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BIOLOGICAL PHENOTYPE AND CORECEPTOR USAGE OF HUMAN IMMUNODEFICIENCY VIRUS*

(A SHORT REVIEW)

EVA MARIA FENYÖ

Microbiology and Tumorbiology Center, Karolinska Institute, 171 77 Stockholm, Sweden

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Introduction

The AIDS epidemic is well into its second decade and up to date has claimed 13.9 million lives. Human immunodeficiency virus (HIV) is the causative agent of AIDS and according to the December 1998 estimates of UNAIDS (United Nations AIDS Programme) 32.4 million people are living with HIV/AIDS worldwide. The main routes of HIV transmission are sexual, from mother to child or by intravenous drug use. At the beginning of the epidemics blood and blood products played an important role, but testing of blood donors and control of blood products have successively minimized the risk of becoming infected by this route.

HIV is a retrovirus, a member of the lentivirus subfamily. Lentiviruses occur in several animal species and cause slow – often fatal – diseases affecting various organ systems depending on the species and the age of the animal at the time of infection (reviewed in [1]). In HIV-infected humans, gradual depletion of CD4+ T cells results in immunodeficiency after several years and leads to the clinical entity of acquired immunodeficiency syndrome (AIDS). The rate of CD4+ cell depletion is highly variable in different individuals, from stable CD4 counts in long-term nonprogressors

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(>10 years) to a decline of 15×10^6 cells/liter/month leading to AIDS in a few years. Also, infection by HIV type 1 (HIV-1) as known in Central Africa, Europe and the Americas, leads to immunodeficiency much faster than infection with HIV type 2 (HIV-2) in West Africa [2]. Still today we do not know what exactly determines the disease progression rate in individual patients. Conceivably, virus-host cell interactions have a decisive role in this process. Here we shall focus on the biological phenotype of the virus which has been demonstrated to vary according to the severity of HIV infection, thus providing a marker for viral virulence.

Biological variation of HIV-1

The original observation some 14 years ago that the rate of HIV-1 replication and the amounts of virus obtained in primary isolation cultures vary according to the severity of HIV-1 infection in the patient suggested that we might be looking at viral determinants of the pathogenic process. This prompted us to further investigate HIV-1 biological phenotype, such as replication rate, cytopathology and cell tropism in tissue culture, in primary cells and established cell lines. Based on biological phenotype, such as replication rate, HIV-1 isolates could be divided into two major groups [3–5]. In one group virus could be isolated within days from peripheral blood mononuclear cells (PBMC) of HIV-1 infected immunodeficient patients, and was able to induce syncytia not only in PBMC but also in cell lines. Hence the designation, rapid/high or syncytium inducing (SI). The other group of viruses was characterized by a prolonged time to isolation (2–3 weeks), slow replication rate in PBMC, absence of or marginal cytopathology (small syncytia or cell killing) and inability to infect established T-lymphoid and monocytoid cell lines. Most primary HIV-1 infections occur with this latter type of virus, called slow/low or non-syncytium inducing (NSI). Those individuals that do become infected with rapid/high or SI virus lose CD4 cells at a faster rate than slow/low or NSI virus infected individuals [6]. Changes in viral phenotype may also occur within the same infected individual undergoing clinical progression, and have been shown to involve switch from NSI to SI [7, 8]. Using syncytium induction in MT-2 cells to test the phenotype of sequential isolates derived from a cohort of 53 HIV-1 infected homosexual men over a period of 5–8 years, we found that no change in NSI phenotype was associated with a better prognosis (Table I) [9]. Taken together, the data from our group and from several others indicate that HIV-1 biological phenotype is a marker for viral virulence. Recently, these phenotypic traits could be translated into molecular terms, such as coreceptor usage, opening new doors in our understanding of HIV-1 pathogenesis.

