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Testing for HIV infection in time: scientific reasons[1]

Summary

The author advocates testing, diagnosis and therapy of HIV infection as soon as possible after contracting the virus and whenever feasible. The arguments are deduced from the results of basic research. The following should be reduced, delayed or inhibited: (1) the viral load in blood plasma and semen; (2) rapid internal propagation of the virus, which is combined with integration of proviruses into cells of unknown life span and compartmentalisation (e.g. the brain may present a sanctuary site); (3) rapid individual formation of quasispecies out of initially homogeneous virus strains of suboptimal fitness, combined with the transition of NSI strains to the more aggressive SI strains and escape from the immune response and therapy; (4) irreversible damage to the immune system; later opportunistic infections; (5) unconscious transmission of possibly drug-resistant virus. Early diagnosis and therapy appear possible in many cases, involving major advantages for individuals and society.

Zusammenfassung

Frühes Testen auf, frühe Diagnose und Therapie von HIV-Infektionen, so bald als möglich nach der Infektion und wenn immer machbar, werden empfohlen. Die Argumente werden aus den Resultaten der Grundlagenforschung abgeleitet. Es geht darum, zu hemmen, zu reduzieren, zu verzögern: (1.) die Konzentration der Viren im Blutplasma bzw. im Sperma; (2.) die rasche interne Ausbreitung der Viren, die einhergeht mit der Integration von Proviren in Zellen unbekannter Lebensdauer und der Bildung von viralen Kompartimenten in Organen (z.B. Gehirn); (3.) das rasche Auftreten von Quasi-Spezies ausgehend von ursprünglich homogenen Stämmen oft suboptimaler Fitness, z.B. mit Varianten, die dem Immunschutz oder einer Therapie entgehen oder aggressiver sind (SI-Varianten); (4.) irreversible Schäden im Immunsystem; spätere opportunistische Infektionen; (5.) unwissentliche Übertragungen, möglicherweise resistenter Varianten. In vielen Fällen scheint eine frühe Diagnose und Therapie möglich und den Betroffenen wie auch der Gesellschaft Vorteile zu bringen.

Introduction

Basic research provides means to appreciate the transmission pathways of the human immunodeficiency virus (HIV), its epidemiology, the outcome of AIDS, or the difficulties encountered with vaccination and chemotherapy. Good arguments in favour of the infected individuals and of society, respectively, for early diagnosis and therapy – as soon as possible after contracting the virus – can be deduced from the results of basic research. They could also be derived from conventional epidemiology and medical rules. Here, I will summarise some of the basic knowledge, focusing mainly on early phases of the disease.

[1] The author is retired professor for biochemistry, especially molecular biology. He would like to thank Drs. M. Kiener in Nidau/Biel and G. Bertoni in Berne for their advice with respect to clarity and scientific accuracy. The Swiss National Science Foundation has for many years supported his group in their virologic research. NB: literature considered until end of 1997.

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Schweiz Med Wochenschr 1998;128: Nr 15

Taxonomy and molecular biology of HIV

Abbreviations and glossary (in alphabetical order)

CCR5, CC-CKR5	a chemokine receptor for beta-chemokines of the family with a cysteine (C-C) pair; binds the C-C chemokines RANTES, MIP-1α, MIP-1β, produced by CD8+ T cells (CTL); serves as a coreceptor for HIV-1/2 on monocytes/macrophages and activated/memory T lymphocytes (NSI phenotype)
Chemokines	a superfamily of small chemotactic cytokines; direct the migration of subsets of white blood cells to sites of inflammation and to their specific niches in lymphoid organs
CTL	cytotoxic T lymphocyte
CXCR4	fusin, LCR-1, HUMSTSR, LESTR, a chemokine receptor for alpha-chemokines of the family with a cysteine-x-cysteine (C-X-C) motif; binds the stromal cell-derived factor 1 (SDF-1); serves as a coreceptor for HIV-1/2 on (naive) T lymphocytes (SI-phenotype)
DC	dendritic cell
Env	surface (envelope) protein of retroviruses; in HIV-1 this viral gene product is a protein with a molecular weight of 160 kilodaltons forming the spikes of the virion surface
FDC	follicular dendritic cell
Gag	group antigen; inner proteins of retroviruses
GCDC	germinal center dendritic cell
IDC	interdigitating dendritic cell
IN	integrase of retroviruses; this viral gene product integrates the double-stranded DNA product of reverse transcription into the host genome
MHC	major histocompatibility complex
myr	myriad, 1 million
NSI	non-syncytium inducing phenotype of HIV
p	protein
PBMC	peripheral blood mononuclear cell
Pol gene	viral polymerase gene of retroviruses, containing the information for the biosynthesis of the reverse transcriptase, of a ribonuclease (H), of IN and of a proteinase, which cleaves the polyprotein gene products into the final proteins
PR gene	the viral gene for a proteinase of retroviruses, which cleaves the polyprotein gene products of the genes for Gag and Pol into the final proteins
RNase H	a viral ribonuclease activity situated in the reverse transcriptase hydrolyzing the genomic RNA of the virion during reverse transcription into a first DNA strand
RT	reverse transcriptase of retroviruses; this enzyme is a RNA-dependent and DNA-dependent DNA-polymerase acting in the virion and after uncoating in the cytoplasm of the host cell
SI	syncytium-inducing phenotype of HIV

HIV is the causative agent of AIDS. It belongs to a large virus family, the *Retroviridae*, containing enveloped, plus-strand *RNA* viruses. Their hallmark is a gene coding for the enzyme reverse transcriptase (RT), which enables them to replicate their RNA genome through a double-stranded *DNA* intermediate. HIV is a member of the genus *Lentivirus*, characterised by accessory genes and proteins not found in simple retroviruses. Five distinct lineages of *primate* lentiviruses have been identified: HIV-1/SIV (chimpanzee), HIV-2/SIV(sooty mangabey, macaques) and 3 others of monkeys. HIV-1 and HIV-2 represent 2 serotypes with partial crossreactivity. HIV-1 can be divided into 2 *distinct clusters*: M (major) and O (outlier). M isolates can be subdivided into at least 10 major genetic subtypes (clades), A–J, on basis of sequences of the envelope (*env*) gene. There are at least 5 subtypes of HIV-2. In view of the rapid global viral traffic, map presentations of clade distributions can only be short-term pictures [1]. Two groups of main phenotypes in cell culture have been described in HIV-1 clades A–E and O: (1) The "rapid-high", syncytium(cell-cell fusion)-inducing (SI), T(lymphocyte)-tropic isolates replicate rapidly to high titers in immortalised tumor cell lines and in primary CD4+ T lymphocytes (T helper cells, Th), but not in monocytes or macrophages. (2) The "slow-low", non-syncytium-inducing (NSI), M-tropic variants replicate more slowly and to lower titers in monocytes, primary (*resting* [2]) macrophages and in primary *activated/memory* CD4+ lymphocytes, but not in established CD4+ T-cell lines. As a rule, NSI-viruses initiate the infection and dominate the early phases of the disease; however, they are always present. In later phases the more aggressive cytopathic SI phenotype may prevail [3]. Viruses of both phenotypes need 2 receptors on the cell surfaces for successful infection. NSI use CD4 and the beta-chemokine receptor CCR5 (CC-CKR5); SI bind to CD4 and to the alpha-chemokine receptor CXCR4 (*fusin*), irrespective of clades. However, many SI isolates can use both coreceptors [4] or even the chemokine receptors CCR3 or CCR2b and others [5]. Important reviews, covering the field, are indicated [6–10]. The CD4 molecule is an immunoglobulin-like cell-surface protein acting together with the T-cell receptor in recognition of antigen-presenting cells such as interdigitating (IDC) and follicular dendritic cells (FDC) of lymphoid organs, Langerhans cells, monocytes/macrophages. CD4 is expressed on

