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HIV-1 INFECTION OF CELLS AND AIDS PROGRESSION

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SUMMARY

• *HIV-1, the etiological agent of AIDS, enters cells within minutes after binding to a receptor molecule, fusing its membrane with the cell membrane and uncoating its envelope to deliver the RNA-protein complex into the cell cytoplasm. The infection cycle then proceeds for hours to days through a series of steps, including reverse transcription of the viral RNA, integration of the resulting DNA into the cellular genome, transcription of the proviral DNA, expression of vims proteins, their assembly and virus budding, leading to production of progeny virions. Virus spread by subsequent cycles of infection occurs within weeks and months, while AIDS develops after years or even decades. This review discusses the causes of this diversity in kinetics, emphasizing the importance of quantitation, and how quantitation of infection kinetics may help in understanding the factors that affect the disease progression.*

INTRODUCTION

• HIV-1, the etiological agent of AIDS (2,21,30), enters cells by binding to a receptor molecule, CD4 (11,29), fusing its membrane with the cell membrane (43) and uncoating its envelope to deliver the RNA-protein complex into cell cytoplasm (24). The infection cycle then procceeds through a series of steps, including reverse transcription of the viral RNA, integration of the resulting DNA into the cellular genome, transcription of the proviral DNA, expression of virus proteins, their assembly and virus budding, leading to production of progeny virions (45). While the HIV entry takes minutes and hours, productive infection of cells requires days to complete. Virus spread by subsequent cycles of infection occurs within weeks and months, while AIDS develops after years or even decades. Understanding the causes of this diversity in time scales is critical for understanding the factors that affect the disease progression. Here I present a current view on virus entry, infection and AIDS progression, emphasizing the quantitation of their kinetics.

HIV-1 ENTRY INTO CELLS

• HIV-1 envelope glycoprotein binding to receptor molecules

Human immunodeficiency virus (HIV-1) enters cells by utilizing a high affinity interaction between the viral glycoprotein (gp!20-gp41 complex) and the cellular receptor CD4 (11,29). There are few studies on binding of intact virions to cells and studies on binding of infected to target cells are lacking. In contrast, there a number of studies with soluble recombinant gp 120 and CD4 (35). It was found that sCD4 binds with high affinity to gp 120 and inhibits viral infection and syncytia formation *in vitro* (9,12,20,26,42,44). The high affinity of the CD4-gpl20 interaction and the presumption that the soluble receptor molecule efficiently interact with a wide variety (theoretically with all) of viral isolates led to human clinical trials (27,41). The trials showed that sCD4 did not change the patient status and cooled the enthusiasm about sCD4. To find out possible reasons for the failure of the clinical trials and to better understand the nature of the CD4-gpl20 binding we studied thoroughly the binding of sCD4 to membrane-associated gp!20-gp41.

We measured the rate constants of sCD4 binding and dissociation (16,17) and found that in contrast to previous work of others (36) the affinity of binding is not affected by temperature (16). Interestingly*, the rate of binding of

sCD4 and inhibition of HIV-1 infection at concentrations used in human clinical trials (about 0.1 Mg/ml) was slow while the dissociation was fast (17,18). We suggested that this property of the gp!20-gp41-sCD4 interaction is one of the causes for the failure of sCD4 as an anti-HIV-1 agent in those trials (17,18).

- **HIV-1 envelope glycoprotein-mediated membrane fusion**

The binding of the membrane-associated HIV-1 envelope glycoprotein to its membrane receptor CD4, initiates a complex cascade of phenomena, which ultimately result in fusion of the viral membrane with the plasma membrane of the cell and virus entry (3,35,43). Cells which express the viral proteins also fuse with CD4 target cells, which in some cases is dramatically manifested by formation of giant cells (syncytia) (31,32). While the expression of the gp!20-gp41 is sufficient to induce fusion after interaction with membrane-associated CD4, a number of factors determine the rate and efficiency of the fusion process. It was found, for example, that many animal cells expressing human CD4 do not fuse with gp!20-gp41 expressing cells (1,5,33). Mutations in and antibodies against the V3 loop of gp!20, which is the principal neutralizing domain of the envelope glycoprotein and does not overlap the CD4 binding site, can abrogate the fusion activity, without affecting binding (reviewed in [35]). It was suggested that the CDR3 in the first domain of CD4 also plays a role in post-binding events (reviewed in [40]), but recent extensive mutational studies (4) questioned this hypothesis. Other domains of CD4, however, are certainly important in post-binding events as demonstrated by mAbs directed to the second (7,10) and third (25) domains of CD4 and chimeric CD4-CD8 molecules (38).

