Human immunodeficiency virus type 1 in faeces and serum: evidence against independently evolving subpopulations

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It is not known whether independent tissue-specific evolution accounts for the differences between human immunodeficiency virus type 1 (HIV-1) subpopulations in intestinal tissue and blood. To study this, sequential serum samples from three persons were analysed for the presence of HIV-1 V3 genotypes which were detected exclusively in faeces at a specific time-point. For two persons the faeces genotype was found in serum samples collected before the time of faeces collection: 7 months for one person and 32 months for the other person. In the third person, serum collected 1 month after faeces collection contained the faeces genotype in abundance. These data indicate that a difference between intestinal tissue and blood HIV-1 subpopulations is not the result of complete compartmentalization and independent HIV-1 evolution in intestinal tissue, but that it reflects an unequal distribution of HIV-1 in different tissues.

Cells infected with human immunodeficiency virus type 1 (HIV-1) have been detected in a wide variety of lymphoid and nonlymphoid tissues, and characterization of HIV-1 from different tissues suggests compartmentalization (Epstein et al., 1991; Barnett et al., 1991; Kuiken et al., 1995; Korber et al., 1994; Chiodi et al., 1989; Keys et al., 1993; Zhu et al., 1996; Overbaugh et al., 1996). Using intestinal biopsy material we have shown that in some patients the HIV-1 subpopulation in intestinal tissue differs from the HIV-1 subpopulation in serum (van der Hoek et al., 1996). Others have shown that HIV-1 isolates recovered from the bowel can differ in biological properties from isolates recovered from blood (Barnett et al., 1991). It has been suggested that a difference in HIV-1 subpopulations in various compartments is the result of independent tissue-specific evolution (Itescu et al., 1994; Wong et al., 1997). To determine whether this is the case for intestinal tissue, we studied HIV-1 in faeces and serum. Analysing faeces instead of intestinal biopsy material is legitimate because faeces contains HIV-1 sequences that are present in intestinal tissue (van der Hoek et al., 1996). We studied three persons for whom sequential serum samples were available and for whom we determined that faeces contained V3 genotypes not detectable in serum collected at the time of collection of faeces (van der Hoek et al., 1998).

For each of the three persons one faeces sample and sequential serum samples were collected. The serum sample collected at the same date as the faeces sample was labelled serum from month 0. The sequential serum samples were collected before and, for two persons, also after the faeces sample. The labelling of the serum samples indicates the time of serum collection in months relative to the time at which faeces was collected. The first serum sample from person 92 was collected within 2 years of seroconversion and for person 73 within 6 months of seroconversion. Person 03 was HIV-1 seropositive on enrolment in the study in 1984, but as the HIV-1 epidemic in Holland started in the early 1980s (van Haastrecht et al., 1992; Lukashov et al., 1996), it was suspected that collection of sera was started not long after seroconversion. Persons 03 and 73 were infected with HIV-1 by homosexual contact and participated in the Amsterdam Cohort Study of homosexual males. Person 92 is a haemophiliac infected by HIV-1-contaminated blood products. All three persons had developed AIDS approximately 20 to 30 months before the time of faeces collection.

HIV-1 RNA was isolated from 50 µl faeces-suspension and 100 µl serum and the V3-encoding region was amplified by RT–PCR as described (van der Hoek et al., 1998). In each person different clusters of virus variants (genotypes) could be identified (van der Hoek et al., 1998) and the nucleotide distances between these genotypes were greater than 0·035. The distribution of genotypes in faeces and serum at month 0 is depicted in Fig. 1. The sequential serum samples were examined for the presence of the V3 genotypes which were at one time exclusively present in faeces (genotypes which were

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Fig. 1. Deduced amino acid sequences of the V3 genotypes in faeces and serum. The consensus sequences of the genotypes are aligned against the consensus sequence of HIV-1 subtype B (CB). The distribution of the genotypes in faeces and serum is given at the far right of the sequence. Dashes indicate identity with the reference sequence, dots indicate deletions. Amino acid numbering in gp120 is indicated at the top of the figure.

assigned genotype A sequences) by Quantitative Homoduplex Tracking Assay (QHTA) (Zhu et al., 1996). By this method, a $^{32}$P-labelled single-stranded PCR product of the faeces-V3 genotype A was hybridized to unlabelled V3 PCR product obtained from the sequential serum samples, to form radio-labelled duplexes. These duplexes were subsequently electrophoresed on a polyacrylamide gel and separated on the basis of the number of mismatched nucleotides, because mismatches between the annealed strands result in a heteroduplex of a slower mobility (Delwart et al., 1993). Duplexes with a mobility similar to the control homoduplex (i.e. the probe annealed to the PCR product used to prepare the probe) represent variants that are thought to belong to the same genotype as the probe, and were categorized as homoduplex-like duplexes.