many cell types (dendritic cells [DC], monocytes and macrophages, T-cells and others).

The genome of HIV-1 is composed of 3 main genes: for the group antigen (*Gag*), the polymerase (*Pol*), and the envelope protein (*Env*). The gene products are (p for protein, numbers for molecular weights in kilodaltons): from *Gag*: p17 (matrix protein), p24 (capsid), p2, p7 (nucleocapsid), p1 and p6; these proteins form the inner part of the virion. From *Pol*: proteinase (PR, p9), RT (p66/p51 heterodimer), ribonuclease H (RNase H, in p66 of RT), integrase (IN, p32); these proteins belong also to the inner parts below the lipid envelope. From *Env*: the envelope precursor glycoprotein (gp) 160, which is cleaved into gp 120 and gp 41; gp 160 is locked into the membrane and forms the spikes of the virion surface. The products of the auxiliary genes are: p23 vif ("viral infectivity factor"), p15 vpr ("viral protein R"), p16 vpu ("viral protein U"), p14 tat ("transactivator of transcription"), p19 rev, p27 nef ("negative factor"). The names have remained without actual meaning. These proteins may play roles in cases of infected persons, who do not develop AIDS ("long-term non-progressors"). Many reports mention, for example, substituted or truncated NEF genes in case of non-progressors.

HIV invades an organism by contact with body fluids. It has been detected in blood, semen, vaginal fluids, breast milk, tears, stool, urine, saliva and cerebrospinal liquor of seropositives ([11], and references therein). In semen, the virion concentration in terms of viral RNA may be very high (up to 10 million [myr] copies per ml); it is strongly correlated with the blood plasma viral load. Potent antiretroviral therapy can rapidly reduce the viral load in semen [12].

In a first step, gp 120 binds to the receptors mentioned; thereafter the viral envelope fuses with the plasma membrane of the host cell, so that the inner parts of the virion are injected into the cytoplasm. The RT polymerises a complementary DNA-strand, using the genomic RNA as a template, which is concomitantly degraded by RNase H; thus, the new DNA is now free to be a template for the second DNA-strand. The product is a cytoplasmic double-stranded DNA, which in chemical terms cannot be distinguished from host DNA. This preintegration complex is targeted through nuclear pores into the nucleus by the matrix protein and vpr. This trick allows HIV to successfully infect also non-dividing, resting cells such as terminally differentiated macrophages. In the nucleus, IN integrates the viral DNA en bloc and covalently into the host genome. From now on, the viral genome mimicks as a *provirus* a gene battery of the host. The host cannot discriminate between own and foreign genes. The provirus cannot be removed; it remains until the cell dies. In activated (dividing) cells, the provirus is copied and given to daughter cells during mitosis. The infection is fixed, irreversible, life-long. Thus, an infected individual probably remains always potentially infectious, irrespective of the clinical stage [12]. In some resting cells the provirus may remain silent (latent); in activated (transcription) cells the provirus may give rise to low or high copy numbers of viral RNA. In this case the *host-coded RNA-polymerase II* synthesises the new genomic RNA and also the mRNAs over and over again. They are transported with the help of rev from the nucleus to the cytoplasm for protein synthesis. The viral proteins are formed as large covalent polyproteins according to the 3 main genes and are tailored to the definite lengths by the viral PR. If these cleavages cannot be performed (PR inhibitors!) non-infectious virions will result. Finally, the various components are put together with the genomic RNA in the plasma membrane, where the new virions bud into the extracellular space.

Accordingly, viral nucleic acids can be found as extracellular RNA (virion RNA), as intracellular integrated DNA without intracellular RNA (silent provirus), as intracellular integrated DNA with intracellular RNA (transcribing provirus), or as intracellular linear or circular DNA not integrated into the host genome.

The virions form a quasispecies in the infected host

Each virion contains *two* quasiidentical genomic RNAs: the viral genome is diploid and heterozygous. During reverse transcription the RT can switch from one template to the other (copy choice), thus fabricating recombinant DNA. This is an important mechanism of diversification and explains the occurrence of genomic mosaics of clades. One reason for this phenomenon is infection of one cell with two strains. It has been demonstrated to occur *in vivo* in a rhesus monkey [13]. Recombinations may generate high level, multidrug-resistant strains in individual treated patients [14]. In addition, RT is error-prone, introducing non-base paired nucleotides (substitutions) into the DNA, which may lead to mutations in the viral

Schweiz Med Wochenschr 1998;128: Nr 15

proteins. RT lacks a correction function; it fails to remove mispaired nucleotides. On average, every new genome (about 10^4 nucleotides) exhibits one substitution, so that many virions do not possess identical RNAs, and a population of virions is never homogeneous: it is a quasispecies, which can be analysed by modern methods (amplification, sequencing). This leads to intriguing conclusions. In later stages of infection every patient carries his individual quasispecies [15, 16]. As virus changes take place only during multiplication in living cells, the probability of new phenotypic traits of HIV is proportional to the number of replication cycles in an individual and to the number of infected persons world-wide [1]. The number of global HIV variants was estimated to total 10^{14} –10^{18} [16]. The actual number of infected persons is at least 24 (30?) myr adults and 1.5 myr children. More than 10,000 new infections occur each day [17]. There is no reason to assume that in the course of the pandemic mitigated variants will prevail. Mutated virions which are non-infectious or strongly attenuated are eliminated from the epidemic spread.

