THE RATIO OF DEFECTIVE HIV-1 PARTICLES TO REPLICATION-COMPETENT INFECTIOUS VIRIONS

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Received July 15, 1993; revised October 13, 1993

Summary. – The ratio of infectious to defective particles has been investigated for human immunodeficiency virus type 1 (HIV-1). Although the concentration of HIV core p24 protein defined by ELISA permits the estimation of a total number of average-sized HIV particles, it does not provide information on the numerical value of infectious particles. This problem was addressed by limiting dilution of viral supernatant derived from various infected cell lines and then comparing the total number of HIV particles to the end-point titers of viral inoculum determined by reverse transcriptase assay and virus-induced cytotoxicity. The results indicate that the ratio of infectious to defective virus particles varies for different virus strains and host cells within a range from 1:1 to 1:100, but for given virus-cell system it remains constant.

Key words: HIV-1; defective particles; infectious virions

Due to the von Magnus effect (von Magnus, 1947), the total number of virions is higher than the number of replication-competent particles. According to accumulated data on many plant and animal viruses (Huang and Baltimore, 1977) it is known that for every infectious particle produced by the host cell there are several defective interfering particles without infectious properties. To establish whether this universal rule of the relationship of infectious particles to defective virions is applicable to human immunodeficiency virus (HIV), one must be able to quantificate viral particles and identify those that are infectious. This problem is not unimportant to the biology of HIV infection. Until recently the quantification of HIV, when determined by limiting dilution assay, posed a problem since it was based on cytotoxicity assays and thus neglected defective virions without cytolytic properties. On the other hand, the estimations based on the number of viral genome copies posed a contrary problem in identification of virions with infectious capacity.

Recently, by means of a simple mathematical calculation, it has been established that since the mass of the p24 fraction in one HIV-1 particle is equal to 1×10^{-16} g, a culture medium containing, for example, 100 pg of p24 will contain the equivalent of 1 million HIV-1 particles (Bourinbaiar, 1991). Hence, the quantity of retroviral particles can be determined according to the mass of viral *gag* gene product p24 determined by ELISA. Levels of p24 protein in a viral culture are so far the only conveniently obtainable values that are derived from a measurement based on the standardized metric system. The correctness of the above figures has also been verified by independent calculations (Bourinbaiar *et al.*, 1991; Bourinbaiar 1992) derived from the mathematical formulas devised by Svedberg and Pedersen (1940) and adapted for viruses by Markham (1962). From these calculations the mass of an individual HIV virion was estimated to be 11.6×10^{-16} g. This places the mass of p24 at 1.29×10^{-16} g. This value appears to be in very close proximity to the value 1×10^{-16} g.

These figures are in agreement with other published estimations. For example, electron microscopy-based counting of HIV particles suggested 10^8 viral particles per ml of H9 culture that usually produce about 10 ng of p24 or the equivalent of 10^8 particles per ml (Popovic *et al.*, 1984). The mass of the core protein of avian retrovirus was reported by Vogt (1965) to be 5×10^{-16} g. This value was based on an original calculation by Bonar and Beard (1959) and was obtained from estimation of the dry weight of viral pellet preparation and electron microscopy counting through its section. Moreover, according to recent electron microscopy measurement of the core of HIV-1 by Hoglund *et al.* (1992)

its mass would be equal to no more than 3×10^{-16} g. Another study (Layne *et al.*, 1992) has estimated that each HIV particle contained 5×10^{-17} g of p24. Finally, direct comparative study based on simultaneous measurement of p24 and number of viral RNA copies by polymerase chain reaction (Piatak *et al.*, 1993) confirmed that theoretical estimations mentioned above were exact. Thus, knowing the total amount of viral particles in a given viral supernatant, and correlating this to the number obtained from functional assays, the ratio of defective versus infectious virions can be established.

The infectivity of the virus or its functional capability is usually expressed in arbitrary TCID units which may depend on many variables such as viral strain, target cell number, susceptibility, viability, culture medium composition etc. However, as opposed to multipartite viruses, one infectious unit of HIV in the absence of defective replication should theoretically correspond to one viral particle. HIV-induced cytopathic effect can be estimated by measuring the absorbancy of violet-colored formazan reduced by live lymphocytes from tetrazolium salts (MTT). This method is not dependent on a subjective bias usually associated with manual counting of syncytial cells or plaques. Because of the simplicity of MTT assay we decided to use it as the TCID indicator by exposing MT-4 susceptible cells to the virus. The measurement of reverse transcriptase (RT) activity associated with the particulate viral matter (Popovic *et al.*, 1984) was chosen as another means of estimating the functional activity of the retrovirus. To arrive at this goal, serial ten-fold dilutions of culture medium from virus-producing T lymphocytic and monocytic lines were measured simultaneously by ELISA for viral p24 antigen, by RT assay for reverse transcription-competent virions, and by MTT assay for virus-induced MT-4 lymphocyte death (Table 1).

Table 1. End-point titration of various strains of HIV-1

p24 or number of virions	Cell line/virus strain	End-point dilution (log)		
		p24	MTT	RT
100ng or 10^9	MOLT-4/HTLV-IIIB	–5	–3	–3
10ng or 10^8	MT-2/HTLV-IIIB	–4	–4	–4
1ng or 10^7	ACH-2/LAV	–3	–3	–2
10pg* or 10^5	U1/HIV-1	–1	–1	–1

*The detection limit of p24 kit used in this study is 7.8 pg/ml.

The results reveal that the number of viral particles as determined by p24 tends to be higher than the number obtained by RT or MTT assays. The end-point doses defined by RT and MTT assays usually correlated and depended on the viral strain and the type of host cell. We conclude that there is from 2 to 0 log difference between the total number of viral particles and the number of functional virions. According to multiple samplings at different days of the host cell cultures (range 1 – 4 days) the ratio of infectious to defective particles seemed to be remarkably stable. In a low-producing lymphocytic line such as ACH-2/LAV one out of 10 particles appeared to be infectious, whereas in a high producing cell line like MOLT-4/HTLV-IIIB only one out of 100 particles is infectious. In the lowest virus-producing U1/HIV-1 monocytic line every particle appeared to be infectious. Surprisingly, the same ratio was found for high producing MT-2 cells despite the fact that they carried the same HTLV-IIIB strain as MOLT-4 lymphocytes. It is possible that such high efficiency of infection is attributed to the synergism with HTLV-I phenotype mixing (Lusso *et al.*, 1990). It appears from these experiments that, *in vitro*, the ratio of a total number of viral particles (comprising defective particles) to infectious virions is constant for given virus-cell system. One may assume that this deliberation can be pertinent only to the artificial environment created *in vitro* and has little or no relevance to *in vivo* situation. However, a similar phenomenon of the stability of the ratio of defective to infectious particles was reported to exist also in vivo for primary HIV-1 isolates derived from the most diverse clinical sources (Lu and Andrieu, 1992; Lu *et al.*, 1992).

Acknowledgements. Special thanks are due to Caroline F. Coleman for editorial assistance. The technical assistance of Raisa Nagorny is very much appreciated. This work was supported in part by a grant from NIH T32 AI07382-03.

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