Human immunodeficiency virus type 1 RNA populations in faeces with higher homology to intestinal populations than to blood populations

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To determine whether human immunodeficiency virus type 1 (HIV-1) in faeces is representative of the HIV-1 population in intestinal tissue, we studied HIV-1 V3 variation in faeces, intestinal biopsies and serum from two individuals. Phylogenic analysis of HIV-1 V3-coding RNA in faeces from one individual showed three distinct genotypes. Viruses belonging to all three genotypes were also present in sigmoidal tissue and in serum. Jejunal tissue contained two of these three genotypes. Analysis of the V3-coding RNA in faeces of the other individual showed five distinct genotypes. One of these genotypes was present in all specimens from this individual. Besides this shared genotype, jejunal tissue and serum contained sequences belonging to one other genotype. In addition, one of the other three V3 variants was detected in sigmoidal tissue. For both persons the shared HIV-1 RNA genotypes in faeces and serum displayed a distinctly different frequency distribution. In one individual, the genotype which was detected in a majority of the clones in faeces (59%) and as a minority in serum (11%), was the most abundant genotype in jejunal and sigmoidal tissue (61% and 80%, respectively). For the other individual the genotype that was present in faeces in a significant number of clones (43%) was detected in serum as a minority (8%), whereas this genotype composed 47% of the clones isolated from jejunal tissue. Taken together these data suggest that faeces contain HIV-1 sequences that are derived from local HIV-1 replication in intestinal tissue.

Introduction

The gastrointestinal tract is one of the organs most affected in human immunodeficiency virus (HIV)-infected persons (Dworkin et al., 1985; Gazzard, 1992; Gillin et al., 1985; Griffin, 1990; Kotler et al., 1984). Gastrointestinal infections by a range of pathogens are often identified in HIV-infected patients (Laughon et al., 1988). In addition diarrhea of unknown aetiology has been found in many HIV-infected patients (Bartlett et al., 1992; Riecken et al., 1990) and can be explained by direct cytopathogenic effects of HIV or by a local immune disorder caused by HIV (Batman et al., 1989; Kotler et al., 1993; Rodgers et al., 1986; Ullrich et al., 1989, 1990; Clayton et al., 1992). In vivo infection of intestinal mucosal cells by HIV has been identified by immunohistochemistry, in situ hybridization and PCR (Kotler et al., 1991; Heise et al., 1991; Jarry et al., 1990; Nelson et al., 1988; Fox et al., 1989). HIV-infected cells in the intestinal mucosa have been identified as lymphocytes, macrophages, dendritic reticulum cells and epithelial cells (Mathijs et al., 1988; Nelson et al., 1988; Levy et al., 1989; Fox et al., 1989; Heise et al., 1991; Jarry et al., 1990). Characterization of HIV-1 isolates recovered from bowel biopsies showed that these strains can differ in biological properties from HIV-1 strains recovered from serum of the same patient (Barnett et al., 1991). This finding indicates a compartmentalization of HIV-1 in the intestinal tract.

Intestinal mucosa contains the largest pool of lymphoid cells, which recirculate from the blood into the intestinal mucosa guided by specific homing receptors (reviewed by Yednock & Rosen, 1989). The intestinal mucosa is most likely the portal of entry for HIV in homosexual and bisexual males.
For the development of an HIV vaccine it would be useful to determine the characteristics of the HIV variants which replicate in the intestinal mucosa.

Recently we have reported a reliable and rapid procedure for the detection of HIV-1 RNA in faeces (van der Hoek et al., 1995). This study showed that the HIV-1 populations in faeces and serum may differ. The difference between the populations might be the result of virus replication in cells present in the intestinal mucosa. Faeces may therefore be a suitable specimen to determine the characteristics of the HIV-1 variants which replicate in the intestinal tract, without the need for invasive sampling techniques. However, it has to be established whether the HIV-1 variants detected in faeces are indeed variants which originate from replication within the intestinal tract.

This study describes the characterization of HIV-1 populations in faeces, intestinal tissue and serum, from two subjects selected for having distinct HIV-1 populations in faeces and serum.

