The History of HIV-1 Biological Phenotypes Past, Present and Future

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HIV-1 primary isolates have been classified according to their behavior in tissue culture into two distinct groups based on their growth kinetics, cytopathology and cellular tropism. Classification by growth kinetics and cytopathology is indicative of replication rate and syncytium induction in activated peripheral blood mononuclear cells (PBMC). Accordingly, the terms slow/low and rapid/high for replication pattern and non-syncytium inducing (NSI) and syncytium inducing (SI) for cytopathology were introduced [1–3]. In general, the terms SI and NSI correspond broadly to the rapid/high and slow/low phenotypes, respectively [4, 5]. A currently used method to distinguish between these HIV-1 phenotypes is based on the ability of a virus to infect and induce syncytia in the MT-2 T cell line [6]. These two groups of viruses are also distinct at the genetic level, SI viruses generally have a greater positive charge within the third variable region of the envelope glycoprotein than NSI viruses [7–9]. A third phenotype is defined by tropism and includes the terms T cell tropic, macrophage-tropic, dual-tropic [10] and T cell line-adapted (TCLA, reviewed in [11, 12]). Classification of primary HIV-1 isolates by tropism is, however, not as clear a classification as by replicative capacity and cytopathology, since conflicting data exist concerning macrophage tropism of several reported HIV-1 isolates. Macrophage tropism, as defined by the ability of the virus to infect and replicate in primary monocyte/macrophage cultures has been described either as a feature of primary viruses that mostly lack T cell line tropism [13] or as a general characteristic of HIV-1 isolates [10, 14]. Another report describes tropism as a continuous spectrum, ranging from viruses that infect macrophages with high efficiency and T cell lines with very low efficiency, to the converse [15]. Clearly, more experimental data are required to clarify this point. Finally, adaptation to growth in T cell lines changes the biological properties of primary HIV-1 isolates resulting in a variety of phenotypic and genotypic changes leading to altered cellular tropism and increased sensitivity to neutralization by antibody and soluble receptor [12, 16, 17].

The classification of SI and NSI, which corresponds broadly to the rapid/high and slow/low phenotypes, respectively, (see Table 1) is not as simple as it first appears. For example, NSI (slow/low) viruses are able to grow as rapidly as SI (rapid/high) viruses and form syncytia in primary cultures of CD4+ T cells depleted of CD8+ T cells ([18, 19] and unpublished results). Rapid/high viruses are similar

to SI viruses in that both are able to replicate in CD4+ cell lines of monocytoid or T-cell origin, and are usually isolated from late-stage, immunodeficient patients. Yet in cases where NSI viruses are isolated from AIDS patients, these viruses grow rapidly in PBMC cultures but do not infect monocytoid or T-cell lines. However, it has to be remembered that in spite of these complicating factors, the biological properties of HIV-1 have allowed the definition of virulence markers [20–22]. A more precise definition of such phenotypic traits associated with increased HIV-1 virulence will be of importance.

Table 1. Classification of HIV-1 biological phenotypes

		previous terminology based on			
				tropism	
Chemokine receptor usage	New Classification	cytopathology in MT-2 cells	replication rate in PBMC	concept 1	concept 2
CXCR4	**X4**	syncytium-inducing	rapid/high	T cell tropic T cell line -adapted	dual-tropic*
CCR5 CCR3/CCR2b	**R5** R3/R2b	non-syncytium-inducing	slow/low	macrophage-tropic	dual-tropic*
CXCR4 & CCR5 and/or CCR3	**X4R5** X4R5R3 X4R3	syncytium-inducing	rapid/high	T cell tropic	dual-tropic*
Bonzo, Bob and others	yet to be named	unclassified	unclassified	unknown	

* Dual tropism for macrophages and lymphocytes as defined by Valentin et al. [10].