In most of the studies on fusion syncytia formation has been used as an indicator of cell fusion and lack of syncytia has been assumed as a proof for lack of fusion. In other studies fusion was measured at one point in time and lack of syncytia at that time point was assumed to indicate lack of fusion. To avoid limitations of other assays we developed a method for measuring membrane fusion based on fluorescence dye redistribution and videomicroscopy, and extensively studied the kinetics of fusion for a number of systems.

We attempted to identify the kinetic and molecular components of the overall fusion process (3). We measured the rate of fusion of cells expressing gp!20-gp41 (15) and found that while the lag times in cell fusion are in the range from 15 min to hours (15,40), the lag times in virus-cell fusion are much shorter (1-2 min) (18). The fusion rate was critically dependent on the level of expression of gp!20-gp41 (13) and the membrane proximal domains of CD4 (23). There was a correlation between the rate of membrane fusion and formation of giant cells (syncytia) (17). We also found that calcium ions are required for efficient fusion (14) and an additional component(s) ofhuman cells confers the ability of animal cells expressing human CD4 to fuse (5). Interestingly, fusion of human B cell lines with gp!20-gp41 expressing cells was enhanced by antigen-specific immunoglobulin receptors, which may indicate existence of a mechanism for specific depletion *in vivo* of B cells expressing anti-gp!20-gp41 antibodies (22). The knowledge gained in studying binding and fision mediated by the CD4-gpl20-gp41 allowed us to develop a novel approach to inhibit HIV-1 infection and syncytia formation which is based on CD4-plasma membrane vesicles (39) and may prove useful in the therapy of AIDS.

- **Uncoating of the virus envelope**

Fusion of the viral membrane with the cell membrane is followed by uncoating of the virus envelope and penetration of the virus core through an opening of 20 to 80 nm in size (24). It seems that core disintegrates and releases the viral nucleoprotein complex through this opening because morphologically intact virus cores were not seen in the cytoplasm beneath the fusion zone. The release of the virus genome into the cell cytoplasm occurs within 3-10 min at 37°C (24). The mechanisms of envelope uncoating are presently unclear and the exact time sequence of events unknown. One may speculate that the virus envelope uncoating is similar to the initial stages of syncytia formation following fusion of the cell membranes. However, what is the role of the interaction of the virus core with the envelope during its uncoating remains to be elucidated.

REPLICATION OF THE VIRAL GENOME AND VIRUS ASSEMBLY

- The replication of the HIV-1 genome can be divided into two main stages: the first phase is carried our principally by enzymes found in the virus and includes reverse transcription of the viral RNA and integration of the resulting DNA into the cellular genome; the second phase, which includes synthesis of progeny genomes and mRNAs, and their translation, is carried out by cellular enzymes. Processing of the virus proteins involves both virus and cellular enzymes, while assembly of progeny virions, including budding, occurs spontaneously and does not seem to require enzymatic activity. It appears that efficient replication of the viral genome requires integration of the virus

DNA in the cellular genome, which can be the limiting factor in the kinetics of virus replication.

- **Synthesis of viral DNA and its integration into the cellular genome**

The synthesis of viral DNA is carried out by the concerted action of a virus-specific RNA-dependent DNA polymerase and a virus-specified ribonuclease H (37), and occurs in the cytoplasm of the cell immediately after the uncoating of the viral envelope within the first 6 h of infection (28). The overall process of reverse transcription leads from a molecule of single-stranded RNA with the structure R-U5-genes-U3-R (R,U5 and U3 being a repeated sequence, and unique sequences, respectively, which are involved in the reverse transcription, and transcription initiation and regulation) to a molecule of double-stranded linear DNA of the structure U3-R-U5-genes-U3-R-U5. The sequence U3-R-U5 thus constitutes a long terminal repeat (LTR) which serves as an HIV-1 promoter.