This individual and global view furnishes arguments for early diagnosis whenever and wherever possible. In the individual, treatment soon after infection has a fair probability to hit a homogeneous and drug-sensitive virus population (see also below). The more the pandemic expands the more virions will be formed, some of which may adopt a more aggressive phenotype due to the diversification phenomenon.

The early phase: mode and risk of transmission

The main modes of transmission are sexual, perinatal and parenteral. Nowadays, the impact on women is rising sharply [17]. There is a clear influence of other sexually transmitted diseases on HIV transmission: genital ulcers caused by syphilis, chancroid or herpes increase the risk four-fold; gonorrhoea, chlamydia and trichomoniasis increase it 2- to 3-fold [17]. However, surface lesions of a mucosa are not prerequisites for infection. Experiments with macaques demonstrated that atraumatic deposition of cell-free SIV onto the *genital* or *oral* mucosa resulted in transmission and AIDS. The minimal virus dose needed to achieve systemic infection after oral exposure was 6,000 times lower than that after rectal exposure [18]. The authors state that unprotected receptive oral intercourse should be added to the list of risk behaviours, though the risk in human adults is impossible to assess because of the recall bias and the inherent problems of interviews. In contrast, oral infection was well documented in neonates after breast feeding.

The early phase: the virus transmitted; viral RNA and proviral DNA

Shortly after infection the population of viral envelope proteins is homogeneous [19]. Therefore, it is probable that a single virion can lead to infection ("single hit") [3, 15, 19]. A competent virion is highly infectious [15, 16]. The initial homogeneity may also be explained by *selection*. In unrelated preseroconverters the *RNA* env genes from plasma were completely homogeneous within a given person, irrespective of the route of transmission [19]. However, in chronically infected partners the well-known individual quasispecies were present, and the sequence of the transmitted viruses matched best with a *minor* variant in the blood of the transmitters [3]. The isolates were exclusively NSI and M-tropic at time of seroconversion [3, 19].

Proviral *DNA* in peripheral blood mononuclear cells (PBMC) was also analysed. The envelope DNA sequences remained homogenous for several months after seroconversion in contrast to the plasma virion RNA samples [19]. The virions in the plasma are not formed by PBMC. Only a small percentage of cells in the blood (1 in 10^2 to 1 in 10^4) at any given time appears to possess HIV-DNA and most of these cells are latently infected, not synthesising HIV-RNA [20]. Envelope DNA sequences in PBMC going back to preseroconversion stages may remain unchanged even *over years* as an increasingly small component of the proviral population [21]. However, corresponding sequences disappear immediately after seroconversion from the plasma virion-RNA cohort. Plasma RNA sequences already in early stages of infection are orders of magnitude more heterogeneous than RNA sequences before seroconversion or the proviral DNA sequences in PBMC several months after seroconversion [19]. These provirus bearing cells are mainly memory cells, a subset of the T-cells [22].

Coinfection with several strains is possible. Donor cells can transmit the virus. In fact, cell-to-cell transmission is much more efficient than infection by free virions (see below). Foreign macrophages or DCs were found to survive in a recipient and were detected during more than a week [23]. A small number of foreign cells in the mucosa is sufficient to initiate the disease [24].

The early phase: *internal propagation* – the viral side

A seronegative asymptomatic volunteer at risk, unaware of his infection, donated blood and inguinal lymphoid tissue. Proviral DNA was present in cells from both sources, HIV-RNA was detected in the plasma, and CD4+ cell levels were reduced by 50%. Within hours of surgery the volunteer developed a severe influenza-like syndrome. In a second blood sample, 12 days later, plasma HIV-RNA had increased 200-fold in tandem with virions of increased growth capacity *in vitro*. Upon seroconversion the HIV burden was suppressed and HIV-specific CD8+ cytotoxic T-lymphocytes (CTL) appeared [25]. Dramatic progress of infection was also demonstrated in macaques, infected atraumatically either intravaginally or orally with single animal infectious doses of a cell-free simian/human chimeric virus. The animals produced high virus loads and CD4+ cell loss within 1 month and died after 6 months. A first burst of replication peaked after 2 weeks. After 2 days cells expressed viral *RNA* and protein in the vagina, uterus, pelvic and mesenteric lymph nodes. By day 4 infected cells had disseminated to the spleen and thymus, by day 5 to the blood, by day 7 to the central nervous system. Activated infected lymphocytes readily cross the blood-brain barrier. By day 15 the global elimination of CD4+ T cells was in full progress. No differences in the courses of the infections were seen after oral infection [24]. Thus, the speed of virus multiplication and internal propagation may be dramatic. This leads to an initial transitory clinical illness with rash, fever, lymphadenopathy, and HIV-1 RNA levels in the plasma of up to 20 myr copies per ml. Then the viremia diminishes by 10- to 200-fold within a few weeks. This suppression is thought to be mediated by CTL. MHC (HLA) class I restricted CTL are a subset of the CD8+ lymphocytes. CTL enforced selection on the virus population during primary infection; escape variants rapidly emerged [26]. All this stresses again the point that early diagnosis and therapy are mandatory. At the beginning, only a few cells are infected with a homogeneous virus of suboptimal fitness. Before seroconversion, reservoirs in organs and in latently infected cells of long lifetime and in the DC network of lymphoid tissues have not yet been fully established. Otherwise, even before seroconversion, HIV replication may cause irreversible damage to the immune system [25].

The early pathogenesis – the host side

After selection and maturation in the thymus, (CD4+)T lymphocytes are thought to differentiate, thereby passing through subsets. Naive cells have not been confronted by an antigen; they are not activated and have no effector (cytokine production, B cell help) function. CD4 and CXCR4 are expressed [6, 27]. They travel between secondary lymphoid organs such as lymph nodes, Peyer's patches, tonsils, spleen. If engaged by an antigen-presenting cell, they become activated and proliferate, exhibit effector functions and express CCR5. They alter the migration behaviour, reach the skin, the intestinal lamina propria, the pulmonary interstitium, the joints and sites of inflammation [27, 28]. If they survive, they return into a resting state as memory cells. They express CD4 and CCR5 and also have a broad tissue distribution. However, exposure of resting CD4+ T lymphocytes to HIV-1 upregulated the expression at the cell surface of a receptor for homing to lymph nodes [20]. The partners of these cells are the antigen-presenting macrophages and DCs. The DCs line mucosal surfaces and are found in blood (blood DC, CD4+, CCR5+, CXCR4+ [29], MHC I and MHC II+ [30]), exsudates, thymus, lung, skin, gut, vagina, oropharynx, tonsils. They seem to be endowed with a long life span. They express adhesion molecules and can activate naive T-cells, with which they aggregate, to extensive proliferation. The related epidermal Langerhans cells (CD4+, CCR3+, CCR5+, CXCR4+) lie on the surfaces of the oral and cervical mucosa and in the skin. Langerhans cells in situ do not express CXCR4. They can be infected

