Greater diversity of HIV DNA variants in the rectum compared to variants in the blood in patients without HAART

Véronique Avettand-Fènoël, Laurent Hocqueloux, Michaela Muller-Trutwin, Thierry Prazuck, Adeline Mélard, Marie-Laure Chaix, Eric Agoute, Christophe Michau, Christine Rouzioux

► To cite this version:

Véronique Avettand-Fènoël, Laurent Hocqueloux, Michaela Muller-Trutwin, Thierry Prazuck, Adeline Mélard, et al.. Greater diversity of HIV DNA variants in the rectum compared to variants in the blood in patients without HAART. *Journal of Medical Virology*, 2011, 83 (9), pp.1499. 10.1002/jmv.22132 . hal-00657572

HAL Id: hal-00657572

<https://hal.science/hal-00657572>

Submitted on 7 Jan 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Greater diversity of HIV DNA variants in the rectum
compared to variants in the blood in patients without
HAART**

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-10-2182.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	08-Apr-2011
Complete List of Authors:	Avettand-Fènoël, Véronique; Hôpital Necker, Laboratoire de Virologie Hocqueloux, Laurent Muller-trutwin, Michaela Prazuck, Thierry Mélard, Adeline Chaix, Marie-Laure; Necker Hospital Agoute, Eric Michau, Christophe Rouzioux, Christine
Keywords:	HIV reservoir, gut-associated lymphoid tissue, rectum, phylogeny, diversity

SCHOLARONE™
Manuscripts

Table 1. Virologic, immunologic and clinical characteristics of patients

		Zenith CD4					CD4+ cell count		HIV DNA, Log		
		Duration	HIV	T					cp/million cells		
		Stage of	of	RNA	cell	Treatment	Blood	Rectum	Plasma		
Pt	Sex	infection	infection,	Log ₁₀	nadir	(tritherapy)	/mm ³	/million	HIV RNA,	PBMCs	rectum
		months	copies	cells			cells		log cp/mL		
				/mL	/mm ³						
A	M	PI	3 *	5.8	267	naïve	322	NA	5.8	3.3	3.3
B	M	PI	1 *	5.2	729	naïve	729	NA	5.1	3.6	4.0
			3.5 *	5.2	729	for 2.5 months	850	NA	<1.7	3.2	3.9
C	M	CI	22	5.7	261	naïve	469	20770	4.6	3.3	3.0
			37	5.7	251	naïve	251	NA	5.7	3.6	3.5
D	M	CI	117	4.9	377	no	425	7310	4.3	3.7	4.0
E	M	CI	122	4.4	105	for 4 years	270	NA	<1.7	3.5	2.8
F	F	CI	64	5.3	222	for 3 years	426	32890	<1.7	3.5	2.8
G	F	AIDS	120	6.0	15	no	15	5600	6.0	3.8	3.3

M: male, F: female, PI: primary infection, CI: chronic infection, PBMCs: peripheral blood mononuclear cells, cp: copy

no: no treatment

NA: not available

* after onset of acute retroviral illness

Fig. 1.

Distribution of pairwise distances among rectal HIV-DNA variants (Fig. 1a) and PBMC HIV-DNA variants (Fig. 1b). These boxplots represent the averages of comparisons of pairwise genetic distances from each clone derived from rectal HIV-DNA (Fig. 1a) or from PBMC HIV-DNA (Fig. 1b) to every other clone from the same tissue/fluid. Distances were computed by using the Kimura model and are displayed as 100 times the raw distance score. The horizontal lines show the median and the boxes comprise the first to third quartiles of calculated average genetic distances. A, B, C, D, E, F and G

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

concern viral clones from patient A, B, C, D, E, F and G, respectively. Significant differences between pairwise rectum HIV-DNA distances and pairwise PBMC HIV-DNA distances were found for each patient ($p<0.01$, Mann-Whitney test), except for patients under treatment (B: 2nd set of samples, E and F).