Methods

Subject. Serum and intestinal biopsy specimens were obtained from the faeces of eight HIV-infected persons. From four persons HIV-1 nucleic acids could not be amplified from faecal or intestinal tissue, or the detection of an HIV-1 population was not reproducible. Of the four remaining persons, two were selected for a detailed study on the HIV-1 population in the different specimens. This selection was based on the detection of different HIV-1 populations in faeces and serum, monitored by direct sequencing of amplified HIV-1 V3 sequences. These two subjects have been described previously (individuals F and N; van der Hoek et al., 1995). One person (individual F) was a male infected by homosexual contact, while the other person (individual N) was a woman who became infected by heterosexual contact with an intravenous drug user. Individual N was diagnosed as having CDC class IV C1 HIV infection and individual F as CDC class II HIV infection (Centers for Disease Control, 1987). Both persons suffered from diarrhoea at the time of sampling, a cryptosporidial infection was identified in individual N, and a gastrointestinal infection by adenovirus in individual F. The search for HIV-1 nucleic acids in the different specimens was performed with informed consent of all subjects.

Patient specimens. Biopsy specimens of about 8 mm² were obtained from jejunum and sigmoid tissue using a colonoscope. Jejunoscopy was performed under sedation and biopsies were obtained under fluoroscopy. Biopsies were collected in vials containing PBS, washed twice in 1 ml PBS, separated into two or three portions of about 3 mm³ and stored in 1 ml lysis buffer L₄ (Boom et al., 1990) at -20 °C. The jejunal biopsies of individual F were microscopically normal, while in sigmoid tissue a moderate increase in the number of lymphocytes was observed. For individual N, the jejunum showed an increased number of lymphocytes, and the sigmoid showed a moderate increase in number of lymphocytes together with mild fibrosis. In both the jejunum and the sigmoid of individual N cryptosporidiosis was detected.

Faeces were collected the day prior to the endoscopy. About 1.5 g faeces was suspended in 3 ml broth (containing nutrient broth no. 2 supplied by Oxoid, 500 IE/ml penicillin, 500 mg/ml streptomycin and 3 mg/ml amphotericin B). Faeces suspension (100 µl) was added to 1 ml lysis buffer L₄ homogenized by vortexing and stored at -20 °C. Serum was collected on the day of the endoscopy and stored at 70 °C.

Extraction of nucleic acids from serum, faeces and biopsy material. Isolation of nucleic acids from serum specimens was performed by protocol Y/SC, as described in detail by Boom et al. (1990, 1991). Nucleic acids were extracted from faeces—broth mixtures or intestinal biopsies by protocol F/SC, which is an adaptation of protocol Y/SC for
Fig. 2. Phylogenetic analysis by the neighbour-joining method of the clones from faeces of individual F. Codes refer to the clonal sequences as shown in Fig. 1. The bootstrap values of the genotypes are given at the root of the cluster.

Faeces and has been described recently (van der Hoek et al., 1995). In each experiment negative and positive extraction controls were included. As a positive extraction control, 10⁶ HIV-1 RNA molecules isolated from a virus culture were used (Layne et al., 1992).

- **RT-PCR and DNA PCR.** Reverse transcription (RT) and PCR amplification of the V3-coding region with primers L10 (RT), L9 and 5'V3NOT in the first PCR and primers 3'V3NOT and 5'KSI in the nested PCR was performed as previously described (van der Hoek et al., 1995). DNA PCR was performed as for RT-PCR but without the addition of reverse transcriptase (van der Hoek et al., 1995).

