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The Role of Virus Biological Phenotype in Human Immunodeficiency Virus Pathogenesis

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Abstract

HIV-1 pathogenesis in children as well as in adults is closely associated with changes in co-receptor use of isolated viruses. The ability to use the CXCR4 receptor appears to be a marker of increased virulence, even if the mechanism(s) leading to an accelerated immunodeficiency awaits understanding. Multi-tropism, in the sense that the virus can use several receptors, is not a sign of increased virulence *per se,* since HIV-2 using many receptors, and presumably entering many different cell types, is less pathogenic than HIV-1. The SIV model is useful in studies of the very early events of virus infection and suggests that the availability of target cells may determine the pattern of co-receptor use.

Key words

HIV. HIV 2. Subtypes. Pathogenesis. Tropism. SIV. Co-receptors.

Definition of biological phenotype

The original observation of differential replication kinetics and cytopathicity of primary human immunodeficiency virus type 1 (HIV-1) isolates in peripheral blood mononuclear cells (PBMC) dates back to the mid-1980s, when two distinct phenotypes were observed[1]. One type of virus was easy to isolate from patient's PBMC, since the virus grew fast and culture supernatants yielded high reverse transcriptase activity (RT) within a few days. Such cultures showed extensive syncytia formation. Isolation of the other type of virus –in the majority of cases– needed more patience and cultures had to be kept for up to four weeks in order to ascertain that the low RT activity detected was indeed the sign of virus replication. Cytopathic effect was absent or consisted of hardly visible small syncytia or single cell killing. This led us to use the descriptive designation rapid/high and slow/low, respectively, for the two types of HIV-1[1]. Intriguingly, the two phenotypes were isolated from patient groups distinguished by the severity of HIV-1 infection. Slow/low viruses were isolated from patients with no or mild symptoms, while rapid/high viruses were obtained from patients showing signs of severe immunodeficiency. This suggested that we might be looking at an important determinant of HIV-1 pathogenesis.

Further work from our laboratory and by others confirmed the original observation[2-4]. Tersmette, et al. called the two types of viruses syncytium inducing and non-syncytium inducing, SI and NSI, respectively. Cheng-Mayer, et al. showed that change from slow/low or NSI to rapid/high or SI may occur in the same HIV-1 infected individual in the course of disease progression. Further analysis of the infectious properties of HIV-1 isolates revealed that slow/low viruses infect fewer cells in donor PBMC cultures than rapid/high viruses and viral RNA is more abundant in individual cells of cultures infected with rapid/high viruses than with slow/low viruses[5]. Tissue culture infectious dose-50 titres in donor PBMC were also dramatically different, low to undetectable with slow/low viruses and in the 3-$\log_{10}$ range with rapid/high viruses[6]. The clearest distinction between the two groups of viruses was achieved by testing replication in established cell lines of T-lymphoid or monocytoid origin[1-4,7].

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Rapid/high or SI viruses replicated in cell lines, while slow/low or NSI viruses did not. This led Koot, et al. to suggest the use of the MT-2 cell line for rapid and efficient screening of HIV-1 phenotype[8].

The riddle of these phenotypic differences was solved with the discovery that HIV-1 entry into cells requires consecutive binding of the virus envelope to at least two distinct cell surface structures. The CD4 molecule was known for over 10 years to function as –what we believed– the receptor of HIV-1 (HIV-2 and SIV as well) for cell entry[9,10]. However, the CD4 molecule promoted HIV-1 entry into human cells only, since hamster, mink or mouse cells carrying human CD4 could not be infected[11-13]. Berger, et al. were the first ones to isolate an additional cell surface structure that allowed entry of T-cell line adapted HIV-1 strains[14]. This original discovery was soon followed by the isolation of another structure that functioned as co-receptor for many of the HIV-1 primary isolates earlier named as slow/low or NSI[15,16]. These cell surface structures were identified as chemokine receptors known to belong to the family of 7-transmembrane G-protein coupled receptors (for a recent review see[17]). The two major co-receptors for HIV-1 fall into two groups of chemokine receptors, named after the primary structure of the chemokine(s) bound, CC and CXC, respectively. CCR5 is the major (and in many cases the only) co-receptor used by primary HIV-1 isolates with slow/low or NSI phenotype, whereas the ability to use CXCR4 enables rapid/high or SI viruses to enter established cell lines[18]. This allowed us to adapt a simple and straightforward nomenclature for HIV-1 phenotypic differences[19]. Viruses using CCR5 only are called R5 and viruses using CXCR4 only are called X4. Dual- or multitropic viruses are able to use two or more receptors, and are denoted according to the pattern of receptor use, for example R5X4 or R3R5X4, respectively.