In Fig. 2, the QHTA analysis is shown for the three patients. The QHTA analysis confirmed our observation that at the time of faeces sampling, genotype A sequences were not present in serum, because homoduplex-like duplexes were not seen in lanes 0 of Fig. 2(A), the lanes representing sera collected at the same date as faeces. Performing QHTA analysis of the PCR products obtained from faeces confirmed our finding that for person 92 only genotype 92A sequences were present in faeces, for person 03 only genotype 03A and 03B, and for person 73 only genotype 73A and 73B (Fig. 2B). For person 03, at month 0, a majority of the clones displayed genotype 03C sequences in serum. However, the QHTA analysis of this serum sample indicated that an extensive proportion of the heteroduplexes are formed with genotype 03B sequences, and heteroduplexes with genotype 03C sequences are hardly seen. It might be that if, as is the case in our system, the probe is not present in excess during hybridization, competition for binding to the probe occurs. As genotype 03C sequences exhibit more nucleotide differences to the genotype 03A sequences in comparison to the genotype 03B sequences (13 ± 2% and 3 ± 5% differences, respectively), it may be that hybridization to genotype 03B sequences by the genotype 03A probe is largely favoured when a mixture of 03B and 03C sequences is offered. This inadequate heteroduplex formation can have consequences in a study which evaluates the appearance of variants other than the variants.
Fig. 2 For legend see facing page.
Fig. 3. Phylogenetic analysis of the sequences from sequential serum samples and the faeces specimen. Phylogenetic analysis was performed using the neighbour-joining method (Kumar et al., 1993). ‘−XX’ or ‘+XX’ indicate the time of serum collection in months relative to the time at which faeces was collected. Consensus sequences of sequence clusters were used in the analysis, except for individual sequences that did not cluster near the other sequences from the same serum sample (indicated by ‘clone’). ‘I’ or ‘II’ indicate whether the variant was detected as a majority or a minority in the serum sample. For serum samples −32 (73), −1 (92), −13 (92) and −3 (03), sequences of non-genotype A origin were not included in the phylogenetic analysis. The bootstrap values of 100 replications are given at the root of the cluster. Sequences from all three patients were analysed together; therefore, the sequences from each patient served as outgroups for the others. Phylogenetic clusters with double lines indicate sequences that include the faeces-specific genotype A sequences. The numerical codes for the persons are given in parentheses.

used to prepare the probe. However, we used the QHTA technique with a faeces-specific genotype A probe to track for genotype A sequences in the sequential serum samples; therefore, inadequate heteroduplex formation would not have had any influence in our study.

In our search for serum samples that did contain genotype A sequences, we selected those sera displaying homoduplex-like duplexes for cloning and sequencing of the V3 region. Phylogenetic analysis (van der Hoek et al., 1998) was performed to determine whether the sequences of the clones clustered with genotype A sequences.

For person 92, the serum samples collected at −13 months and +1 month (i.e. 13 months before and 1 month after faeces collection) displayed homoduplex-like duplexes. One of eleven clones from the −13 sample clustered with the genotype 92A sequence (−13: clone 92-39, Fig. 3). Of the +1 serum sample, six of fourteen clones clustered with the genotype 92A sequence (consensus sequence: +1:II, Fig. 3). For person 03, the PCR products from the −7 serum sample showed extensive homoduplex formation, and eight of thirteen clones clustered with genotype 03A (consensus sequence: −7:I, Fig. 3). For person 73, from the first available serum sample onward, a heteroduplex was seen with a mobility similar to the homoduplex. Sequencing clones from the first two serum samples revealed that the sequence with a mobility similar to the homoduplex (sequence −93:clone73-16) did not cluster with the genotype 73A sequences (Fig. 3) and showed extensive differences from genotype 73A (nucleotide distance 0.065). Since the mobility of this heteroduplex made it difficult to pinpoint a serum sample likely to contain genotype 73A sequences, direct sequencing of the PCR products of the sera was performed (data not shown). Based on the QHTA and the direct sequences, the −32 serum sample was selected for cloning and sequencing. Five of eleven clones clustered with genotype 73A (consensus sequence −32:I, Fig. 3).

The appearance of the same genotype at one time-point in faeces and at another time-point in serum suggests that HIV-1 passed between intestinal tissue and blood. Passing of virus between blood and the central nervous system has also been described (Korber et al., 1994). Recently, a study was published concerning the evolutionary dynamics of HIV-1 in various tissues (Hughes et al., 1997). In that study, calculations were performed which used the diversity at synonymous nucleotide positions to determine the moment of spread of HIV-1 to a tissue. Such a study can only be performed when new variants are not permanently introduced into a compartment after the initial introduction of HIV-1 into that compartment. Based on our findings, we suggest that the heterogeneity of a subpopulation cannot be used to determine the moment of HIV-1 to intestinal tissue. For instance, HIV-1 might have been in intestinal tissue for a long time, but a newly introduced variant may (1) replace the original subpopulation in this compartment.
or (2) replicate in this compartment together with the variants of the older population. In the former case the time of HIV-1 spread to intestinal tissue is underestimated; in the latter case the time of HIV-1 spread is overestimated.

Previous studies suggest that independent evolution is operational in lung and brain (Itescu et al., 1994; Wong et al., 1997). These conclusions were based on phylogenetic analysis of HIV-1 sequences in tissue samples that were collected at one time-point. Similar to those studies, we noted large differences between faeces- and serum-specific genotypes at a given time-point, but by studying sequential serum samples we concluded that, in fact, the faeces-specificity of a genotype is lost at other time-points. Therefore, we conclude that independent evolution of HIV-1 within intestinal tissue does not account for the difference observed at a given time between HIV-1 subpopulations in faeces and serum.

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