- **Cloning and sequencing.** For direct sequencing or cloning of the PCR products, 10 µl of nested PCR product was purified from agarose gel slices as described previously (van der Hoek et al., 1995). Purified DNA was eluted in either 15 µl H₂O (for direct sequencing) or in 6 µl H₂O (for cloning). Direct sequencing of 6 µl purified PCR product was performed using Sequenase 2.0 (USB) as described previously (van der Hoek et al., 1995). Two microlitres of the purified PCR fragments was cloned using a TA cloning kit (Invitrogen) according to the manufacturer's recommendations. Screening for clones containing a plasmid with an insert was performed by PCR. Colonies were suspended in 75 µl of brain heart infusion medium (Life Technologies) and 2.5 µl of this suspension was used as input in a PCR of 25 cycles, using a reaction mixture containing 25 ng of SP6 primer (5' GATTAGGTTGACACTATAG 3') and 25 ng of T7 primer (5' TAATACGACTCACTATAGGG 3'), 2.7 mM-MgCl₂, 0.5 U Taq polymerase (Perkin-Elmer Cetus), 50 mM-KCl, 0.2 mM each dNTP and 0.1 mg BSA (Boehringer) in a total volume of 25 µl. PCR products were analysed by agarose gel electrophoresis, and PCR products of the expected size were used for sequencing with dye-labelled Sp6 and T7 primers (Taq dye primer cycle sequencing kit; Perkin Elmer/Applied Biosystems) according to the manufacturer's protocol. The products were analysed on an automatic sequencer (Applied Biosystems). Seven to 12 clones were sequenced for each PCR product.

- **Detection of HIV-1 nucleic acids in faeces, intestinal tissue and serum.** HIV-1 nucleic acids could be detected in faeces, intestinal tissue and serum of individuals F and N. For faeces and serum, the V3 RT-PCR was positive, whereas a V3 PCR without a reverse transcription reaction was negative, indicating that the amplified product in the RT-PCR originated from HIV-1 RNA. For jejunal and sigmoidal tissue the V3 PCR without reverse transcription (DNA PCR) was positive, so these specimens contained HIV-1 DNA. For this reason, HIV-1 RNA and DNA, which are both amplified by RT-PCR from intestinal tissue, are referred to as total HIV-1 nucleic acids.

- **Reproducibility of amplification from the patient specimens.** In order to examine the possibility that differences in HIV-1 populations could be due to sampling errors, we determined whether cloning could be performed using the product from a single PCR reaction, or whether cloning should be performed on more than one PCR amplification (obtained from independent extractions). Consequently, the extraction, amplification and sequencing of the PCR products from the different specimens and direct sequencing of the PCR product was performed at least twice. From the specimens, direct sequencing of which showed matching sequences (less than 2% of nucleotide positions showing differences), one or two PCR products were used for analysing
**Table 1. Frequency of the different genotype V3 regions in the patient materials of individual F**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Individual F</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>Unassigned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces RNA (n = 27)</td>
<td>16 (59%)*</td>
<td>6 (22%)*</td>
<td>5 (19%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jej RNA + DNA (n = 28)</td>
<td>17 (61%)</td>
<td>0</td>
<td>5 (18%)</td>
<td>4 (14%)</td>
<td>1 (3.5%)</td>
<td>0</td>
<td>1 (3.5%)†</td>
<td>0</td>
</tr>
<tr>
<td>Jej DNA (n = 19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (11%)</td>
<td>12 (63%)</td>
<td>5 (26%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sig RNA + DNA (n = 10)</td>
<td>8 (80%)</td>
<td>2 (20%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sig DNA (n = 12)</td>
<td>6 (50%)</td>
<td>3 (25%)</td>
<td>3 (25%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum RNA (n = 9)</td>
<td>1 (11%)</td>
<td>6 (67%)</td>
<td>1 (11%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (11%)†</td>
<td>0</td>
</tr>
</tbody>
</table>

* P = 0.011 compared with the frequency distribution of these genotypes in serum.
† Variants which cluster outside the clusters of genotype F1 to F6, shown in Fig. 3.
Fig. 4. For legend see facing page.
clones. The PCR products were not pooled, but clones were sequenced from the independently obtained PCR products. In those cases where the independent extractions did not show the same extent of heterogeneity (3–10% of nucleotide positions showed differences), clones were sequenced from two or three PCR products, without pooling of the PCR products.