The discovery that HIV-1 uses members of the seven transmembrane domain chemokine receptor family as co-receptors for membrane fusion and entry (reviewed in [23]) has changed our perception about the properties of both T cell line-adapted and primary HIV-1 isolates. The two most well-defined HIV-1 co-receptors are CXCR4 and CCR5, members of the CXC and CC chemokine receptor subfamilies, respectively (reviewed in [24]). CXCR4 was the first HIV-1 co-receptor to be characterized, and was shown to be required for the fusion of T cell line-adapted viruses with non-human cells expressing human CD4 [25]. It is expressed on many cell types including transformed T cells, fibroblasts, primary T cells, and macrophages. Subsequently, CCR5 was shown to be the principal co-receptor for primary HIV-1 isolates with the NSI phenotype [26–30], whereas the SI phenotype was associated with the use of CXCR4 alone or in combination with CCR5 [31–33]. Other members of the CC chemokine receptor family, such as CCR2b and CCR3, may also function as co-receptors for NSI virus entry, although generally in a less efficient manner than CCR5 [26, 28, 33]. The almost complete resistance to infection by slow/low, NSI-type viruses of individuals carrying a deletion of 32 base pairs in both alleles of the CCR5 gene confirms the importance of this molecule in HIV-1 transmission *in vivo* [34–36].

The previous classification systems based on replicative and syncytium inducing capacities of primary HIV-1 isolates in PBMC or MT-2 cells can be translated into classification based on co-receptor usage, thereby creating a molecular basis for defining HIV-1 biological phenotype. Thus primary and T cell line adapted HIV-1 isolates can now be grouped on the basis of co-receptor use. The classification systems presently used and their relationship to co-receptor usage are shown in Table 1. Thus viruses previously termed SI (rapid/high) which infect activated PBMC and readily infect CD4+ T cell lines are defined by their use of the CXC-chemokine receptor CXCR4. NSI (slow/low) viruses which preferentially infect activated PBMC would be defined by their use of members of the CC-chemokine

receptor family, principally CCR-5. According to the terminology based on co-receptor usage, CXCR4 using viruses would be termed X4 and CCR5-using viruses R5 [37]. Viruses that were previously defined as SI (rapid/high), and which use both CXCR4 and CCR5 (and/or CCR3) receptors, would be termed X4R5 (-R3) viruses. Since novel members of the chemokine receptor family which can function as HIV co-receptors continue to be cloned and characterized [38–43] it will be important to allow room for expansion within this classification scheme. A classification system, based on the clustering of a particular virus isolate or clone by the molecules required for virus entry and virus-induced cell-cell fusion should remain a clear and flexible system for the future.

Studies in which HIV-1 envelope genes have been expressed, either in the form of recombinant glycoproteins or as infectious virus, in the presence of CD4 and the appropriate co-receptor have demonstrated that both SI and NSI viruses are equally able to induce syncytium formation. Thus the terms syncytium-inducing and non-syncytium-inducing are not absolute and may only be relevant in terms of co-receptor expression levels on target cells. Similarly, both rapid/high and slow/low viruses appear to have comparable replication kinetics in transformed cell lines expressing both CCR5 and CXCR4 together ([44] and unpublished results) or separately [33, 45]. It is becoming clear that both CD4 and chemokine receptor expression levels are important factors in determining susceptibility of cells to different viruses.

The phenotypic classification system for describing the biological properties of viral isolates continues to be used in parallel with the adaptation of the new nomenclature, and should be interpreted in the context of the underlying molecular interactions. However, many aspects of the molecular relationships relating to viral phenotype are yet to be resolved. We know today that the basis of PBMC suitability for the isolation of a wide variety of HIV isolates is that activated CD4+ T lymphocytes contain populations that express either CCR5 or CXCR4 that can be infected with viruses using either or both molecules [47, 48]. In virus isolation cultures involving cultures with mixed donor PBMC [46], HIV-1 replication patterns still appear as slow or fast, low- or high-titre, respectively, and the level of expression of the relevant co-receptor in the PBMC cultures appears to determine the rate of replication [49]. Phenotyping viruses with the MT-2 cell line remains an efficient way of defining the SI/NSI phenotype. Similarly to other immortalized CD4+ T-cell lines, MT-2 cells express CXCR4 and are susceptible to CXCR4-using viruses. When using MT-2 cells to study viral phenotype, the assay should be indicated (for example, by specifying that the viruses are MT-2 negative or MT-2 positive), along with the more descriptive SI/NSI designation. Furthermore, the T-tropic and M-tropic classification should be used with care, such that a virus should only be called macrophage tropic when tested in primary monocyte/macrophage cultures. It is important to appreciate that classification by co-receptor utilization in cell lines expressing high levels of CD4 and co-receptors may not necessarily indicate that such viruses will use these co-receptors to infect primary resting cells *in vivo*. Thus, until a more complete understanding of the molecular basis for tropism is attained, we suggest that viruses should be classified according to the experimental system employed, in conjunction with the second receptor utilization patterns.

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