar to that in the cells removed by panning. The provirus load in the cells removed by panning was similar to the level in the DC preparation. Since these cells represented no more than 2% of the cells in the DC preparations, they could not account for the bulk of provirus detected in the purified DC.

The virus load in the CD3+ cell population, 90–95% pure after magnetic bead separation, was measured and the amount of virus in the T helper fraction estimated from the percentage of CD3+ cells labelling for CD4. Estimated virus load in CD4 lymphocytes ranged from 45 to 2200 provirus copies per 10000 cells. There was no correlation between the virus load in the DC and T helper cell population (r = 0.2, Fig. 1a). DNA from the blood of uninfected controls was negative for HIV-1 provirus.

DC number in PBMC was estimated by flow cytometry in 13 patients. Since there is no known membrane marker unique to human DC they were identified by the absence of markers specific for T, B, NK and mononuclear cells and by expression of

Table 1. Virus load in DC, CD4+ T cells and cocktail + ve cells

The number of HIV provirus copies per 2 × 10^4 copies of the β globin gene is shown. The number in parentheses is the estimated number of infected CD4 lymphocytes calculated from the number of CD4+ cells in the CD3+ population and assumes that CD8+ cells are not infected. Estimates were based on replicates of 20 except values indicated by an asterisk (*), which were based on doubling dilutions of replicates of 4.

<table>
<thead>
<tr>
<th>Patient</th>
<th>CD4/μl</th>
<th>CDC stage</th>
<th>T cells</th>
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<th>DC number (× 10^3/ml blood)</th>
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MHC DR antigen (Fig. 2). The number of DC in the non-adherent PBMC population was reduced for the patient group (Figs 2 and 3). The mean number of DC per ml of blood was $2.10 \times 10^3$ ($n = 13, SE \pm 0.58$) and $7.20 \times 10^3$ ($n = 22, SE \pm 1.32$) for patient and controls, respectively ($P < 0.01$). The reduced DC number did not merely reflect a fall in the mononuclear cell population (Fig. 3a) since the percentage of DC in the mononuclear fraction was also reduced (Fig. 3c). DC constituted $0.36\%$ ($n = 13, SE \pm 0.07$) of the mononuclear cell population in the patient group and $0.72\%$ ($n = 22, SE \pm 0.12$) in the control group ($P < 0.05$). There was a slight trend towards increased virus load in DC as DC number fell, but this was below the level of statistical significance ($r = 0.38$, Fig. 1b).

The nucleotide sequence of the HIV-1 envelope V3 loop and flanking regions was determined for three patients showing the greatest reduction in DC number (patients 3, 4 and 5), the sequence obtained starting some 23 amino acids before the amino cysteine (residue 24) of the V3 loop, and extending for 22 amino acids beyond the carboxy cysteine (residue 58), a total of 84 residues. Nine or ten clones were isolated by limiting dilution PCR and were compared with sequences in clones isolated from CD3+ cells. Inspection of the
deduced amino acid sequence suggested that for patients 4 and 5 there may be different populations of provirus in the DC and T cells (Fig. 4). The deduced amino acid sequences were aligned and phylogenetic dendrograms constructed by three alternative methods (neighbour-joining, maximum likelihood and parsimony; Felsenstein, 1989). Each method gave qualitatively similar results; the results for neighbour-joining are shown. Bootstrap re-sampling was then used to assess the probability of the branching order shown (Fig. 5). The phylogenetic analyses indicate that the sequences are unique to the patients from which they derive. Furthermore, despite the presence of several notable distinctions between the DC and CD3+ populations, the branching order of the overall dendrogram shows considerable intermingling between the two populations of sequences (Knight & Patterson, 1997). It was noted that there was a non-significant elevation in the mean pairwise distances between samples in the CD3+ groups in each patient compared to the corresponding DC group.

Signature pattern analysis was used to identify those residues which could distinguish between the DC and CD3+ populations (Korber & Myers, 1992; Grez et al., 1994). Given the observation from the phylogenetic analysis that the patient sequence sets were significantly different from one another, no attempt was made to assess an overall pattern across the entire group. For patient 3, only three positions in the data set were found (11, 67 and 77), and none of the imbalances between the DC and CD3+ patterns was significant (as assessed by Fisher’s exact test, corrected for repeated sampling using Sidak’s multiplicative inequality). A similar pattern was seen for patient 5, where only positions 34 and 82 were detected and neither position was in significant imbalance between the DC and CD3+ groups. However in patient 4 six positions were seen in which a discrepancy between DC and CD3+ groups was noted (positions 41, 62, 63, 64, 70 and 71). Of these, two positions, 41 and 62, showed highly significant departure from a random distribution. At position 41, six of ten sequences had a serine (S) in the DC group, the other four sequences being arginine (R), whereas all 10 of the sequences from the CD3+ group had an R codon (Fisher’s exact test, \( P = 0.0054 \); Sidak’s correction for six repeated samples, \( P = 0.008 \), implying significance at the 5% level). Similarly the CD3+ sequence set shows only two occurrences of lysine (K) at position 62, whereas the DC group is made up of 10 occurrences of the K residue (Fisher’s exact test, \( P = 0.0004 \). It is possible that the glutamine (Q) and lysine (K) polymorphism at position 63 may also be different between the two groups (three Q in DC, eight Q in CD3+) but the associated probability (0.0349) is higher than that required to satisfy the repeated sampling correction.