PI: primary infection, CI: chronic infection

Patients A, B (1st sample), C are naïve to HAART; Patients D and G had received HAART but, at the time of sample collection, were not receiving treatment.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Fig. 2. Phylogenetic relationship of viral clones derived from the blood and rectum (maximum-likelihood trees, using FR-HXB2 as root). The numbers near the nodes indicate the percentage of bootstrap replicates (1000). Samples at inclusion: rectal HIV-DNA (open circle), blood HIV-DNA (open triangle), blood HIV-RNA (open square); second set of samples for two patients: rectal HIV-DNA (full circle), blood HIV-DNA (full triangle), blood HIV-RNA (full square). A, B, C, D, E, F and G illustrate viral clones from patient A, B, C, D, E, F and G, respectively. All the clusters identified with the maximum-likelihood approach were confirmed using a neighbor-joining analysis.

**Greater diversity of HIV DNA variants in the rectum compared to variants in the blood
in patients without HAART**

Véronique Avettand-Fenoel* ^{1,2}, Laurent Hocqueloux ³, Michaela Müller-Trutwin ⁴, Thierry Prazuck ³, Adeline Melard ^{1,2}, Marie-Laure Chaix ^{1,2}, Eric Agoute ³, Christophe Michau ⁵, Christine Rouzioux ^{1,2}

¹ AP-HP, Hôpital Necker-Enfants Malades, Laboratoire de Virologie, Paris, 75015, France

² Université Paris Descartes, EA3620.

³ CHR d'Orléans - La Source, Orléans, France.

⁴ Institut Pasteur, Unité de Régulation des Infections rétrovirales, Paris, France.

⁵ CHR Saint-Nazaire, France.

Running head

Rectal HIV reservoir

***Corresponding author**

Véronique Avettand-Fènoël, Laboratoire de Virologie, Hôpital Necker - Enfants Malades,
149 rue de Sèvres, 75015 Paris. Phone number: 33 1 44 49 49 61,
Fax number: 33 1 44 49 49 60 veronique.avettand@nck.aphp.fr

Abbreviations: HAART: highly active antiretroviral treatment, HIV: human immunodeficiency virus, PBMCs: peripheral blood mononuclear cells

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

26 The gut-associated lymphoid tissue represents the largest reservoir of HIV-1. Improving
27 knowledge of this reservoir by studying the diversity of viral population is a key step towards
28 understanding the pathogenesis and dynamics of HIV. Obtaining samples is difficult and
29 little information is available on gut viral quasispecies during the course of infection in
30 humans.

31 The aim of the study was to characterize rectal viral strains and their diversity and to
32 investigate the relationships between the rectal tissue reservoir and viral variants in the blood.
33 Phylogenetic analyses were performed on the *env* sequences for rectal HIV DNA, blood HIV
34 DNA and HIV RNA clones, with maximum likelihood and neighbor-joining methods on
35 seven patients. Genetic diversity was assessed.

36 Higher diversity of HIV DNA clones was noted in the rectum compared to blood in 4 out of 5
37 patients without HAART. Viral diversity was present in the rectum from time of the primary
38 infection. Similar degrees of diversity were observed in the rectum and blood during HAART.
39 Rectal and blood HIV variants were interspersed partially or totally in the seven patients. A
40 certain number of rectal HIV DNA clones were clustered together in six patients.

41 These results suggest that variants in the rectum were more heterogeneous than variants in the
42 blood from patients without HAART, probably because the activated milieu of gut-associated
43 lymphoid tissue may provide an improved environment for viral replication, and indicate
44 exchange of viral populations between blood and rectal tissues, reflecting the dynamics of
45 HIV during course of infection.