with NSI- and SI-HIV types [31, 32]. The motile DCs can transport HIV without being productively infected and transmit it to local activated or memory T cells [33, 34]. Finally, they migrate to the paracortical region (T cell zone) of the draining lymph nodes as IDC (CD4low). In contrast, FDC (CD4–) are located exclusively in the B cell follicles. They have the capacity to trap antigen in the form of immune complexes for long periods of time. The germinal center DCs (GCDC, CD4+) in the B cell follicles are strong antigen presenters for T cells. These constellations may explain the initial selection of NSI and M-tropic strains: DCs and macrophages at the port of viral entry transport the virus to the peripheral tissue homing CD4+ CCR5 T lymphocytes – the activated and memory T cells which are the main targets during acute viremia before seroconversion. The majority of these T cells is destroyed [22] as shown by the transient drop in CD4+ cell counts. Surviving cells differentiate into resting memory cells with inactive provirus and may circulate for *years* together with their descendants [19–22]. Memory seems to be maintained by long-lived *clones* rather than by individual cells with a long life span [35]. In contrast, naive cells may survive for up to 10 years. The role of DCs in the pathogenesis of the HIV infection has recently been reviewed [36, 37]. In addition, cell proliferation is not required for HIV-1 replication in macrophages, whereas HIV-1 replication in T lymphocytes needs activation and cell division. Virus can enter quiescent T cells, but reverse transcription and nuclear import are inefficient [38].

In the rectum, a gateway may lead through M cells. This is an epithelial cell type for transepithelial transport of macromolecules, particles, and microorganisms. The basolateral membrane of M cells forms an intraepithelial pocket containing CD4+ Th and memory cells, B lymphocytes, macrophages and DCs. Microorganisms are generally transcytosed intact and alive [39].

Clinical latency is not biological latency: turnover, viral load and prognosis

After seroconversion the level of CD4+ T cells climbs to about 700/µl and then slowly drops to 100/µl during the clinical latency. As stated, the decrease of plasma viremia within weeks of infection is correlated with the appearance in peripheral blood of anti-HIV CTL. A significant number of these monoclonally expanded cells involved in the primary immune response rapidly disappear [40]. Clonal exhaustion may be the reason. HIV-1 does not infect CD8+ T cells. However, CD8+ thymocytes contain a population infected with HIV-1 provirus; the infection may take place during times of intrathymic T cell maturation when cells express CD4 as well as CD8 [41].

For the kinetic data, see table 1. After the initial drop of viremia, the concentration of plasma virions is set at a value which remains constant for many years (set point of viral *RNA* in steady state). Later on, the titer increases to a maximum until death after 11 to 12 years. Several papers [15, 42–44] demonstrate that in asymptomatic individuals there are many infected, virus producing cells in secondary lymphoid organs (Waldeyer ring, lymph nodes, appendix, Peyer's patches, spleen). Heavy crowds of virions lie in the spaces between the cells. Activation of the cells may be triggered by antigens being presented by IDCs/GCDCs which can activate memory *and* naive CD4+ T cells [45]. The virus progeny is then juxtaposed to large numbers of uninfected cells recruited by the same antigen [15]. Such a mechanism may explain the virus bursts in plasma after antigenic stimuli. The viral *tat* protein seems to play a major role also in continual activation from the large pool of quiescent T cells. *Tat* is secreted by infected cells and activates primary uninfected, quiescent T cells *in vitro* and *in vivo*; these cells then become highly permissive for productive HIV-1 infection [46]. Large amounts of HIV can also be detected on FDC of secondary lymphoid organs. A peculiar fact is that the virus is bound to the surface of these cells in form of an immune complex containing neutralising antibodies; yet, HIV on FDC is highly infectious. FDC can convert neutralised HIV into an infectious form and transfer it to activated CD4-T cells without being infected themselves [47].

In a series of papers [48–54], the enormous dynamics of viral and cellular turnover (biosynthesis versus destruction in unit time) was depicted (table 1). The authors used different methods for analyses of tissues or blood (kinetic analysis of the viral clearance in blood after initiating a potent therapy [49, 50, 54]; serial biopsies of lymphoid tissue and evaluation by *in situ* hybridisation and computer-assisted quantitative image analysis [48, 51]; measurements of infectious virus and viral RNA in plasma, and of infectious virus, integrated vi-

Table 1

Determinations, calculations and estimations of HIV-1 viral and cellular dynamics in a 70 kg individual [48–57].

	total	blood	tissue
reference values			
lymphoid tissue mass	700 g		
total number of lymphocytes	10^{12}		
per gram of lymphoid tissue	1.5×10^{9}		
total number of activated CD4+ T cells	1.5×10^{11}		
per gram of lymphoid tissue	2×10^{8}		
activated, producing cells [48, 51, 52]			
activated CD4+ T lymphocytes with integrated viral genome: producing (and dying) cells	0.02% = 3×10^{7}		
per gram of lymphoid tissue	5×10^{4}		
(intracell.) copies of viral RNA per gram of lymphoid tissue			10^{7}–10^{8}
intracell. copies of viral RNA per producing cell			≥75
total body burden in lymphoid tissues (FDC)			10^{11}
half-life in steady-state, producing cells, days			0.9
total turnoverrate of producing cells per day			8×10^{7}
net loss of CD4+ T cells/day			0.01% = $\geq2\times10^{7}$
activated, non-producing cells [48, 51, 52]			
activated CD4+ T lymphocytes with unintegrated viral genome: probably non-producers	2% = 3×10^{9}		
resting cells, latent state [48–52, 54, 56]			
resting CD4+ T lymphocytes with integrated viral genome (memory, replication-competent)	0.02% = 0.5–3×10^{7}		
half-life in steady state, stably infected PBMC, days		24–433	
viral dynamics [48–51, 53, 56, 57]			
virus production per CD4+ T cell per day	$\leq10^{3}$		
total virus production, steady state, per day	$\leq3\times10^{10}$		2×10^{10}
mean daily release of virions into extracell. fluids, steady state	$\leq10^{10}$	$\leq10^{10}$	
virions per mL plasma regardless of clin. state		10^{2}–10^{7}	
half-life of virions in steady state, days		0.6–2	1
number of replication cycles per year	≥300		
therapy [49–51, 54]			
doubling time, replacement by resistant mutants, days (nevirapine/ritonavir) (monotherapy)		≤2	
half-life of HIV-1 RNA on FDC, days			1.7
clearance rate for FDC pool, virions per gram/day			2×10^{7}
viral RNA copies/cell, mononuclear cells, 3 weeks of treatment			>20
viral RNA copies/mononuclear cell with HIV-1 DNA, 6 months of treatment			<10

Note: Data are compiled from many papers. The principle of the methods is indicated in the text. As far as possible, values are given for the compartments investigated.

ral DNA, non-integrated viral DNA in form of preintegration complexes, and viral messenger RNA species in infected cells of lymph nodes [52]). The procedures yield conformable results.