The phenotypic differences between HIV-1 isolates observed in PBMC cultures can be explained by the expression pattern of these receptors *in vitro*[20]. CXCR4 is expressed on a majority of T-lymphocytes soon after culture allowing rapid replication and syncytium induction by viruses using this receptor. CCR5 expression is delayed and present on a minority of T-lymphocytes at any time during culture. Consequently, HIV-1 isolates using this receptor replicate slowly to low levels and show very little, if any, syncytium formation in PBMC.

The two types of chemokine receptors signify different functions within the immune system[21]. CXCR4 participates in regulation of so called house-keeping functions, it has an important role in re-circulation of lymphoid cells from tissues to secondary lymphoid organs. In contrast, CCR5 participates in immune defence mechanisms as an important component of inflammatory processes. CXCR4 and CCR5 are present on different cells in most organs with perhaps one exception the thymus, where double positive cells have been observed[20,22,23]. It is intriguing that HIV-1 not only adapted to use these two functionally different receptors but evolution of co-receptor use is an ongoing process in many HIV-infected individuals tightly coupled with disease progression.

Indicator cell lines for testing co-receptor use of primary HIV-1 isolates

Two human cell lines, a malignant glioma, U87, and an osteosarcoma, HOS, were engineered to express CD4 and one of the chemokine receptors used by HIV-1 (HIV-2 or simian immunodeficiency virus, SIV)[15,24,25]. Thus a series of cell lines became available from both U87 and HOS and can be used to dissect co-receptor use of primary HIV-1 isolates. Infection of U87.CD4 cells results in syncytium formation and productive infection in a co-receptor dependent manner. Syncytial cells appear 2-3 days after infection of cell lines carrying the major co-receptors, CCR5 and CXCR4, while syncytium formation is less extensive and is delayed for up to 10 days in cell lines carrying minor co-receptors (CCR3 and CCR2b)[18]. Syncytial cells are infected cells and express the HIV-1 p24 capsid antigen[26]. In this respect, it is interesting to note that all primary HIV-1 isolates are capable of inducing syncytia provided the cells carry the particular co-receptor used by the virus. Likewise, the differences in replication rate can no longer be observed in the indicator cell lines, R5 viruses replicate as efficiently as viruses using CXCR4. Thus the designations slow/low or NSI and rapid/high or SI apply for PBMC cultures and not for engineered indicator cell lines.

GHOST (3) cells carry, in addition to CD4 and one or another co-receptor, the gene for the green fluorescence protein (GFP) driven by the HIV-2 LTR[27]. Infection by HIV-1 (HIV-2 or SIV) induces GFP expression and infected cells appear green 2-3 days after infection. Cultures can be scored under a fluorescence microscope and the fluorescence quantified by flow cytometry[28,29]. Productive infection may also be documented with both series of cell lines on day 6 post-infection by measuring the HIV-1 p24 antigen content of supernatant culture fluids.

The U87.CD4 cell lines tested in one series of experiments include the parental cells and cells expressing each of the chemokine receptors CCR1, CCR2b, CCR3, CCR5 or CXCR4. In addition to the same series of cell lines, GHOST (3) cells expressing Bonzo, the receptor for a transmembrane CXC chemokine[30], or the orphan receptor BOB are also available. These indicator cell lines are generally available from international repositories, NIBSC in Europe and NIH in USA[31,32].

Evolution of HIV-1 co-receptor use with increasing severity of infection

During the late 1980s and early 1990s large studies were conducted by several research groups on the change that might occur in HIV-1 phenotype during clinical progression. The consensus of these studies was that in approximately one-half of HIV-1 infected individuals the virus phenotype changes from NSI to SI[7,33,34], but clinical progression might

also occur without apparent change in virus phenotype. The use of a single established T-lymphoid cell line, MT-2, was instrumental in these studies[33,34]. SI viruses but not NSI viruses could infect and induce syncytia in MT-2 cells. Syncytia were very easy to score, thus the two types of viruses could be distinguished with a simple tissue culture assay. With the recognition of the molecular basis of HIV-1 biological phenotype it was important to formally prove that MT-2 positivity (or, in general, the ability to infect and replicate in established cell lines) translates into the ability to use CXCR4 as co-receptor. Indeed, this was the case. The U87.CD4 indicator cell lines were then used to test sequential isolates from HIV-1-infected adults and children undergoing disease progression. The results showed that HIV-1 isolates obtained during the asymptomatic phase of infection generally use the CCR5 co-receptor[18,35,36], as exemplified in table I. Such R5 isolates are sensitive to inhibition by CC-chemokines (such as RANTES, MIP-1α and MIP-1β)[18,36,38]. Disease progression is in the majority of cases accompanied by a shift of co-receptor use, often to CXCR4 use. Aquisition of CXCR4 use early in infection is predictive of a poor prognosis in case of transmission sexually[39,40] or from mother to child[36,41].