46 **Key words:** HIV reservoir, gut-associated lymphoid tissue, rectum, phylogeny, diversity

Introduction

The mucosal-associated lymphoid tissue contains 40-60% of the body's lymphocytes [Cerf-Bensussan and Guy-Grand, 1991; Mowat and Viney, 1997]. The gut-associated lymphoid tissue represents the largest reservoir of human immunodeficiency virus type 1 (HIV-1) [Brenchley et al., 2004; Chase et al., 2006; Clayton et al., 1997; Veazey et al., 1998; Veazey and Lackner, 2005]. Although HIV-1 replication decreases in gut-associated lymphoid tissue with Highly Active Antiretroviral Treatment (HAART), HIV RNA and replication-competent HIV-1 can be detected in gut-associated lymphoid tissue of patients under successful HAART, thus confirming that the intestinal mucosa can act as a residual HIV reservoir despite HAART [Belmonte et al., 2007; Poles et al., 2006].

Improving knowledge of this intestinal reservoir is a key step towards the understanding of the pathogenesis of HIV and better assessment of the efficacy of treatment. Studying the diversity of the viral population within-, and analyzing the potential differences between blood and the intestine may provide an insight into the dynamics of HIV-1.

Previous studies on HIV quasispecies have described HIV compartmentalization in various tissues and fluids, including the brain, lung, semen and cervicovaginal secretions [Chomont et al., 2007; Delwart et al., 1998; Korber et al., 1994; van't Wout et al., 1998; Wong et al., 1997; Zhu et al., 1996]. Due to the difficulty of obtaining samples, little information is available on gut viral quasispecies during the course of infection in humans. Colon-derived HIV-1 variants were studied from autopsied samples from a patient who died of AIDS, but these variants were not compared to circulating blood variants [Wang et al., 2001]. Based on *Nef* analysis, Van Marle et al. [2007] showed compartmentalization of the gut HIV reservoir in patients infected chronically prior to HAART. However, results were less clear with reverse transcriptase sequences. Poles et al. [2001] have observed the presence of some HIV RNA variants resistant to non-nucleosidic reverse transcriptase inhibitor in gut-associated

1
2
3 72 lymphoid tissue and in peripheral blood mononuclear cells (PBMCs) which were different
4
5 73 from those observed in the plasma. Evidence of cross-infection between gut-associated
6
7
8 74 lymphoid tissue and PBMCs during HAART has been suggested as a possible mechanism for
9
10 75 persistence of HIV [Chun et al., 2008].
11

12 76
13
14
15 77 In this context, the objective of the study was to characterize rectal viral populations, to
16
17 78 compare their diversity to the diversity of blood variants, in patients receiving treatment or
18
19 79 not, and to investigate the relationship between viral variants in the rectum and those
20
21 80 circulating in PBMCs and in the plasma. This study was based on rectal biopsies because they
22
23 81 are the easiest gut biopsies to obtain in humans.
24
25
26

27 82
28
29 83 **Study design**
30

31 84 *Patients and samples*
32
33
34 85 Seven patients, enrolled in a study described previously [Avettand-Fenoel et al., 2008], were
35
36 86 examined. Two of the patients were at the time of the primary infection within four months of
37
38 87 the onset of the acute retroviral illness, four patients were infected chronically and one patient
39
40 88 had AIDS (Table 1). Blood samples and four punches of rectum including the lamina propria
41
42 89 were collected on the same day. In two patients, a second set of samples was taken several
43
44 90 months later (B, C) (Table 1). In total, six sets of samples were collected in patients without
45
46 91 HAART (A, B first set of samples, C first set of samples, C second set of samples, D, G) and
47
48 92 three during HAART (B second set of samples, E, F) (Table 1). Patients A, B (1st sample), C
49
50 93 were naïve to HAART; Patients D and G had previously received HAART but were not
51
52 94 receiving treatment at the time of sample collection (Table 1). Rectal endoscopic biopsies
53
54 95 were undertaken at the same site to avoid potential variations among patients. Rectal tissue
55
56
57
58
59
60

was also collected from the same site for histopathological assessment. The local ethics committee approved the study and patients gave their written informed consent.