The DNA analysis in blood and lymph nodes of asymptomatic individuals in order to investigate the latent tissue reservoirs has shown that at any time only a minute fraction (0.02%) of the lymphocytes is stably infected with a properly integrated provirus from which infectious virus can be obtained [52]. However, in view of the fact that a single productively infected CD4-T cell can synthesise up to thousand virions per day [53], the modest number of cells is sufficient to deliver the daily virus load. These cells contain the highest intracellular concentration of viral RNA with more than 75 copies of HIV-1 RNA per cell [51]. *Resting memory* CD4+ *T cells* (5–30 myr) with an *integrated* genome present the latently infected T cells with replication-competent provirus; they are left over during therapy. Thus, the most prevalent form of HIV-DNA in *resting* and *activated* CD4 lymphocytes is a full-length, linear, not integrated HIV-DNA that is not replication-competent per se (labile, inducible reservoir). Due to the error-prone nature of replication part of this type of DNA will not be able to integrate, being defective or leading

to defective proviruses. It has been shown that defective genomes accumulate in PBMC of infected persons [55]. Due to the same fact the infectivity of free virions (infectious virions per particles released) is only about 1 in 10^3. However, in case of cell to cell transmission the infectivity is 1 in 10 [53].

The concentration of viral RNA on FDCs exceeds that in plasma by large factors; even, when viral RNA in plasma was below detection level under therapy, there were more than 1 myr copies per gram of lymphoid tissues. Under combination therapy (ritonavir, zidovudine [AZT], lamivudine [3TC]) biopsies were obtained and the loss of virus from FDCs was followed. After 3 weeks of treatment many mononuclear cells containing lower RNA copy numbers (more than 20) were detected. After 6 months lymphoid tissues still harboured infected cells (HIV-1 DNA) and in some of these cells low levels of viral gene expression took place (fewer than 10 copies of HIV-1 RNA per cell). However, within 6 months, triple therapy eliminated more than 99% of the lymphoid cells producing the virus [51].

In summary, about *300 virus replication cycles* take place every year [56, 57]. The vast majority of circulating plasma virions is derived from continuous rounds of *de novo* infection, replication and cell turnover. These dynamic events explain readily as to why major changes of HIV quasispecies occur so quickly in response to selective processes. The unavoidable sequelae of the rapid and massive production under uncontrolled conditions are viral escape from immune reactions and from therapy and the formation of more aggressive virions with altered tropism. It is also clear that tiny volumes of fluid containing competent virus ($1–10^3$ virions per µl) or producing cells may be infectious.

Furthermore, latently infected cells with unclear life span are resistant to all usual drug or immunological treatments. Inhibitors of RT cannot affect transcription after activation, because transcription is performed by a host-coded enzyme, not by RT. Therapy has to go on until the latently infected cells die.

All this demonstrates again the necessity of early diagnosis and therapy.

The set point of the concentration of viral RNA in plasma is the result of a dynamic equilibrium. In the steady state it is constant. Therefore, this concentration (the viral "load", the "viremia") has a highly predictive value after 6–12 months of infection [58, 59]. Advancing disease can be predated by a quantitative change of measurable viral RNA production. Also, the prognostic value oft post-seroconversion HIV-1 RNA measurement has been convincingly shown [58]. A single plasma HIV-1 RNA determination obtained after infection seems to predict clinical events years later [59].

The extremely high mutation rates result in virus populations with altered phenotypes

Considering the huge production of virions, the inherent stochastic substitution rate of 1 per 10^4 nucleotides and per replication, and a genome length of 10^4 nucleotides, soon after infection every possible mutation will be probed 10^4 times or more daily. It is important to note that substitutions appear spontaneously. Therapy will not induce substitutions, but it will select in favour of a resistant mutant. Many mutants may be disabled and grow slowly, so that their concentration within the quasispecies is very low. Distributions of fitness variants *in vivo* have been demonstrated [60]. This has also been shown in case of therapy-resistant mutants. In 12 infected persons 246 viral PR genes were examined; resistant mutants were found at low concentrations before therapy. This fact reflects reduced fitness. However, during therapy additional mutants were introduced into the PR and the catalytic activity of the enzyme improved [61]. Under the selective influence of indinavir multiple variants arose which were cross-resistant and exhibited 11 or more amino acid changes in PR. The lesson is clear: if mutants are present before therapy starts, their replication goes on, modestly at the beginning, more rapidly later on, and substituted provirus can be given to daughter cells. Similar findings were published in case of nevirapine [50], or other RT inhibitors, or even in case of multi-drug therapy [62]. Obviously, the same mechanisms lead to escape of viruses from humoral or cellular immune responses [26, 63]. Again, development of resistance calls for an early diagnosis and therapy. The longer one waits, the higher the probability that resistant mutants evolve out of an initially non-resistant virion population. Until now, every monotherapy has selected resistant mutants. Correspondingly, the danger exists that resistant mutants are transmitted ([60, 64] and many others).

Thus, there are two fundamental mechanisms which allow HIV to circumvent immunologic

and chemotherapeutic actions: provirus formation in latently infected cells and mutation. There is a third mechanism: reservoir formation in organs.

Reservoir formation and compartmentalisation of quasispecies

The infection potential, which is not restricted to CD4 bearing cells, and homing mechanisms of circulating cells lead to a wide distribution of infected cells within the organism and within organs. Variants will also be selected by the microenvironments of different anatomic compartments. Many observations support the concept of anatomically distinct, independently evolving quasispecies ("virodemes") [65]. Examples have been mentioned [15, 21]. Compartments have been identified in sigmoidal and jejunal tissues (faeces) [66], and in the upper and lower human female reproductive tract, where multiple cell types including epithelial cells were shown to be targets for infection [67]. Macrophages may serve as a hidden reservoir; infected macrophages seem to persist for months, still releasing virus. Very high levels of virus are shed into the blood, even when CD4+ cells are almost exhausted and lymph nodes are devastated. Macrophages may become the major site of production, e.g. in the lung, with independent evolution of the virions [68]. Macrophages, both uninfected and infected with opportunistic pathogens, have been identified as highly productive sources in coinfected lymph nodes; common pathogens may dramatically increase the production of virus [69]. Particularly intriguing is the infection of the brain which may be an early event [70]. Here, quasispecies go also their own ways [65, 71]. The principal target cells are the monocyte-derived microglial cells [65, 70], which express CD4 and CCR5. Entry into the brain may be mediated by monocytes and macrophages or by infection of brain capillary endothelial cells [72]. Direct infection of neurons, astrocytes and oligodendrocytes may contribute to the brain disease. AZT remains the only antiretroviral agent of proven benefit in the treatment of AIDS encephalopathy; in several cases it took up to 31 months until resistant mutants appeared in the brain [65].