The linear DNA molecules associated with matrix proteins and other virus components are transported to the nucleus of the host cell in a process which is independent of cell division but requires ATP: features which are indicative of an active transport process (6). Circular DNA molecules are detectable in the nucleus 8 to 12 h after the virus entry and increase in number during the next 20 h (28). The next event is the integration of the viral DNA into that of the host. The integration is mediated by a virus-associated enzyme, the integrase, which has three functions: it trims the ends of the duplex DNA; it cleaves the host DNA; and finally it covalently joins the freshly cut ends of the virus and host DNA (8). This reaction occurs in the cell nucleus, where nuclear enzymes repair the site of insertion. Once integrated, viral DNA (provirus) remains permanently associated with the cellular genome. It is replicated regularly along with the cell DNA and loss of provirus (if occurs) seems to be a consequence of random deletion events, rather than a result of provirus instability.

- **Expression of viral genes and their regulation**

Expression of virus components begins with the synthesis of a complete RNA copy of the proviral DNA. The rate of transcription is controlled by cellular proteins. A large family of proteins binds to sequences near the site of RNA initiation of transcription and either increase or decrease the rate of initiation or/and elongation. For example, proviral DNA is not transcribed in resting T cells. As most of the CD4 positive cells in the circulation are resting, little virus is made by this population of cells. However, specific or nonspecific mitogenic stimulation of the CD4 cells results in active RNA transcription. The ability of the virus to remain silent in the cell account in part for the long latent period of the HIV-1 disease.

After activation of the HIV-1 provirus by cellular factors, the first viral genes to be expressed are those which encode nonstructural proteins with regulatory functions (45). The most important of the viral factors is the Tat protein which is needed for efficient transcription of the viral RNA. Tat is thought to act by two possible mechanisms: by helping to initiate the RNA transcription or/and by ensuring the production of full-length RNA transcripts during the RNA elongation step. The Tat protein is a powerful transactivator and inscreases tremendously the number of infections virions produced by infected cells, which leads to rapid spread of the virus.

The full-length RNA transcripts are then multiply spliced and transported to the cytoplasm where the HIV-1 regulatory proteins are expressed. At this stage another regulatory protein, Rev, which affects the transport of unspliced and singly spliced mRNA from the nucleus to the cytoplasm, becomes essential. These unspliced and singly spliced mRNAs encode the structural and enzymatic proteins of HIV-1 which are needed for assembly of virions.

The temporal aspects of RNA synthesis show a complex pattern (28). HIV-1 specific RNA appears first at 12 to 16 h postinfection and increases thereafter. There are early and late transcriptional phases. Early mRNAs are predominantly the smaller one: presumably the 2-kb spliced mRNAs for the low-molecular weight regulatory proteins (tat, rev) and the 4.3-kb spliced mRNAs which represents predominantly env, and possibly others. However, full-size transcripts (9.2 kb), which act both as the genome for the virus and as the mRNA for the gag and pol genes, were also evident in albeit small amounts. The late phase correlates with the appearance of Rev. After 24 h only 9.2-kb mRNA is made. The significant delay in the full-level expression of the full (9.2 kb), and to a lesser extent, the 4.3 kb mRNA species is somewhat similar to the asymptotic stage in the progression of the HIV-1 disease. A hypothesis (28) suggests that a controlled shift from the early to the late transcriptional phases may represent the transition from the latent to the acute infection *in vivo.*

- **Virus assembly and release**

The assembly and release of virions by budding is an unusu-; al process in at least two aspects. First, assembly and budding occur simultaneously. Second, analysis of mutants shows that only a relatively small fraction of the virion proteins are required. Mutants lacking pol, env and genome