The presence of basic amino acids at positions 34 and 48 in the V3 loop is associated with variants showing an SI phenotype (Fouchier et al., 1992, 1995). None of the clones sequenced, whether from DC or T cells, had nucleotides encoding basic amino acids at these positions. In addition, there was no extensive deviation from the V3 loop consensus of North American isolates (LaRosa et al., 1990) as would be expected of SI isolates (Chesebro et al., 1992; Milich et al., 1993).

Discussion

Our investigation on HIV-1-infected symptomatic patients confirmed that DC are targets for HIV-1 infection in vivo, and for the first time provided evidence that virus variants in DC can differ from those in T cells. Several lines of evidence argue against the possibility that provirus detected in the purified DC preparations reflects contamination with T cells or monocytes. The panning procedure used to purify DC removes about 98% of contaminating T, B, NK and monocyctic cells from the DC-enriched LDC preparations (Patterson et al., 1995). Provirus load in the cells removed by panning was, in most cases, similar to the virus load in the purified DC. Thus the low level of contaminating cells in the purified DC preparations cannot account for the level of provirus in these cells. Other studies have shown only a very low level of monocyte infection in peripheral blood (Bagasra & Pomerantz, 1993; Innocenti et al., 1992; Schnittman et al., 1989) and, therefore, it is likely that most infected contaminating cells would be T cells. The finding that virus load in the DC fraction was independent of the number of provirus copies in the T cells also suggests that most viral DNA in the DC preparations is not derived from T cells. Finally, the sequencing studies show differences in the virus populations that infect DC and T cells. Such differences would not be observed if the bulk of the viral DNA detected in the purified DC originated from the T cell population.

The level of CD4 lymphocyte infection in symptomatic patients in different investigations ranges from less than 1% (Livingstone et al., 1996) to around 10% (Hsia & Spector, 1991; Jurriaans et al., 1992), and in one study of 17 CDC stage IV patients a mean infection level of 30% was observed (Bagasra et al., 1993). Some of these apparent discrepancies may reflect differences in patients and in the stage of disease. For most of the patients in this study estimates fell in the 0.5-10% range. These estimates were based on assays of DNA from purified CD3 cells and assume that CD4 rather than CD8 lymphocytes are infected. These estimates may require revision if the recent report of CD8 lymphocyte infection is confirmed (Livingstone et al., 1996). The possibility that some DC co-purified with T cells as DC–T-cell conjugates cannot be excluded. However, since CD4 T cells are thought to constitute the largest sub-population of infected cells in the blood, small numbers of contaminating DC are unlikely to alter significantly the estimated number of infected T cells, and furthermore the differences in virus nucleotide sequences in the two cell populations argue against the presence of large numbers of contaminating DC.

In earlier studies, overnight cultures of PBMC were centrifuged over a metrizamide gradient to separate small lymphocytes from the larger LDC which consist mainly of
monocytes and DC (Macatonia et al., 1990). DC in the LDC fraction were identified by their morphology in light microscopy and were found to be reduced in number in asymptomatic and symptomatic patients (Macatonia et al., 1990). It may be argued that morphological assessment is too subjective for quantitative studies, particularly when the cells constitute only a minor fraction of the mononuclear cell population, and that some DC may pellet into the lymphocyte

Fig. 4. Amino acid sequences of the HIV-1 envelope V3 loop and flanking regions from proviral clones isolated from DC and T cells. For each patient the combined DC and T-cell consensus sequence is shown.

Fig. 5. The phylogenetic tree shown is an unrooted dendrogram generated by the PHYLIP programs PROTDIST and NEIGHBOR (Felsenstein, 1989), the pairwise distances between protein sequences were computed using the Dayhoff PAM matrix to measure differences (Dayhoff, 1979). The assignment of the three major branches was highly significant when assessed by bootstrap re-sampling (PHYLIP programs SEQBOOT and CONSENSE). The percentage of resamplings which support the tree shown is indicated by the labels on the branches. Essentially identical results were obtained using the neighbour-joining algorithm of Saitou & Nei (1987) implemented in the CLUSTALW program (Thompson et al., 1994) which uses the Kimura approximation for estimation of protein differences (Kimura, 1983). The deletion found in four of the sequences (patient 3, sequences DC3, DC5, DC7 and DC9) led us to ignore this residue in the computations presented here; a second analysis in which these four sequences were omitted gave essentially identical results (data not shown).
fraction on the metrizamide gradient. Here we show the occurrence in symptomatic patients of a reduction in the numbers of DC identified by FACS analysis on overnight cultures of mononuclear blood cells not subjected to separation on metrizamide gradients. There is an overall loss of mononuclear cells which might suggest a non-specific loss of DC. However, there is a specific reduction in DC number, since the percentage of DC in the mononuclear fraction is also reduced. There are several possible mechanisms that could account for the loss of DC. There may be a direct virus cytopathic effect or cytotoxic T lymphocyte (CTL) killing of infected cells. CD34 + stem cells cultured in vitro with GM-CSF and TNF-α differentiate into DC (Caux et al., 1992; Reid et al., 1992). Our current studies show that after 24 h in culture these cells start to express CD4 and become susceptible to HIV-1 infection (J. Gilmour and others, unpublished). Infection does not appear to affect differentiation of DC, but may render them susceptible to CTL killing and thereby decrease the number of circulating DC. DC exposed to HIV-1 for 2–3 days become targets for specific CTL (S. E. Macatonia, B. A. Askonas & S. C. Knight, unpublished data).