Is an early diagnosis possible?

The goal of an early diagnosis is the protection of the community and the initiation of a combination therapy [73]. Early diagnosis, even before seroconversion, is possible in many cases. The diagnostic tools have been refined, such as EIAs for IgG and IgM HIV-1 specific antibodies. The limit of detection for the p24 antigen is 8 pg per ml [74], and a boosted assay for the quantitation of HIV-1 RNA levels in plasma for routine clinical analysis with a lower detection limit of 20 copies per ml has been described [75]. The viral load test is a powerful prognostic tool [58, 59] notwithstanding the exceptions. However, the levels of viremia become predictive for the clinical outcome only after 6–12 months of infection. The initial levels do not predicate the prognosis [76].

The early symptoms and the differential diagnosis have been depicted [77]. At the time of seroconversion the initial manifestations may be protean; however, they have been described as a "mononucleosis-like illness of acute onset occurring 2–6 weeks after HIV-1 infection, usually resolving after 1–2 weeks, though occasionally lasting considerably longer". Many gay men undergo an acute seroconversion illness (50–90%). Maculopapular exanthemas and mucosal ulcers affecting the mouth and genital areas imply a syphilis. The pathophysiology of the infection at the time of seroconversion or afterwards has been described above. However, triple drug therapy with AZT, 3TC and a proteinase inhibitor can lead to a more than 10^4-fold reduction of the load and can in many patients maintain plasma virus below detection limit for the whole duration of treatment [78]. Therefore, early initiation of a highly active antiretroviral therapy (HAART) has been advocated [73, 77, 79, 80]. The question of partner notification has also been taken up in this context [81]. Treatment should be started as soon as the diagnosis is made and maintained for at least six months to limit the fatal initial peak of virion production [77]. Post-exposition prophylaxis (PEP) without diagnosis should not be performed, unless a putative transmitter has contracted the infection (nonprotected genital, anal or oral intercourse, condom rupture, sharing of injection material, rape, hospital accidents). In cases of unknown serostate of both partners PEP should not be initiated. When indicated, a combination treat-

ment with AZT, 3TC and indinavir or nelfinavir is to be started immediately or after 72 h at the latest and to be maintained for several weeks. Tests should be done at the start and after 3, 6, and 9 months after exposition.

Late triple therapy leads only to an incomplete immune reconstitution [82]; probably, the increase of CD4+ T cells is due to peripheral expansion of existing cells and not to stem cell regeneration. Late therapy may even provoke reactivation or infection by opportunistic pathogens. On basis of mathematical models it will take 2–3 years to rid the body of the virus; extinction seems to be difficult [54, 78, 83]. However, very recent findings suggest that the time required for virus eradication, if possible, is considerably longer than previously predicted [54]. In four studies [84–87] PBMC and/or lymph node biopsies during HAART were examined (13 patients, 10 months; 10, 36–52 weeks; 6, up to 2 years; 22, up to 30 months). A few patients were newly infected and treated within 10 weeks of the onset of primary infection. Therapy was successful: HIV-RNA in plasma was mostly below detection limit. However, CD4+ T cells carried integrated and unintegrated HIV-1 DNA and produced infectious virus upon activation in vitro; viral RNA in lymph nodes and viral DNA in lymph nodes and PBMC remained detectable. These cells are latently infected resting (memory) CD4+ T lymphocytes. Their frequency among resting CD4+ cells is low (not more than 16 per 10^6); but they do not decrease with increasing time of HAART. The decay rate of this compartment is very slow, and it is established early after infection. On the other hand, the combination therapy was effective in suppressing viral replication, so that no new substitutions associated with drug resistance were identified in the viruses recovered *in vitro* after 2 years of therapy. Production of viruses is not attributable to drug failure, but rather to the persistence of long-lived and latently infected T cells. These findings again favour early diagnosis and therapy.

After questionable contacts, before seroconversion, it is an issue of personal responsibility to ask for tests. At that time a combination therapy could at least mitigate the consequences which have been discussed above by restricting the viral load. The prerequisite of early diagnosis is education and correct information of society. Testing of patients during the phase of sexual activity in the practice and/or hospitals is recommended; the expenses are neglectable as in Switzerland the overall expenses of a HIV infection amount to SFr. 600,000.– at least [88]. However, an anamnesis concerning sexual life is rarely made and the initiative to advise for HIV-testing is taken by the doctors in only one third of the cases [88], a situation which must be improved.