RNA still yield normal-appearing virions, except for the absence of surface knobs. Thus capsid formation and budding are determined by only core and matrix proteins. The capsid precursor protein and the capsid replicative enzyme precursor proteins coassemble at the inner surface of the cell membrane. These proteins insert themselves into the cell membrane by virtue of a myristic acid which is attached to the amino terminus of the capsid precursor protein. The complex of capsid protein, replicative enzyme precursor, , and viral RNA assemble into a closed spherical particle which buds through the cell membrane. The newly assembled particle appears to remain attached by a tether to the surface of the cell before release. What is the time duration of the virus assembly and release is unknown but it appears to be relatively rapid and ptobably occurs within minutes provided appropriate conditions as, e.g. high concentration of virus components.

HIV-1 SPREADING KINETICS IN VITRO Km AIDS PROGRESSION

• The first description of the acquired immune deficiency syndrome (AIDS) appeared in 1981, and described 26 patients with Kaposi' s sarcoma and 5 - with oral thrush and pheumocystis pneumonia. The connection between the two groups was that the patients were all young male homosexual and their diseases were usually associated with iatrogenic immunosuppression. It was soon recognized that the epidemiology or distribution of cases is characteristic of a blood associated virus transmission via blood contact, via sexual intercourse and perinatally. Initially the definition of AIDS required the clinical diagnosis of conditions that were moderately predictive of cellular immunodeficiency, without an underlying cause, but later this definition was revised to include serological evidence of HIV infection. In an attempt to ease the problem of definition, the Centers for Disease Control (CDC) in Atlanta developed a classification system, which details four mutually exclusive categories of HIV-1 infection as shown in Table 1. This classification system is shown to demonstrate the complexity of the HIV-1 infection *in vivo,* but is not meant to suggest that every individual has to progress through all stages. However, patients having reached a particular stage do not revert to earlier stages if the symptoms settle.

I	Acute infection with seroconversion
II	Asymptotic infection
III	Persistent generalized lymphadenopathy
IV	A Constitutional disease
	B Neurological disease
	C Immunodeficiency
	C1 CDC definition of AIDS
	C2 Infections not in the original difinition of AIDS
	D Tumors in CDC definitions of AIDS
	E Other, e.g. Hodgkin's, carcinoma, lymphoid, interstitial pneumonia

Table 1. *Classification of effects of HIV-1 infection.*

• **HIV-1 infection kinetics in tissue cultures**

According to CDC classification system there are three major stages in the kinetics of HIV-1 infection leading to AIDS: (i) acute infection, (ii) asymptomatic phase, and (iii) AIDS-related complex and AIDS. The acute infection stage is characterized by an initial short period (days to weeks) without detectable virus; the viral load then sharply increases reaching a peak after several weeks and then slowly decreases in parallel with an increase in the concentration of antibodies against the virus components. As AIDS progresses the antibody titer decreases and the viremia increases. Interestingly, the infection of permissive T cells by HIV-1 in tissue cultures resembles some of the features of the acute infection *in vivo* and the late stages of AIDS. It typically proceeds in three stages: (i) no detectable virus production, (ii) a sharp increase in virus concentration reaching a peak, and (iii) a decline in virus production. The lag times without detectable virus production and the time periods to reach the peak of virus infection are critically dependent on the number of infectious virions produced by an infected cell and the time needed for the virus replication. While the replication times can be estimated by infecting cells with high multiplicities of infection (MOIs), measurement of the number of infectious virions during the virus transmission is more complicated if not impossible with the current assays, because of the cell-to-cell spread of the virus and its rapid inactivation.