**Sequence analysis.** By sequencing the individual clones, a 303–318 bp fragment (HIV-1\textsubscript{gag} nucleotide positions 7060–7366; Myers et al., 1990), including the V3-coding region of gp120, was analysed. The nucleotide consensus sequences from the clones from faeces were determined by taking at every nucleotide position the nucleotide which was most frequent. At positions which displayed a 50% distribution, one of the two nucleotides was randomly chosen. Phylogenetic analysis was carried out using the PHYCLIP package (Felsenstein, 1993) and the neighbour-joining method of the MEGA program (Kumar et al., 1993). The nucleotide distance matrix input for the neighbour-joining program was generated by Kimura’s two-parameter estimation (Kimura, 1980). Bootstrap resampling (100 replications) was employed to place approximate confidence limits on individual branches. P values were determined using a two-tailed Fisher’s exact test.

**Results**

**Sequences of the V3 clones from individual F**

A total of 105 clones were sequenced from the different specimens from individual F. None of the sequences contained inactivating frameshifts or stop codons. In addition to nucleotide substitutions resulting in silent and non-silent mutations, length polymorphism was observed in the V3 loop (deduced amino acids are shown in Fig. 1).

Phylogenetic analysis of the clones derived from faeces showed three distinct genotypes of V3, with bootstrap values of 100, 99 and 97 of 100 replications (genotype F1, F2 and F3, shown in Figs 1 and 2). Sequences with high similarity to these three genotypes were detected in serum and sigmoidal tissue (shown in Figs 1 and 3), whereas two of these three genotypes were present in the jejunal HIV-1 nucleic acids (genotype F1 and F3, shown in Figs 1 and 3). The HIV-1 DNA isolated from the jejunal tissue did not contain HIV-1 nucleic acids F1, F2 and F3, but instead three other genotypes were detected (F4, F5 and F6, shown in Figs 1 and 3).

Genotype F1, the main variant in faeces (16 of 27 clones) was also the main variant in the sigmoidal tissue (6 of 12 clones in the HIV-1 DNA population and 8 of 10 clones obtained from total HIV-1 nucleic acids, shown in Table 1). In addition the HIV-1 nucleic acids of the jejunal biopsy also contained the genotype F1 as a majority (17 of 28 clones). In serum, a genotype F1 sequence was only detected in 1 of 9 clones (Table 1). Genotype F2, the main variant in serum (6 of 9 clones) was present in 6 of 27 clones from faeces. This variant was not detected in jejunal biopsy, and was detected in the sigmoidal biopsy as a minority (2 of 10 clones of HIV-1 DNA and 3 of 12 clones of the total HIV-1 nucleic acids of sigmoidal biopsy). Genotype F3 was detected as a minority in faeces, intestinal tissue and in serum (shown in Fig. 1).

So, the majority V3 variant in faeces (genotype F1), differed from the majority genotype of V3 sequence in serum (genotype F2). The distribution of the genotypes F1 and F2 in faeces and serum was significantly different ($P = 0.011$; Table 1).

Although the genotypes obtained from individual F displayed a high degree of diversity, phylogenetic analysis of the sequences from individual F clustered tightly together with a bootstrap value of 99 when compared with several outgroups (sequences of individual N and the consensus sequence of subtype A, B, C, D and E; data not shown).

**Sequences of the V3 clones of individual N**

Inactivating frameshifts were not observed in any of the total 81 clones which were sequenced of individual N. However, in three clones a stop codon was present, indicating a non-functional virus, or being the result of mutations introduced by amplification with Taq polymerase (deduced amino acid sequences are shown in Fig. 4). Substantial V3 sequence heterogeneity was observed, which was most striking in the clones from faeces (Fig. 4).