References

1 Koblet H. New ecological niches for viruses in a future Europe. Part II: Changing ecology and viral traffic. Med Microbiol Lett 1992;1:185–94.
2 Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC, Popovic M. The role of mononuclear phagocytes in HTL-VIII/LAV infection. Science 1986;233:215–9.
3 Zhu T, Mo H, Wang N, Nam DS, Cao Y, et al. Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. Science 1993;261:1179–81.
4 Zhang L, Huang Y, He T, Cao Y, Ho DD. HIV-1 subtype and second-receptor use. Nature 1996;383:768.
5 Rucker J, Edinger AL, Sharron M, Samson M, Lee B, et al. Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. J Virol 1997;71:8999–9007.
6 Unutmaz D, Littman DR. Expression pattern of HIV-1 coreceptors on T cells: implications for viral transmission and lymphocyte homing. Proc Natl Acad Sci USA 1997;94: 1615–8.
7 McNicholl JM, Smith DK, Qari SH, Hodge T. Host genes and HIV: the role of the chemokine receptor gene CCR5 and its allele (Δ 32 CCR5). Emerging Inf Dis 1997;3:261–71.
8 Moore JP, Trkola A, Dragic T. Co-receptors for HIV-1 entry. Curr Op Immunol 1997;9:551–62.
9 Gallo RC, Lusso P. Chemokines and HIV infection. Curr Op Inf Dis 1997;10:12–7.
10 Doms RW, Peipert SC: Unwelcomed guests with master keys: how HIV uses chemokine receptors for cellular entry. Virology 1997;235:179–90.
11 Zhang H, Bagasra O, Niikura M, Poiesz BJ, Pomerantz RJ. Intravirion reverse transcripts in the peripheral blood plasma of HIV type 1-infected individuals. J Virol 1994;68:7591–7.
12 Gupta P, Mellors J, Kingsley L, Riddler S, Singh MK, et al. High viral load in semen of HIV type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. J Virol 1997;71:6271–5.
13 Wooley DP, Smith RA, Czajak S, Desrosiers RC. Direct demonstration of retroviral recombination in a rhesus monkey. J Virol 1997;71:9650–3.
14 Burke DS. Recombination in HIV: an important viral evolutionary strategy. Emerging Inf Dis 1997;3:253–9.
15 Cheynier R, Henrichwark S, Hadida F, Pelletier E, Oksenhendler E, et al. HIV and T cell expansion in splenic white pulp is accompanied by infiltration of HIV-specific cytotoxic T lymphocytes. Cell 1994;78:373–87.
16 Wain-Hobson S. The fastest genome evolution ever described: HIV variation in situ. Curr Op Genetics Development 1993;3:878–83.
17 Quinn TC. Global burden of the HIV pandemic. Lancet 1996;348:99–106.
18 Baba TW, Trichel AM, An L, Liska V, Martin LN, et al. Infection and AIDS in adult macaques after nontraumatic oral exposure to cell-free SIV. Science 1996;272:1486–9.
19 Zhang LQ, MacKenzie P, Cleland A, Holmes EC, Leigh Brown AJ, et al. Selection for specific sequences in the external envelope protein of HIV type 1 upon primary infection. J Virol 1993;67:3345–56.

20 Wang L, Robb CW, Cloyd MW. HIV induces homing of resting T lymphocytes to lymph nodes. Virology 1997;228: 141–52.

21 Simmonds P, Zhang LQ, McOmish F, Balfe P, Ludlam CA, et al. Discontinuous sequence change of HIV type 1 env sequences in plasma viral and lymphocyte-associated proviral populations in vivo: implications for models of HIV pathogenesis. J Virol 1991;65:6266–76.

22 Schnittman SM, Lane HC, Greenhouse J, Justement JS, Baseler M, Fauci AS. Preferential infection of CD4+ memory T cells by HIV type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc Natl Acad Sci USA 1990;87:6058–62.

23 Cafruny WA, Bradley SE. Trojan horse macrophages: studies with the murine lactate dehydrogenase-elevating virus and implications for sexually transmitted virus infection. J Gen Virol 1996;77:3005–12.

24 Joag SV, Adany I, Li Z, Foresman L, Pinson DM, et al. Animal model of mucosally transmitted HIV type 1 disease: intra-vaginal and oral deposition of simian/human immunodeficiency virus in macaques results in systemic infection, elimination of CD4+ T cells, and AIDS. J Virol 1997;71: 4016–23.

25 Ferbas J, Daar ES, Grovit-Ferbas K, Lech WJ, Detels R, et al. Rapid evolution of HIV strains with increased replication capacity during the seronegative window of primary infection. J Virol 1996;70:7285–9.

26 Price DA, Goulder PJR, Klenerman P, Sewell AK, Easterbrook PJ, et al. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc Natl Acad Sci USA 1997;94:1890–5.

27 Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc Natl Acad Sci USA 1997;94:1925–30.

28 Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science 1996;272:60–6.

29 Granelli-Piperno A, Moser B, Pope M, Chen D, Wei Y, et al. Efficient interaction of HIV-1 with purified dendritic cells via multiple chemokine coreceptors. J Exp Med 1996;184: 2433–8.

30 Watts C. Inside the gearbox of the dendritic cell. Nature 1997;388:724–5.

31 Dittmar MT, Simmons G, Hibbitts S, O'Hare M, Weiss RA, et al. Langerhans cell tropism of HIV type 1 subtype A through F isolates derived from different transmission groups. J Virol 1997;71:8008–13.

32 Pope M, Frankel SS, Mascola JR, Trkola A, Isdell F, et al. HIV type 1 strains of subtypes B and E replicate in cutaneous dendritic cell-T-cell mixtures without displaying subtype-specific tropism. J Virol 1997;71:8001–7.

33 Cameron PU, Freudenthal PS, Barker JM, Gezelter S, Inaba K, Steinman RM. Dendritic cells exposed to HIV type 1 transmit a vigorous cytopathic infection to CD4+ T cells. Science 1992;257:383–7.

34 Pope M, Betjes MG, Romani N, Hirmand H, Cameron PU, et al. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. Cell 1994;78:389–98.

35 Michie CA, McLean A, Alcock C, Beverley PCL. Lifespan of human lymphocyte subsets defined by CD 45 isoforms. Nature 1992;360:264–5.

36 Weissman D, Fauci AS. Role of dendritic cells in immunopathogenesis of HIV infection. Clin Microbiol Rev 1997;10: 358–67.

37 Grouard G, Clark EA. Role of dendritic and follicular dendritic cells in HIV infection and pathogenesis. Curr Op Immunol 1997;9:563–7.

38 Schmidtmayerova H, Nuovo GJ, Bukrinsky M. Cell proliferation is not required for productive HIV-1 infection of macrophages. Virology 1997;232:379–84.

39 Neutra MR, Frey A, Kraehenbuhl J-P. Epithelial M-cells: gateways for mucosal infection and immunization. Cell 1996;86:345–8.

40 Pantaleo G, Soudeyns H, Demarest JF, Vaccarezza M, Graziosi C, et al. Evidence for rapid disappearance of initially expanded HIV-specific CD8+ T cell clones during primary HIV infection. Proc Natl Acad Sci USA 1997;94:9848–53.

41 Lee S, Goldstein H, Baseler M, Adelsberger J, Golding H. HIV type 1 infection of mature CD3hi CD8+ thymocytes. J Virol 1997;71:6671–6.

42 Embretson J, Zupancic M, Ribas JL, Burke A, Racz P, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993;362:359–62.

43 Pantaleo G, Graziosi C, Demarest JF, Butini L, Montroni M, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 1993;362:355–8.

44 Frankel SS, Wenig BM, Burke AP, Mannan P, Thompson LDR, et al. Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. Science 1996;272:115–7.

45 Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. Nature 1997;387:713–7.

46 Li CJ, Ueda Y, Shi B, Borodyansky L, Huang L, et al. Tat protein induces self-perpetuating permissivity for productive HIV-1 infection. Proc Natl Acad Sci USA 1997;94:8116–20.

47 Heath SL, Tew JG, Tew JG, Szakal AK, Burton GF. Follicular dendritic cells and HIV infectivity. Nature 1995;377: 740–4.