To gain insight into the kinetic mechanisms of HIV-1 infection and to quantitate the critical parameters of a spreading infection, we developed a new approach, based on a combination of empirical data and theoretical modeling (19). We measured the kinetics of cell aggregation, cytopathicity and virus production at different MOI and described it by empirical equations. Then we derived those equations by using a model of virus spread by subsequent cycles of infection, which allowed us to calculate the time required for one cycle of infection and the number of infectious virions produced by one infected cell during this cycle. Interestingly, we found that the number of infectious virions is much higher (10 ^ to ICP-fold) under conditions which favor cell fusion than the HIV-1 infectivity in culture supernatants. Another important conclusion from our

analysis is that the slow infection kinetics of an HIV-1 tat minus mutant is not due to a longer replication time but reflects the low number (approximately 10) of infectious particles produced per cycle. While the infective process *in vivo* is much more complex than in tissue cultures (34), the evaluation of the critical parameters of a spreading infection by acutely infected cells may provide a basis for further insight into the mechanisms of AIDS progression.

- **Acute infection in patients**

Primary infection with HIV-1 may be associated with acute clinical manifestations, for example, sore throat, non-specific flu-like symptoms and gastrointestinal disturbances. The incubation period, defined as the time from exposure to clinical symptoms of acute infection, is variable, ranging from a few days to up to a few months. It is felt that the incubation period may be longer in sexually transmitted infection possibly because of a smaller dose of inoculum (see above for a discussion of an *in vitro* analogue) and the non-parenteral route of transmission. However, incubation periods of up to 6 months have been documented via the parenteral route. Symptoms of the illness last in average 1-2 weeks and up to 3-4 weeks and are accompanied by viremia. Specific antibodies can be detected (seroconversion) from 8 days to 10 weeks after the onset of acute illness. Antibodies to gp!20, gp!60 and p24 develop first and antibodies to gp41 and other viral antigens develop about 4 weeks later.

Severe immunodepression may occur during acute HIV-1 infection. This is probably related to the selective loss of antigen-primed T-memory cells, as well as defects which are present in the B cells and monocyte compartments. The number of CDS cells increases, while the number of CD4 cells decreases in parallel with the increase in viral load. This pattern is preserved following recovery from the acute infection. The return to normality in the CDS and CD4 cells is incomplete.

- **Asymptotic phase**

The asymptotic phase of HIV-1 infection may last for many years. The CD4 cell number declines almost linearly with time during the early stages of the disease at an average rate of 60-100 cells/mm^ per year. The slope of CD4 decline varies within individual patient population and seems to be related to the biological properties of individual viral isolates. Syncytia-inducing isolates were associated with steep decline and early development of symptomatic disease, while slowly replicating non-syncytia-forming strains were associated with low rates of short-term progression. Based on the analysis of the HIV-1 infection kinetics in tissue cultures, this can be attributed to the different number of infectious particles produced by the two types of viral strains.

- **AIDS-related complex and AIDS**

With the loss of CD4 cells the symptoms of opportunistic infections rise steadily. Some studies have reported that the CD4 trend may enter a phase of accelerated and rapid decline immediately prior to the development of AIDS, although other cohorts have failed to confirm this trend and report a linear falls throughout the natural history. The rate of CD4 decline is variable between individuals, and the level at which an AIDS-defining infection or tumor occurs is also variable. There is an approximate hierarchy of opportunistic infections present at different stages of the accumulating immunodeficiency. For example, herpes zoster occurs on average 5 years prior to development of AIDS. Oral Candida occurs later and is associated with CD4 numbers below 300/mm^. The most frequent opportunistic infection, *Pneumocystis* pneumonia, is common after the CD4 count has persistently fallen bellow 200/mm^3.

The viral load increases with the progression of AIDS both as cell-free virus and provirus. The viremia in the blood serum can reach 1(P or more infectious units per ml, and the number of CD4 cells containing provirus compared to uninfected CD4 cells is in the range of 1:10 to 1:100. The titer of the antibodies, especially against structural proteins, e.g.p24, decreases, but antibodies against the envelope glycoproteins are still present in relatively high concentrations. Under those conditions the virus probably spreads by cell fusion. How exactly the interplay between the immune system and virus behavior leads to the steady loss of the CD4 cells, disease and death remains to be elucidated. Quantitation of the time course of HIV-1 infection and its interpretation based on studies *in vitro* and theoretical modeling is one of the major approaches which can provide such knowledge needed for understanding the mechanisms leading to AIDS progression.

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