Nucleic acids extraction

RNA was extracted from plasma using the QIAamp RNA Mini Kit (Qiagen, Courtaboeuf, France) and used to synthesize cDNA with the Reverse transcriptase core kit (Eurogentec, Angers, France). Total DNA was extracted from PBMCs using the QIAamp DNA mini kit and from rectal tissues using the QIAamp tissue kit (Qiagen). The extracts of the four rectal biopsies collected on the same day from each patient were pooled.

Cloning and sequencing

The *env* regions encoding C2-V5 were amplified using a previously described nested PCR [Delwart et al., 1993]. Viral sequences were amplified using an Expand High Fidelity^{PLUS} PCR system (Roche Diagnostics, Meylan, France). To avoid a PCR-based bias, at least two PCRs from the pooled extracts were performed on each sample. Rectal HIV DNA, blood HIV DNA and blood HIV RNA from each patient, as well as the sequential samples, were amplified on different days to avoid contamination. To prevent any inter-sample contamination, water and one sample from each patient were amplified on one day. Moreover, the different amplifications of the samples from each patient were separated by at least two weeks.

Phylogenetic analyses

A direct cloning of PCR products was performed as described previously [Briat et al., 2005]. Clones were sequenced separately from each PCR amplification. Sequences were analyzed with Sequence Navigator software and aligned with ClustalX software [Thompson et al., 1997]. Gap-stripped sequence alignments were performed for each patient. Distances between pairs of sequences were estimated based on Kimura's two-parametric model of nucleotide substitution (DNAdist module - PHYLIP package, v3.67) [Felsenstein, 2001]. The means of

1
2
3 121 all comparisons of pairwise genetic distances from each clone derived from rectal HIV DNA,
4
5 122 from PBMC HIV DNA or from plasma HIV RNA to every other clone from the same
6
7
8 123 tissue/fluid were calculated for each patient. Maximum-likelihood trees were constructed
9
10 124 using the HKY model (PHYML v2.4.4) [Guindon and Gascuel, 2003]. The reliability of the
11
12
13 125 branching orders was confirmed by performing 1,000 bootstrap resamplings. In order to
14
15 126 confirm the topology of the resulting trees, the distance matrix was employed for
16
17 127 phylogenetic analysis with the neighbor-joining method. Bootstrap values >70% were
18
19
20 128 considered significant.

21
22 129 *Statistical considerations*

23
24 130 The differences detected between pairwise distances among variants from the rectum, variants
25
26
27 131 from PBMCs and variants from plasma were assessed using Mann-Whitney test.

28
29 132
30
31 133 **Results**

32
33 134 Seven patients were included: patients A and B were at the time of the primary infection;
34
35 135 patients C, D, E, and F at chronic infection, and patient G with AIDS (Table 1). No
36
37
38 136 confounding condition such as inflammation was observed during histopathological analyses
39
40
41 137 of rectal tissue.

42
43 138 Overall, phylogenetic analyses were conducted on 522 sequences from clones derived from
44
45 139 nine rectal samples, four plasma samples and nine PBMC samples (six to 30 clones per
46
47
48 140 sample, median: 25) (GenBank accession numbers: GQ859192-GQ859245; GQ904239-
49
50 141 GQ904706).

51
52 142 The phylogenetic analysis showed that all sequences of rectal clones and of blood clones from
53
54 143 each patient clustered together, with no contamination between the samples from different
55
56
57 144 patients (bootstrap=100%) (data not shown).

58
59
60 145

Pairwise distances within HIV DNA quasiespecies in the rectum, within HIV DNA variants in PBMCs and within HIV RNA variants in the plasma were calculated. These seven patients with diverse history of infection had very different levels of genetic diversity, with medians of pairwise distances within HIV DNA quasiespecies in the rectum ranging from 0.1 to 2.2% depending on the patient taken into account (Fig.1a), from 0.02 to 1.8% within HIV DNA quasiespecies in the PBMCs (Fig.1b) and from 0.04 to 1.95% within HIV RNA quasiespecies in the plasma (data not shown).