Phylogenetic analysis revealed that in faeces probably five distinct genotypes were present (genotype N1 to N5, shown in Fig. 5). Bootstrap values of genotype N1, N2 and N3 were respectively 94, 100 and 100 for 100 replications. Sequences with similarity to two genotypes were present in serum (genotype N1 and N2; Figs 4 and 6). N1 and N2 genotype V3 sequences were also detected in the jejunal biopsy HIV-1 nucleic acids (Figs 4 and 6). HIV-1 DNA in the jejunal biopsy did not exhibit the N1 genotype, indicating that the N1 genotype probably originated from HIV-1 RNA in virions. In the sigmoidal intestinal tissue, variant N1 was not detected (Fig. 4). Besides genotype N2, N3 genotype sequences were detected in the sigmoidal biopsy nucleic acids (Figs 4 and 6).

The majority sequence in faeces was genotype N1. This genotype was present in 10 of 23 clones (shown in Table 2). Genotype N1 was detected in serum in 1 of 12 clones. In jejunal tissue 9 of 19 clones contained the N1 genotype, whereas this genotype was not present in any of the clones.

![Fig. 4. Deduced amino acid sequences of the V3 region of the clones of individual N. The consensus sequence of the clones from faeces is shown (FNcons), the sequences of the clones from the different patient specimens are aligned against the FNcons sequence. Sources of the sequences are indicated as described in the legend of Fig. 1. The genotype of the sequence is shown at the end of the clonal sequence. The V3 loop is separated, dashes indicate identity with the reference sequence, dots indicate deletions, stars indicate stop codons and amino acid positions involved in SI capacity are marked (1). Numbering is according to the nucleotide numbering of HIV-1\textsubscript{gag} (Myers et al., 1990).](image-url)
obtained from sigmoidal tissue. The majority genotype in serum, genotype N2, was detected in 11 of 12 clones (Table 2). This dominant serum variant was also present with a relatively high frequency in faeces (8 of 23 clones). In the tissue of the sigmoid, genotype N2 was detected in most of the clones (7 of 7 clones of HIV-1 DNA and 8 of 11 clones of the total HIV-1 nucleic acids obtained from sigmoidal tissue). HIV-1 DNA extracted from jejunal tissue also consisted of genotype N2 (9 of 9 clones), whereas total HIV-1 nucleic acids extracted from the jejunal tissue contained genotype N2 in 10 of 19 clones.

Although the genotype N2, which was present in a majority of the clones in serum, was also present at a relatively high frequency in faeces, the distribution of genotypes N1 and N2 was significantly different for faeces and serum ($P = 0.018$). Besides faeces, the only material in which V3 genotypes N1 and N2 were detected with high frequency was the jejunal material, when total HIV-1 nucleic acids were amplified.

The high diversity of sequences observed in individual N was not likely to be due to contamination with a laboratory strain or sequences from another person, since the sequences of the different genotypes of one patient clustered together (bootstrap value 100) when compared with several outgroups (data not shown).

The V3 genotypes N1 to N5 displayed amino acid differences which indicate a difference in phenotype of the corresponding viruses. Genotype N2 displayed positively charged amino acids at positions 11 and 25 within the V3 loop (Fig. 4), a characteristic highly correlated with syncytium-inducing (SI) capacity in culture. In serum this SI variant N2 was the majority V3 variant, and in all specimens from individual N, V3 variant N2 could be detected. Although variant N2 was present in faeces, the majority of the sequences did not display an SI phenotype (genotypes N1, N3, N4 and N5; Fig. 4 and Table 2).
Table 2. Frequency of the different genotype V3 regions in the patient materials of individual N

<table>
<thead>
<tr>
<th>Individual N</th>
<th>Genotype</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces RNA (n = 23)</td>
<td></td>
<td>10 (43%)*</td>
<td>8 (35%)*</td>
<td>2 (9%)</td>
<td>2 (9%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Jej RNA + DNA (n = 19)</td>
<td></td>
<td>9 (47%)</td>
<td>10 (53%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jej DNA (n = 9)</td>
<td></td>
<td>0</td>
<td>9 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sig RNA + DNA (n = 11)</td>
<td></td>
<td>0</td>
<td>8 (77%)</td>
<td>0</td>
<td>0</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>Sig DNA (n = 7)</td>
<td></td>
<td>0</td>
<td>7 (100%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serum RNA (n = 12)</td>
<td></td>
<td>1 (8%)</td>
<td>11 (92%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* P = 0.018 compared with the frequency distribution of these genotypes in serum.