The association of CXCR4-using viruses with severe immunodeficiency suggests that these viruses are more virulent than R5 viruses –a conclusion similar to that drawn earlier on the basis of SI/NSI definition in MT-2 cells. If so, what is the mechanism of HIV-1 virulence? Since CCR5 and CXCR4 are expressed on different cells in most organs[20,22,23], viruses using different receptors may selectively damage or eliminate one or the other cell type. Conceivably, infection of a certain sub-population(s) of cells, presumably with CXCR4 expression, like *naïve* CD4+ T-cells[42], is crucial in pathogenesis. Indeed, along with our increasing ability to distinguish functional subsets of T-cells by testing for phenotypic markers the picture of HIV pathogenesis is unfolding. *Naïve* (CD45RA+/CD62L+) and memory CD4+ T-cells (lacking one or both of these markers), although both infected with HIV-1 *in vivo*, show differences in provirus content and in the amount of virus produced when cultured *in vitro*. Memory cells harbour more provirus and produce virus more readily than *naïve* CD4+ T-cells[43-45]. Virus with R5 phenotype seems to be responsible for establishments of latent HIV-1 reservoir shown to be present in resting memory cells (lacking activation markers HLA-DR, CD25 and CD69)[46]. Using limiting dilution

Table Ia. *Sequential HIV-1 isolates from adults*

Patient no.	Time from first positive sample (months)	Patient clinical data		Phenotype of the virus isolate
		CD4+ T cell count (per mm^3)	Therapy*	
1276	46	320	—	R5
	62	270	—	R5
	76	180	AZT	R5
	95	220	AZT	R5
	110	140	AZT	R5
	126	40	AZT	R5
1865	49	340	—	R5
	55	360	—	R5
	67	20	—	R3X4
	70	8	AZT	R3X4
	94	10	AZT	X4
	109	NA	AZT	X4
2282	10	350	—	R5
	21	230	—	R5
	33	330	AZT	R5
	41	184	AZT	R5
	47	180	AZT	R5X4
	58	90	—	R5X4
	66	180	ddI	R5X4
	70	40	ddI	R5X4
2289	14	490	—	R5
	33	290	—	R5
	46	310	AZT	R5
	63	230	AZT	R5
	73	370	—	R3R5X4
	81	240	—	R5X4
	93	70	—	(R3)R5X4
	115	40	—	R5X4
	119	30	—	(R3)R5X4
	123	70	—	R5X4

*The total number of samples tested was higher than included in table Ia: from patient 1276, 1865, 2282 and 2289 nine, eight, 15 and 26 samples were tested, respectively.

Table Ib. *Sequential HIV-1 isolates from children**

Child no.	Age (months)	Patient clinical data		Phenotype of the virus isolate†
		CD4+ T cell count (per mm^3)	Therapy**	
3	6	2920	—	R5
	40	1512	—	R5
	54	645	AZT	X4 (R5R3)
	65	183	AZT + ddC	X4 (R5)
	76	36	AZT + ddC	X4
32	3	2092	—	R5
	10	NA	—	R5
	42	264	AZT	R5
	65	152	AZT + ddC	R5
	77	437	AZT + ddC, death	R5
136	3	3723	—	R5
	34	1117	—	R5
	60	359	—	R5X4
	64	270	—	R5X4
	67	412	AZT	X4 (R5R3)
	76	378	AZT	R5X4
	83	198	AZT + ddC	R5
	92	345	AZT + 3TC	R5
145	1	2386	—	R5
	5	1769	—	R5
	48	331	—	X4
	67	28	—	X4
	74	11	—	X4
	83	6	AZT + 3TC	X4

*The data up to 65-67 months of age were included in[36].
**AZT, zidovudine; ddI, didanosine; ddC, zalcitabine; 3TC, lamivudine.
†Co-receptor use was tested in the U87.CD4 series of indicator cells.