The proportion of infected DC was less than that for T cells. However, this may not necessarily reflect the real rate of DC infection relative to other cell populations. In contrast to T cells, some of which are thought to have a long lifespan, the life of DC is relatively short, with estimates for the mouse ranging from 3 days to 4 weeks (Fossum, 1989; Salomon et al., 1994). Thus there would be little time for accumulation of infected DC. Although there is evidence for a rapid turnover of infected T cells (Ho et al., 1995; Perelson et al., 1996; Wei et al., 1995), latent or defective HIV-1 genomes may accumulate in the resting memory T cell population and partly account for the greater virus load in these cells. If, as a consequence of antigen presentation by infected DC, virus cycles through the DC and T cell compartments, then identical or nearly identical populations of virus in both cell types would be predicted. Phylogenetic analysis of the whole V3 region shows considerable intermingling between the two sequence populations; only in one case (patient 4) were there significant differences between the sequences in the DC and CD3 populations. The presence of amino acid substitutions unique to one or other cell type may indicate that transmission from an infected DC to a T cell, or vice versa, is an infrequent event, or that only certain variants can be efficiently transmitted between the two cells. Alternatively, it may be argued that we have predominantly sampled latently infected memory T cells rather than those T cells showing rapid turnover and which are thought to contain the actively replicating virus population. However, this second conclusion is not supported by the phylogenetic dendrogram (Fig. 5), since there is no evidence for compartmentalization of the sequences into clades according to tissue of origin. Analysis of virus in serum and of the cellular tropisms of the unique variants may explain these findings.

The selective transmission or expansion of subpopulations of virus shown to occur as infection is initiated (Roos et al., 1992; Zhang et al., 1993; Zhu et al., 1993) could reflect selective pressure exerted by the cells first exposed to the infectious inoculum. In monkeys infected intravaginally with simian immunodeficiency virus, Langerhans’ cells appear to be the first cells infected (Spira et al., 1996). Surprisingly, similar selective pressures appear to be exerted in haemophiliacs infected parenterally (Zhang et al., 1993). DC may also be responsible for selection of variants in this situation, since most circulating lymphocytes in healthy individuals are in a non-activated state and virus replication in these cells would be abortive. Furthermore, resting T cells do not express the CCR-5 receptor used by NSI variants (Granelli-Piperno et al., 1996). Therefore, DC may be one of a limited number of cell types able to support productive HIV-1 infection. For future vaccine design, it will be important to determine whether unique variants found in DC are preferentially transmitted. Since in vitro studies also suggest that DC are more susceptible to infection with NSI macrophage tropic virus than lymphotropic SI variants, we anticipated finding NSI variants in the DC and some SI variants in the T cells. Basic amino acids at positions 34 and 48 in the V3 loop, shown to be associated with SI variants (Fouchier et al., 1992, 1995), were not found in either cell population. It is possible that insufficient patients have been studied. However, similar investigations of a larger group of patients and a wider range of tissues also failed to detect SI variants (Donaldson et al., 1994). For two patients the level of variation in the amino acid sequence was greater in the DC cells than in the DC. This latter observation may reflect cumulative infection events.

The finding of HIV-1 infection in vitro in DC has at least two possible consequences for understanding the pathogenesis of AIDS. Firstly, infection and depletion of DC could lead to impaired antigen presentation and a failure to recruit naïve T cells into the recirculating memory pool. Secondly, infected DC could transmit virus to T cells as they form clusters round these cells during antigen presentation. Since productive infection of T cells is restricted to activated cells, DC may constitute an essential component in maintaining repeated cycles of T cell infection. However, as discussed above, the possible implications of the presence of unique virus variants in the two cell types must be borne in mind. The possibility of transmission of virus from the surface of an uninected DC also cannot be excluded (Cameron et al., 1992a, b). Provirus was barely detectable in the DC of a small number of symptomatic patients. This may reflect the high turnover of DC. Alternatively, resting DC are known to express macrophage inhibitory proteins MIP-1α, MIP-1β and RANTES (Zhou & Tedder, 1995) which could block infection via the co-receptor CCR-5, and thus variation in the levels of expression of these chemokines may explain differences in the number of DC infected with HIV-1 (Deng et al., 1996; Dragic et al., 1996). Future studies aimed at the further characterization of HIV-1
variants that infect DC and their effect on antigen presentation may facilitate vaccine development and therapeutic intervention.

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