The genetic diversity within HIV variants in the rectum was then compared to the diversity within HIV variants in the blood for each patient. There was a correlation between the diversity of HIV DNA variants in the gut and the diversity of HIV DNA variants in PBMCs among the 9 pairs of samples ($R=0.66$, $p=0.05$). Data were presented i) for patients without HAART, ii) for patients with HAART. i) In four out of five patients without HAART, the diversity among HIV DNA variants in the rectum was significantly greater than among HIV DNA variants in PBMCs ($p<0.0001$). Differences between the median distance within variants in the rectum and the median distance within variants in PBMCs are presented in Figure 1 (patients A, B first set of samples, D, G). Surprisingly, this was observed from the primary infection stage (A, B first set of samples). The diversity among HIV DNA variants in the rectum was also significantly greater than among HIV RNA variants in the plasma ($p<0.0001$). Differences between the medians distances within variants in the rectum and within variants in the plasma were +0.07%, +0.46%, +0.31% for the first set of samples of patient B, patient D and patient G respectively.

ii) During HAART, the genetic diversity within HIV DNA variants in the rectum and within HIV DNA variants in PBMCs for each patient was not statistically different ($p=0.14$; $p=0.35$ and $p=0.56$ for patients B (second set of samples), E and F, respectively) (Fig. 1). It was also

1
2
3 171 noticeable for patient B that the genetic diversity within HIV DNA quasispecies in the rectum
4
5 172 was significantly higher before treatment (median 0.25%) than during HAART (median
6
7
8 173 0.12%) ($p=0.001$).
9

10 174
11
12 175 Another objective was to check whether the HIV variants in the rectum and in the blood were
13
14 176 interspersed or separated. Phylogenetic analyses showed that variants in the rectum and in the
15
16 177 blood were interspersed partially or totally in all the seven patients (Fig. 2). In patients with
17
18 178 sequential samples, most of the variants from the first set and variants in the blood from the
19
20 179 second set were interspersed (B, C), showing the persistence of some archived variants in the
21
22 180 body over time (Fig. 2). Nevertheless, clusters grouping at least some variants were observed
23
24 181 in the rectum in patients A, B, C, E, F, G (bootstrap values $>70\%$) (Fig. 2). It was also
25
26 182 noticeable that all the clusters identified with the maximum likelihood analysis were
27
28 183 confirmed using a neighbor-joining approach.
29
30
31
32
33

34 184
35
36 185
37
38
39 186 **Discussion**

40
41 187 Studies carried out on the viral quasispecies in the different tissues could provide useful
42
43 188 information enabling understanding of virus trafficking. Very little is known about the gut-
44
45 189 associated lymphoid tissue in patients infected with HIV, due to the small size of samples and
46
47 190 the difficulty in obtaining such samples. For the first time, the genetic diversity of HIV DNA
48
49 191 in the rectum was compared to the genetic diversity of variants in blood, for well-
50
51 192 characterized patients either at the time of the primary infection, or during chronic infection
52
53 193 and or with AIDS. The HIV DNA variants include archived viral strains accumulated over
54
55 194 time, as well as strains from recent infections; while the HIV RNA variants represent recently
56
57
58
59
60