cultures and monoclonal antibodies to the two isoforms of CD45, RA and RO, Blaak, et al.[47] were able to demonstrate that infection of *naïve* (CD45RA+) CD4+ T-cells *in vivo* occurs primarily by CXCR4-using viruses. On the other hand, memory cells (CD45RO+) were infected with a mixture of CCR5- and CXCR4-using viruses. The fact that the frequency of HIV-1-infected *naïve* cells correlated with the rate of overall CD4+ T-cell decline in the patients, allowed the authors to suggest that infection of the *naïve* CD4+ T-cell population with CXCR4-using virus is responsible for the accelerated T-cell decline. In addition, HIV-1 infection of *naïve* CD8+ T-cells has also been demonstrated[48]. Infection of *naïve* cells is likely to occur early in T-lymphocyte ontogeny, perhaps during maturation in the thymus, and may lead to destruction of cells destined to become CD8+ lymphocytes. If so, this may explain the observed decline in CD8+ lymphocyte frequencies and function in HIV-1-infected individuals with progressive immunodeficiency. Conceivably, disturbances in the function of the CD8+ T-cells[49] may contribute to disease progression. Many more markers that distinguish functional subsets of T-cells await testing. It is hoped that delineation of key populations of T-cells, will increase our understanding of HIV-1 pathogenesis.

Another line of thoughts deals with differences in signalling through the different receptors. Signalling through CXCR4 after binding the natural ligand, SDF-1[50,51] is essential for re-circulation of lymphocytes between tissues and secondary lymphoid organs and disruption of this immune network by HIV-1 infection may severely damage the "house keeping" immune functions. Still another possibility is that signalling through CXCR4 is different from signalling through CCR5[52]. Recent reports describe that binding of different HIV-1 envelopes by chemokine receptors may or may not involve signalling in a cell type dependent manner[53-55]. HIV-1 virulence may therefore be the result of virus-cell interaction, rather than the property of a particular virus strain. This is in line with the thinking of Casadevall & Pirofsky[56] who define virulence, in general, as the outcome of the interaction between host and invading microorganism.

Co-receptor use of different HIV-1 genetic subtypes

The evolution in co-receptor use just discussed is based on observations with one genetic subtype –subtype B– of HIV-1. Genetic variability of HIV-1 has been extensively studied (for a recent review see[57-59]. Based on nucleotide sequence information HIV-1 has been divided into three groups: M, N and O. The HIV-1 M group is responsible for the major AIDS epidemic and is further subdivided into several subtypes (A through K) and circulating recombinant forms (CRF). A common measure of differences between these groups of viruses is the divergence between *env* genes, the most variable of the HIV-1

structural genes. The different envelope sequence subtypes differ by approximately 30-35% at the nucleotide and amino acid sequence level. It is therefore important to consider whether this genetic variation has any impact on biological properties of viruses belonging to different subtypes.

Using the U87.CD4 indicator cell lines, we tested co-receptor use of HIV-1 isolates of genetic subtypes A through E (E renamed CRF01_AE) derived from children and adults in different geographic regions. Table II summarises our results for the adults[26,60,61]. Our data together with those from other groups[62,63] show a similar pattern for subtype A, B, D and E isolates, in that non-AIDS patients yield, as a rule, virus with R5 phenotype or with the R5 phenotype compatible V3 genotype, while the majority of isolates obtained from AIDS patients uses CXCR4 (X4 monotropic, dual- or multi-tropic viruses). The results thus confirm the pattern established with HIV-1 of subtype B that viruses with CXCR4 use are more common in individuals with severe immunodeficiency. However, subtype C viruses seem to be at variance with this general pattern, in that CXCR4 use is rare among HIV-1 isolates of subtype C. In fact, none of the AIDS patients carrying subtype C virus yielded other than R5 isolates in our hands[61]. Similar observations were reported by other groups[64-67]. Since the isolates tested were obtained from different geographic regions, this difference seems to be a common characteristic of subtype C isolates.

Among the 23 children's isolates nine were from children with severe immunodeficiency (Table III). Four of these isolates, two of subtype A, one D and one CRF01_AE (the former E subtype) could use CXCR4 as co-receptor. All other children yielded virus with R5 phenotype. The results further confirm the general pattern seen with viruses obtained from adults that CXCR4 use is associated with an increased severity of infection. Moreover, evolution of co-receptor use in children infected with HIV-1 subtype A, D or CRF01_AE could be observed during clinical progression[41]. Again, no CXCR4-using subtype C isolate was obtained, supporting the notion that HIV-1 of subtype C maintains the R5 phenotype during the entire course of HIV-1 infection.