48 Haase AT, Henryk K, Zupancic M, Sedgewick G, Faust RA, et al. Quantitative image analysis of HIV-1 infection in lymphoid tissues. Science 1996;274:985–9.

49 Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, et al. Viral dynamics in HIV type 1 infection. Nature 1995;373: 117–22.

50 Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123–6.

51 Cavert W, Notermans DW, Staskus K, Wietgrefe SW, Zupancic M, et al. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science 1997; 276: 960–4.

52 Chun T-W, Carruth L, Finzi D, Shen X, Di Giuseppe JA, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 1997;387:183–8.

53 Dimitrov DS, Willey RL, Sato H, Chang L-J, Blumenthal R, Martin MA. Quantitation of HIV type 1 infection kinetics. J Virol 1993;67:2182–90.

54 Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 1997;387: 188–91.

55 Sanchez G, Xu X, Chermann J-C, Hirsch I. Accumulation of defective viral genomes in peripheral blood mononuclear cells of HIV type 1-infected individuals. J Virol 1997;71: 2233–40.

56 Coffin JM. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 1995;267:483–9.

57 Plikat U, Nieselt-Struwe K, Meyerhans A. Genetic drift can dominate short-term HIV type 1 nef quasispecies evolution in vivo. J Virol 1997;71:4233–40.

58 Mellors JW, Rinaldo C, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167–70.

59 Feinberg MB. Changing the natural history of HIV disease. Lancet 1996;348:239–46.

60 Goudsmit J, de Ronde A, de Rooij E, de Boer R. Broad spectrum of in vivo fitness of HIV type 1 subpopulations differing at reverse transcriptase codons 41 and 215. J Virol 1997;71:4479–84.

61 Lech WJ, Wang G, Yang YL, Chee Y, Dorman K, et al. In vivo sequence diversity of the protease of HIV type 1: presence of protease inhibitor-resistant variants in untreated subjects. J Virol 1996;70:2038–43.

62 Iversen AKN, Shafer RW, Wehrli K, Winters MA, Mullins JI, et al. Multidrug-resistant HIV type 1 strains resulting from combination antiretroviral therapy. J Virol 1996;70:1086–90.

63 McNicholl JM, McDougal JS. Immunologic escape as a mechanism of viral persistence in HIV-1 infection. Sem Virol 1994;5:307–17.

64 Borman AM, Paulous S, Clavel F. Resistance of HIV type 1 to protease inhibitors: selection of resistance mutations in the presence and absence of the drug. J Gen Virol 1996;77: 419–26.
65 Wong JK, Ignacio CC, Torriani F, Havlir D, Fitch NJS, Richman DD. In vivo compartmentalization of HIV: evidence from the examination of pol sequences from autopsy tissues. J Virol 1997;71:2059–71.
66 van der Hoek L, Sol CJA, Snijders F, Bartelsman JFW, Boom R, Goudsmit J. HIV type 1 RNA populations in faeces with higher homology to intestinal populations than to blood populations. J Gen Virol 1996;77:2415–25.
67 Howell AL, Edkins RD, Rier SE, Yeaman GR, Stern JE, et al. HIV type 1 infection of cells and tissues from the upper and lower human female reproductive tract. J Virol 1997;71: 3498–506.
68 Balter M. HIV's other immune-system targets: macrophages. Science 1996;274:1464–5.
69 Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections. Science 1997;276: 1857–61.
70 Strizki JM, Albright AV, Sheng H, O'Connor M, Perrin L, Gonzalez-Scarano F. Infection of primary human microglia and monocyte-derived macrophages with HIV type 1 isolates: evidence of differential tropism. J Virol 1996;70:7654–62.
71 Hughes ES, Bell JE, Simmonds P. Investigation of the dynamics of the spread of HIV to brain and other tissues by evolutionary analysis of sequences from the p17gag and env genes. J Virol 1997;71:1272–80.
72 Moses AV, Stenglein SG, Strussenberg JG, Wehrly K, Chesebro B, Nelson JA. Sequences regulating tropism of HIV type 1 for brain capillary endothelial cells map to a unique region on the viral genome. J Virol 1996;70:3401–6.
73 Ho DD. Time to hit HIV, early and hard. N Engl J Med 1995; 333:450–1.
74 Phair JP, Margolick JB, Jacobson LP, Phillips J, Rinaldo C, et al. Detection of infection with HIV type 1 before seroconversion: correlation with clinical symptoms and outcome. J Inf Dis 1997;175:959–62.
75 Schockmel GA, Yerly S, Perrin L. Detection of low HIV-1 RNA levels in plasma. J Acq Immune Def Syndr Hum Retrovirol 1997;14:179–83.
76 Pantaleo G, Demarest JF, Schacker T, Vaccarezza M, Cohen OJ. The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression independent of the initial level of plasma viremia. Proc Natl Acad Sci USA 1997;94:254–8.
77 Jolles S, Kinloch de Loes S, Johnson MA, Janossy G. Primary HIV-1 infection: a new medical emergency? Br Med J 1996; 312:1243–4.
78 Bonhoeffer S, May RM, Shaw GM, Nowak MA. Virus dynamics and drug therapy. Proc Natl Acad Sci USA 1997;94: 6971–6.
79 Stephenson J. The art of "HAART": researchers probe the potential and limits of aggressive HIV treatments. JAMA 1997;277:614–6.
80 Carpenter CCJ, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, et al. Antiretroviral therapy for HIV infection in 1997. Updated recommendations of the international AIDS society-USA panel. JAMA 1997;277:1962–9.
81 Fenton KA, Peterman TA. HIV partner notification: taking a new look. AIDS 1997;11:1535–46.
82 Autran B, Carcelain G, Li TS, Blanc C, Mathez D, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112–6.
83 Cohen J. Advances painted in shades of gray at a D. C. conference. Science 1997;275:615–6.
84 Chun T-W, Stuyver L, Mizell SB, Ehler LA, Mican JAM, et al. Presence of an inducible HIV-I latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci USA 1997;94:13193–7.
85 Wong JK, Günthard HF, Havlir DV, Zhang Z-Q, Haase AT, et al. Reduction of HIV-I in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc Natl Acad Sci USA 1997;94: 12574–9.
86 Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, et al. Identification of a reservoir for HIV-I in patients on highly active antiretroviral therapy. Science 1997;278:1295–300.
87 Wong JK, Hezareh M, Günthard HF, Havlir DV, Ignacio CC, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278: 1291–5.
88 Grüninger U. Vom Stellenwert der Beratung in der ärztlichen Prävention. Schweiz Ärztezeitung 1997;78:140–6.