1
2
3 195 produced viral particles. Despite the low number of patients, statistically significant
4
5 196 differences were observed.
6
7
8 197 These data suggest that variants in the rectum were more heterogeneous than variants in the
9
10 198 blood in patients without HAART. To our knowledge, this is the first report to document that
11
12 199 a viral genetic diversity seems to exist in the rectum from the time of the primary infection.
13
14
15 200 For patient B (first set of data), the greater genetic diversity was mainly linked to two
16
17 201 sequences of HIV DNA that clustered together. Unfortunately, other rectal HIV DNA
18
19 202 sequences could not be recovered in patient B due to the low number of CD4+ T cells in
20
21 203 relation to the small size of the rectal biopsies, and further studies will be necessary to
22
23 204 confirm that point. One limitation of this study could be the bias introduced by the strategy of
24
25 205 PCR cloning. However a recent publication again discussed this point and concluded that this
26
27 206 method of cloning is relevant if an adequate number of PCR templates is analysed [Jordan et
28
29 207 al., 2010]. In the present study, the number of PCR templates was high (table 1), therefore the
30
31 208 strategy of PCR cloning could be considered with a limited bias. Although the existence of
32
33 209 undetected minor variants which could influence the viral diversity in PBMCs and in the
34
35 210 rectum cannot be excluded, the results may be explained by the activated milieu of the gut-
36
37 211 associated lymphoid tissue which may provide an improved environment for the replication of
38
39 212 HIV. In a larger group of patients [Avettand-Fenoel et al., 2008], we quantified the
40
41 213 proportion of CD4+ rectal cells by immuno-staining in available biopsies for some patients
42
43 214 and calculated the HIV DNA load per million of CD4+ rectal cells. It was noted that the HIV
44
45 215 DNA load per million of CD4+ rectal cells was higher systematically than the HIV DNA
46
47 216 level per million PBMCs, as described by Chun et al. [2008], Chomont et al. [2009] and Yukl
48
49 217 et al. [2010]. The microenvironment in the gut-associated lymphoid tissue can be explained
50
51 218 by the differences in the local immune selection pressures, the HIV-specific immune
52
53 219 response, the constraints on viral entry and the replication rates due to the availability of
54
55
56
57
58
59
60

1
2
3 220 activated target cells. The increased error prone replication would result in greater viral
4
5 221 diversity.
6
7
8 222 These data suggest that heterogeneity was not significantly different in the rectum and in the
9
10 223 blood during HAART, as described previously [Chun et al., 2008]. This observation suggests
11
12 224 that HAART played a role in equalizing the dynamics of HIV in the gut-associated lymphoid
13
14 225 tissue and PBMCs, although some clusters persisted.
15
16 226
17
18 227 In the cases described, the fact that the HIV variants in the rectum, in PBMCs and in the
19
20 228 plasma were interspersed underlines that large exchanges of virus particles and/or infected
21
22 229 cells occur between these tissues/fluids, as already shown in patients with HAART [Chun et
23
24 230 al., 2008]. This is in line with the positive correlation observed between the HIV DNA levels
25
26 231 in the rectum and in PBMCs at different stages [Avettand-Fenoel et al., 2008]. As observed
27
28 232 previously with the variants of HIV in faeces [van der Hoek et al., 1998] and in the gut-
29
30 233 associated lymphoid tissue [van Marle et al., 2010], complete, independent evolution of HIV-
31
32 234 1 was not observed in the intestinal tissue.
33
34 235 Nevertheless, in most cases, at least some clones of HIV DNA in the rectum could be
35
36 236 differentiated from variants in the blood, as described recently in the esophagus, the stomach,
37
38 237 the duodenum, the colon and the blood with phylogenetic and drug mutation analysis [van
39
40 238 Marle et al., 2010]. This suggests the existence of local replication foci which could play a
41
42 239 major role in the immunopathogenesis of HIV-1 in the altered mucosa.
43
44 240
45
46 241 Finally, these results indicate exchange and trafficking of viral populations between blood and
47
48 242 lymphoid rectal tissues. The results also suggest that viruses present in the rectum were more
49
50 243 heterogeneous than variants in the blood in patients without HAART. Viral diversity is
51
52 244 present in the rectum from the time of the primary infection. Studies on the gut-associated

lymphoid tissue may improve understanding the pathogenesis of HIV, the assessment of treatment efficacy and the development of new therapeutic strategies, particularly those aiming at reducing the reservoir.