Discussion

HIV-1 V3 variation in faeces, intestinal biopsies and serum from two individuals was studied. For both persons the distribution of HIV-1 RNA genotypes in faeces and serum was different. In one individual, the genotype which was detected in a majority of the clones in faeces (59%) and as a minority in serum (11%), was present in jejunal and sigmoidal tissue in the bulk of the clones (respectively 61% and 80%). For the other individual the genotype which was present in faeces in a significant number of clones (43%) was detected in serum as a minority (8%), whereas this genotype composed 47% of the clones isolated from jejunal tissue. As discussed earlier, the detection of HIV-1 RNA in faeces indicates the presence of HIV-1 virions and not HIV-1-infected cells (van der Hoek et al., 1995). The results presented here suggest that faeces contain sequences that are most probably the result of HIV-1 replication in the intestinal tract. Therefore faeces seem to be a suitable specimen for the study of HIV-1 variants that are present in intestinal mucosa, obviating the need for invasive sampling techniques.

In faeces of individual N the HIV-1 population showed a high degree of diversity, which was not observed in any of the other patients’ specimens. HIV-1 variants detected in faeces can theoretically be produced and excreted at different locations of the intestinal tract. It might be that variant N1 in
faeces was produced in the jejunum and that variant N5 originates from the sigmoid.

In faeces of individual F, genotypes F4, F5 and F6 were not present. These variants were exclusively found in jejunal tissue, and as these variants were most dominantly detected in the HIV-1 DNA of the jejunal biopsy, it is most likely that these genotypes are present in HIV-infected cells (proviral DNA). It is unlikely that faeces from which HIV-1 RNA is amplified would contain genotypes F4, F5 and F6.

Previously, a non-homogeneous distribution of HIV-1 proviral sequences in the spleen has been described (Delassus et al., 1992). Like the spleen, the intestine is a lymphoid organ. Along the intestinal tract, a difference in the distribution of sequences was also observed in this study, exemplified by the differences in the HIV-1 populations in jejunal and sigmoidal intestinal biopsies. However we did not observe differences in HIV-1 populations within the jejunum. Extraction, amplification by RT-PCR and sequencing of HIV-1 from biopsy material which was taken from two different sites of the jejunum of individual N showed the same direct sequence of the PCR products (data not shown).

One patient harboured SI- and non-SI-(NSI) characteristic V3 regions in the jejunal and sigmoidal biopsies (individual N). The genotype with SI features (genotype N2) was predominantly present in HIV-1 DNA of the intestinal biopsy specimens, whereas the sequences obtained from the total HIV-1 nucleic acids of the intestinal biopsies contained also the NSI characteristics (genotype N1 for jejunal biopsy and genotype N5 for the sigmoidal biopsy). Besides N1 and N5, genotypes N3 and N4 from faeces also displayed the NSI phenotype. These four V3 variants with NSI characteristics represented the majority of the HIV-1 sequences in faeces. These findings suggest that the cells of the intestinal mucosa that produce HIV-1 are different from the cells producing virus in the blood. These NSI variants of individual N could well be replicating in macrophages (Hwang et al., 1991; Westervelt et al., 1992; Chesebro et al., 1992; Shiota et al., 1992), which are present in the intestinal mucosa. Macrophage-tropic HIV-1 variants are also the viruses most frequently detected early in the HIV-1 infection (van’t Wout et al., 1994).

With regard to the transmission of HIV-1 via the intestinal mucosa, those HIV-1 genotypes that replicate most efficiently in the intestinal mucosa are of enormous interest. Knowledge of these features is of importance to the development of a vaccine which is meant to reduce transmission via the intestinal mucosa. More extensive studies on the comparison between HIV-1 variants in faeces and serum in a greater number of patients are required to determine whether there are certain common features in the HIV-1 RNA variants present in the intestinal mucosa.

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References


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