Table I

HIV-1 biological phenotype and CD4+ cell decline

MT-2 tropism	No. of patients	CD4+ counts $\times 10^6$ cells/liter	
		End of study	Baseline
Neg/Neg	26	327	489
Neg/Pos	20	104	397
Pos/Pos	6	57	308

Table II

Classification of HIV-1 biological phenotypes

Chemokine receptor usage	New classification	Previous terminology based on	
		cytopathology in MT-2 cells	replication rate in PBMC
CXCR4	X4	SI	rapid/high
CCR5	R5	NSI	slow/low
/CCR3/CCR2b	R3/R2b		
CXCR4 and CCR5	R5X4	SI	rapid/high
and/or CCR3	R3R5X4 or R3X4		

It has long been recognized that HIV-1 uses the CD4 receptor for cell entry [10, 11]. It has also been known that CD4 alone is not enough, since transfection of CD4 into non-human cells did not allow viral entry and infection of cells [12, 13]. By functional cDNA cloning an HIV-1 entry cofactor has recently been identified as a member of the seven transmembrane G-protein coupled receptor family [14]. Discovery of the first cofactor, shown to function as coreceptor for HIV-1 isolates able to infect established cell lines, was soon followed by several others [15–17]. The two phenotypically different groups of HIV-1, while both using CD4, could be distinguished by their coreceptor usage, inasmuch rapid/high or SI viruses were shown to use CXCR4, and slow/low or NSI viruses CCRS [18, 19]. This major pattern was straightforward and allowed the establishment of a new nomenclature (Table II) [20].

What are these cell surface structures that HIV learned to use as keys to enter cells? Recent development in the field of immunology has disclosed, in addition to

traditional cytokines (like interferons and interleukines) the existence of chemoattractant substances – called chemokines – that serve as mediators in cell-to-cell signalling (reviewed in [21]). Chemokines belong to a superfamily and show similarities in their primary structure, characteristically a conservation of 4 cysteines in a molecule composed of 65–95 amino acids. Depending on whether the first two cysteines are intercalated by an amino acid or not, chemokines fall into two major groups, CXC or CC chemokines. From the point of view of HIV-1, SDF-1 (stromal cell-derived factor-1) [22, 23] is the prototype for CXC chemokines and RANTES (regulated on activation normal T cell expressed and secreted), MIP-lα and MIP-1β^2 (32 represent CC chemokines [24]). While SDF-1 is produced in many different tissues and apparently has a house keeping function, RANTES, MIP-lα and MIP-1β are involved in inflammatory processes [25]. Chemokines exert their effect on cells by binding to specific receptor molecules followed by intracellular signalling. Chemokine receptors belong to the family of seven transmembrane G-protein coupled receptors, members of which have been identified as coreceptors for HIV-1. There are at least two consequences of this coincidence: i) chemokines may inhibit HIV-1 replication [24] by preferentially binding to the same receptor(s), ii) receptor availability may select for certain viral variants.

Phenotypic differences correlate with distinct coreceptor usage

To test chemokine receptor usage by primary HIV-1 isolates, human cell lines, such as the U87 glioma and the HOS osteosarcoma, were engineered to stably express CD4 and coreceptors [15, 18, 26]. Following HIV-1 infection, the U87.CD4 cell lines expressing CCR1, CCR2, CCR3, CCR5 or CXCR4 were scored for syncytia and p24 antigen production at day 3–7. GHOST(3) cells expressing the chemokine receptors CCR3, CCR5 or CXCR4 or the orphan receptors Bonzo or BOB, contain the green fluorescence protein (GFP) driven by the HIV-2 long terminal repeat. HIV-infected GHOST(3) cells express GFP and the fluorescence can be observed in a UV microscope and quantitated by flow cytometry. Using the U87.CD4 cell system we could show that HIV-1 isolates of different biological phenotype are distinguished by their ability to use the chemokine receptor CXCR4 for entry into target cells [18, 19]. Slow/low viruses formed syncytia on CCR5-expressing cells only (RS viruses), while rapid/high viruses used CXCR4 either alone or in combination with CCR5 (X4 or RSX4 dual tropic, respectively). Syncytium induction by an R5 and R5X4 virus is illustrated in Figure 1. It has to be pointed out that CXCR4-using viruses could often use several receptors, not only CCR5 but CCR3 and some even CCR2, suggesting that

a broader cell tropism might enable the virus to infect many different cell types and this may conceivably contribute to the increased virulence of these viruses. Moreover, we found that all HIV-1 isolates are syncytium inducing – provided the target cells carry the receptor required for infection by the particular virus. The receptor not only has to be present on the target cell surface but it has to be expressed in a high enough concentration to trigger syncytium formation. We know today that low CCR5 expression on PBMC allowed slow replication but rarely syncytia formation by viruses using this receptor (the so-called slow/low or NSI viruses), whereas high concentrations of CXCR4 in the same cultures allowed fast replication and extensive syncytia formation of viruses using CXCR4 (rapid/high or SI).