Acknowledgments

We are particularly indebted to the volunteers who agreed to participate in this project.

We thank Hayete Djarech who performed rectal biopsies with Eric Agoute, and we acknowledge the participants of the AC32 "Reservoirs" of the ANRS (Agence Nationale de Recherches sur le SIDA et les hépatites virales) for helpful discussions.

Funding

This work was supported by Paris-Descartes University and promotes by Orleans Hospital.

The authors have declared no conflict of interest in this publication.

Ethical approval

The local ethics committee (Tours, France) approved the study and patients gave their written informed consent.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

Avettand-Fenoel V, Prazuck T, Hocqueloux L, Melard A, Michau C, Kerdraon R, Agoute E, Rouzioux C. 2008. HIV-DNA in rectal cells is well correlated with HIV-DNA in blood in different groups of patients, including long-term non-progressors. *Aids* 22:1880-1882.

Belmonte L, Olmos M, Fanin A, Parodi C, Bare P, Concetti H, Perez H, de Bracco MM, Cahn P. 2007. The intestinal mucosa as a reservoir of HIV-1 infection after successful HAART. *Aids* 21:2106-2108.

Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC. 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 200:749-759.

Briat A, Dulioust E, Galimand J, Fontaine H, Chaix ML, Letur-Konirsch H, Pol S, Jouannet P, Rouzioux C, Leruez-Ville M. 2005. Hepatitis C virus in the semen of men coinfectd with HIV-1: prevalence and origin. *Aids* 19:1827-1835.

Cerf-Bensussan N, Guy-Grand D. 1991. Intestinal intraepithelial lymphocytes. *Gastroenterol Clin North Am* 20:549-576.

Chase A, Zhou Y, Siliciano RF. 2006. HIV-1-induced depletion of CD4+ T cells in the gut: mechanism and therapeutic implications. *Trends Pharmacol Sci* 27:4-7.

Chomont N, Hocini H, Gresenguet G, Brochier C, Bouhlal H, Andreoletti L, Becquart P, Charpentier C, de Dieu Longo J, Si-Mohamed A, Kazatchkine MD, Belec L. 2007. Early archives of genetically-restricted proviral DNA in the female genital tract after heterosexual transmission of HIV-1. *Aids* 21:153-162.

Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio F A, Yassine-Diab B, Boucher G, Boulassel M R, Ghattas G, Brenchley JM, Schacker TW, Hill BJ, Douek DC, Routy JP, Haddad EK, Sekaly RP. 2009. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 15:893-900.

Chun TW, Nickle DC, Justement JS, Meyers JH, Roby G, Hallahan CW, Kottlilil S, Moir S, Mican JM, Mullins JI, Ward DJ, Kovacs JA, Mannon PJ, Fauci AS. 2008. Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. *J Infect Dis* 197:714-720.