Fig. 1. Syncytium induction by an R5 and R5X4 virus

Sequential isolates from patients with progressive HIV-1 disease may differ in coreceptor usage

Changes in HIV-1 phenotype during clinical progression – often measured as the capacity to induce syncytia in MT-2 cells – have been observed by several groups over the years. Early work has shown that the change involved a phenotypic switch from NSI to SI, as a rule, and lead to the suggestion that SI virus is more virulent than NSI virus [7, 27]. Since we know today that the ability of HIV-1 to infect and induce syncytia in MT-2 cells is dependent on usage of the CXCR4 coreceptor, the phenomenon of viral phenotypic evolution could be revisited in terms of evolution of coreceptor usage. Our results have shown that both in adults and children who acquired HIV-1 infection from their mother, an R5 virus is present early in infection [19].

Clinical progression and decline in CD4 counts is often accompanied by either a switch from R5 to X4 or by a broadening of coreceptor usage yielding multitropic viruses (R5X4 or R3R5X4). In parallel with evolution of coreceptor usage, there is a change in the virus sensitivity to CC chemokines-mediated inhibition [28]. Replication of R5 viruses, but not those using CXCR4 (X4 or multitropic viruses), can be inhibited by RANTES, MIP-lα and often by MIP-1β as well [18]. Evolution to resistance by CC chemokine mediated inhibition occurs in about half of the AIDS patients, while R5 viruses with apparently preserved sensitivity to inhibition by CC chemokines can be recovered from the other half [29]. The impact of this change on the pathogenic process is not well understood. It is an attractive idea that the microenvironment, including cytokines, chemokines and available target cells, in the different organs of an infected individual, selects for virus with different biological properties.

Biological variation appears to be an universal property of HIV-1 isolates across genetic subtypes

Extensive genetic variation of HIV has been well documented (for latest update see ref. [30]). HIV-1 and HIV-2 show an overall difference of 50% in nucleotide sequences. A common measure of differences between viruses and groups of viruses is the divergence between env genes, the most variable of the HIV structural genes. Envelope homology between HIV-1 and HIV-2 is less than 50%. HIV-1 itself can be divided into three distantly related groups; the Major (M) and the Outlier (O) groups, and the newly identified N group of viruses [31]. The M group, which is by far the most widespread, is further subdivided into distinct “clades” or "envelope sequence subtypes", differing by approximately 30–35% at the nucleotide and amino acid

sequence level. The clades are phylogenetic groupings that probably represent a founder effect, that is, the historical beginnings of HIV-1 epidemics in different groups of people around the world. Clade determination is a convenient means of tracking the spread of the virus. *env* clade B, for example, was initially identified in viral isolates from Europe and North America. It has now spread to many other parts of the world. Clade C is the most frequently encountered world wide, mounting to 48% of all HIV-1 infections, while clade A represents 23.5% (UNAIDS 1998 estimates). Emergence of recombinant viruses composed of sequences from different clades in different parts of the virus have been described. For example, an A/B recombinant (A in *gag* and B in *env*) is held responsible for the HIV-1 epidemics in the Kaliningrad area of Russia [32]. The immediate question which arises is what impact has HIV genetic variation on the biology of the virus? Do different clades differ also in virulence? in transmissibility? Is the emergence of recombinant viruses a continuous threat to produce viruses with increased virulence and thereby accelerate the epidemics? Initial studies carried out within the framework of WHO Network involving replication and syncytium induction in PBMC and cell lines, showed no major differences in biological properties of subtypes A–E [33, 34]. More recent work not only confirmed that biological variation is a universal property of HIV-1 isolates across genetic subtypes [35] but showed that coreceptor usage varies with severity of HIV-1 infection. Like in subtype B infections, CXCR4-using viruses were frequently recovered from AIDS patients infected with subtype A, D and E, while individuals in earlier stages of HIV-1 infection yielded predominantly R5 viruses. However, subtype C appears to be at variance with this general pattern, in that isolates are R5, regardless of the severity of HIV-1 infection in the patient (Table III) [36]. Since this is true for subtype C isolates obtained in Sweden (15 isolates), Ethiopia (0/9) and South Africa (1/9) it cannot simply be the result of a founder effect in a certain geographic area. The results suggest that subtype-dependent differences in frequency of usage of certain coreceptors may exist. This opens up the possibility that genetic subtypes, subtype C in particular, may differ in important biological properties such as virulence, tissue tropism and transmissibility.

The promiscuous relative: HIV-2

HIV-2 is less pathogenic than HIV-1 and therefore lends itself for studies on coreceptor usage in relation to pathogenesis. Like HIV-1, a majority (10 out of 11) of HIV-2 isolates use CCR5. Among AIDS patients two out of seven isolates obtained used CXCR4 and showed syncytium induction. Similarities between HIV-1 and HIV-2 end here, however, because unlike HIV-1, most HIV-2 isolates use CCR1, CCR2,

CCR3, BOB and/or perhaps Bonzo as well [37]. Interestingly, nearly all HIV-2 isolates replicate in MT-2 cells, although they do not induce syncytia. In addition to CXCR4, MT-2 cells express BOB mRNA and HIV-2 infection most probably occurs by the orphan receptor BOB. The results indicate that while HIV-1 often evolve to multitropism in the course of the pathogenic process and multitropism is most often associated with CXCR4 usage, multitropism is a general property of HIV-2 isolates. Consequently, multitropism *per se* cannot be held responsible for differences between HIV-1 and HIV-2 pathogenesis.

Table III

Coreceptor usage of HIV-1 genetic subtypes A–E and severity of infection

Subtype	AIDS	No. of isolates	Coreceptor usage	
			CCR5	CXCR4
A	–	12	10	2
	+	8	4	4
B	–	13	13	0
	+	7	1	6
C	–	23	23	0
	+	13	13	0
D	–	5	3	2
	+	9	3	6
E	–	7	6	1
	+	2	0	2

To further investigate the *in vivo* relevance of multitropism, we infected PBMC from individuals homozygous for the deletion in the ccr5 gene (deletion of 32 nucleotides, the so-called Δ32 mutation [38, 39], with 11 HIV-2 and 10 HIV-1 isolates. Taking extracellular antigen production as evidence for productive infection, only cultures infected with viruses using CXCR4 showed signs of virus replication. Testing the antigen negative cultures by PCR revealed that HIV-2 DNA was present in most cases, whereas HIV-1 infected cultures were negative. In HIV-2 infected cultures that were initially antigen negative but PCR positive, virus replication could in some cases be detected several weeks later. Although preliminary, these results suggest that even if the main coreceptors are CCR5 and CXCR4, in the absence of CCR5 the multitropic HIV-2 may utilize, albeit less efficiently, other receptors for cell entry.

Conclusions

Viral biological phenotype is a key player in HIV-1 pathogenesis. Initially, biological phenotype has been defined by replication rate and cytopathology in primary cells and cell lines, but can today be translated into molecular terms, such as usage of different coreceptors at cell entry. CXCR4 usage determines the biological phenotype for viruses of A, B, D and E genetic subtypes and is often associated with AIDS. The fact that the change in virus biological phenotype may occur in the same individual over time and is associated with progressive disease has suggested that CXCR4 using viruses are more virulent. It is tempting to speculate that increased virulence following entry by the CXCR4 receptor is due to differences in receptor-mediated signalling. It remains to be seen whether signalling – if it occurs at all following HIV attachment through the CXCR4 receptor may cause a more severe perturbance of immune functions than signalling through the CCR5 receptor, explaining the differences between viruses using different receptors.

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