- Clayton F, Snow G, Reka S, Kotler DP. 1997. Selective depletion of rectal lamina propria rather than lymphoid aggregate CD4 lymphocytes in HIV infection. *Clin Exp Immunol* 107:288-292.
- Delwart EL, Mullins JI, Gupta P, Learn GH, Jr., Holodniy M, Katzenstein D, Walker BD, Singh MK. 1998. Human immunodeficiency virus type 1 populations in blood and semen. *J Virol* 72:617-623.
- Delwart EL, Shpaer EG, Louwagie J, McCutchan FE, Grez M, Rubsamen-Waigmann H, Mullins JI. 1993. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. *Science* 262:1257-1261.
- Felsenstein J. 2001. PHYLIP, Phylogeny Inference Package, version 3.6 (alpha). Seattle: Department of Genetics, University of Washington.
- Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696-704.
- Jordan MR, Kearney M, Palmer S, Shao W, Maldarelli F, Coakley EP, Chappey C, Wanke C, Coffin JM. 2010. Comparison of standard PCR/cloning to single genome sequencing for analysis of HIV-1 populations. *J Virol Methods* 168:114-120.
- Korber BT, Kunstman KJ, Patterson BK, Furtado M, McEvilly MM, Levy R, Wolinsky SM. 1994. Genetic differences between blood- and brain-derived viral sequences from human immunodeficiency virus type 1-infected patients: evidence of conserved elements in the V3 region of the envelope protein of brain-derived sequences. *J Virol* 68:7467-7481.
- Mowat AM, Viney JL. 1997. The anatomical basis of intestinal immunity. *Immunol Rev* 156:145-166.
- Poles MA, Boscardin WJ, Elliott J, Taing P, Fuerst MM, McGowan I, Brown S, Anton PA. 2006. Lack of Decay of HIV-1 in Gut-Associated Lymphoid Tissue Reservoirs in Maximally Suppressed Individuals. *J Acquir Immune Defic Syndr* 43:65-68.
- Poles MA, Elliott J, Vingerhoets J, Michiels L, Scholliers A, Bloor S, Larder B, Hertogs K, Anton PA. 2001. Despite high concordance, distinct mutational and phenotypic drug resistance profiles in human immunodeficiency virus type 1 RNA are observed in gastrointestinal mucosal biopsy specimens and peripheral blood mononuclear cells compared with plasma. *J Infect Dis* 183:143-148.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876-4882.
- van der Hoek L, Goudsmit J, Maas J, Sol CJ. 1998. Human immunodeficiency virus type 1 in faeces and serum: evidence against independently evolving subpopulations. *J Gen Virol* 79:2455-2459.
- van Marle G, Church DL, Nunweiler KD, Cannon K, Wainberg MA, Gill MJ. 2010. Higher levels of Zidovudine resistant HIV in the colon compared to blood and other gastrointestinal compartments in HIV infection. *Retrovirology* 7:74.
- van Marle G, Gill MJ, Kolodka D, McManus L, Grant T, Church DL. 2007. Compartmentalization of the gut viral reservoir in HIV-1 infected patients. *Retrovirology* 4:87.
- van't Wout AB, Ran LJ, Kuiken CL, Kootstra NA, Pals ST, Schuitemaker H. 1998. Analysis of the temporal relationship between human immunodeficiency virus type 1 quasispecies in sequential blood samples and various organs obtained at autopsy. *J Virol* 72:488-496.
- Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA. 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 280:427-431.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

359 Veazey RS, Lackner AA. 2005. HIV swiftly guts the immune system. *Nat Med* 11:469-470.

360 Wang TH, Donaldson YK, Brettle RP, Bell JE, Simmonds P. 2001. Identification of shared

361 populations of human immunodeficiency virus type 1 infecting microglia and tissue

362 macrophages outside the central nervous system. *J Virol* 75:11686-11699.

363 Wong JK, Ignacio CC, Torriani F, Havlir D, Fitch NJ, Richman DD. 1997. In vivo

364 compartmentalization of human immunodeficiency virus: evidence from the

365 examination of pol sequences from autopsy tissues. *J Virol* 71:2059-2071.

366 Yukl SX, Gianella S, Sinclair E, Epling L, Li Q, Duan L, Choi AX, Girling V, Ho T, Li P,

367 Fujimoto K, Lampiris H, Hare CX, Pandori M, Haase AX, Nthard HX, Fischer M,

368 Shergill AX, McQuaid K, Havlir DX, Wong JX. 2010. Differences in HIV Burden and

369 Immune Activation within the Gut of HIV-Positive Patients Receiving Suppressive

370 Antiretroviral Therapy. *J Infect Dis* 202:1553-61.

371 Zhu T, Wang N, Carr A, Nam DS, Moor-Jankowski R, Cooper DA, Ho DD. 1996. Genetic

372 characterization of human immunodeficiency virus type 1 in blood and genital

373 secretions: evidence for viral compartmentalization and selection during sexual

374 transmission. *J Virol* 70:3098-3107.