Table II. *Co-receptor use of HIV-1 subtypes A-D isolated from adults*

Genetic subtype	Country of origin*	Clinical status	Virus phenotype†				CXCR4-users
			R5	X4	dual/multitropic		
A	SE, BL, TZ	non AIDS	16‡	1	3	R5X4, R3R5X4, R3R5	15%
		AIDS	4	1	3	R5X4	50%
B	SE, RU, IN	non AIDS	17	1	4	R5X4, R3X4	23%
		AIDS	1	1	5	R3X4, R3R5X4, R2R3R5X4	86%
C	SE, TZ, ZA, IN, ETH	non AIDS	33	–	3	R3R5, R5X4	6%
		AIDS	13	–	–		0%
D	SE, TZ	non AIDS	5	4	1	R3X4	50%
	SE	AIDS	3	6	–		67%

*SE, Sweden; BL, Belorussia; TZ, Tanzania; RU, Russia; IN, India; ZA, South Africa; ETH, Ethiopia. Isolates from countries other than Sweden were collected through the UNAIDS Network for HIV Isolation and Characterisation.
†The U87.CD4 series of indicator cells were used.
‡Number of isolates.

Table III. *Co-receptor use of HIV-1 subtype A-D and CRF01_AE isolated from children infected perinatally**

Genetic subtype	Clinical status	Virus phenotype†			
		R5	X4	dual/multitropic	
A	Non-AIDS	5‡	-	-	
	AIDS (B3, death)	2	-	2	R5X4
B	Non-AIDS	3	-	-	
	AIDS (N3,B3)	2	-	-	
C	Non-AIDS	3	-	-	
	AIDS (A3)	1	-	-	
D	Non-AIDS	2	-	-	
	AIDS (C3)	-	1	-	
CRF01_AE	Non-AIDS	1	-	-	
	AIDS (B3)	-	-	1	R5X4

*Children were born in Sweden. The mothers of children carrying subtype A virus originated from Central Africa or Eastern Europe. The mothers of children carrying subtype B were born in Sweden, while those carrying subtype C and D were from Sub-Saharan Africa. The mothers of children carrying CRF01_AE were from Asia.
**Tested on both the U87.CD4 and GHOST (3) series of indicator cell lines.
‡Number of isolates.

Subtype C is responsible for more than 50% of the global HIV-1 epidemic[68]. It is therefore important to explore the differences to other genetic subtypes of HIV-1. In absence of demonstrable evolution of virus co-receptor use, other mechanisms of immunodeficiency are sought for. One hypothesis is that subtype C is particularly sensitive to TNF-α induction due to several (≥3 sites) NFκB sites in the LTR[69-71]. Cloning of the subtype C NFκB site upstream of different promoters resulted in an enhancer gain-of-function when compared to NFκB sites obtained from other subtypes[72]. This in conjunction with elevated levels of TNF-α in AIDS patients[73] and particularly those carrying subtype C viruses[74] has been suggested to be responsible for clinical progression in absence of detectable CXCR4-using virus[60,61,65]. However, in a recent work[75] not all subtype C viruses were found to have a high number of NFκB sites. Another mechanism that could conceivably lead to accelerated immunodeficiency is *in vivo* evolution to increased replicative capacity *in vivo,* without change in co-receptor use[76]. Blaak, et al.[77] demonstrated that in those individuals who develop immunodeficiency in the absence of apparent change in virus (HIV-1 subtype B) co-receptor use, the virus replicative capacity increases over time and is concomitant with increase in plasma viral load. Whether this is the case with subtype C viruses has not been tested yet. Still other contributing factors to clinical progression could be the continuous high level of immune stimulation, like in Ethiopians with concurrent helminthic infections[78,79]. Interestingly, ethnic Ethiopians in Israel have a higher CCR5 expression on blood lymphocytes and monocytes and low production of CC chemokines[80]. Conceivably, increase in availability of CCR5 expressing target cells may favor replication of R5 virus.

Taken together, evolution of co-receptor use seems to be the rule with HIV-1 subtype A, B, D and CRF01_AE during progressive disease. Subtype C apparently does not follow this rule, in that the great majority of isolates obtained at different geographic sites are R5 monotropic regardless of the severity of HIV-1 infection. To clarify the pathogenic mechanisms operating in subtype C infection may be a key question with potential to elucidate HIV-1 pathogenesis in general.

The maze of macrophage tropism

Macrophage tropism of HIV-1 is an important question since macrophages have been implicated as the first line of cells infected, as carriers of HIV-1 infection to many different organs of the infected host, and as viral reservoirs for the persistent HIV-1 infection. Yet, classification of primary HIV-1 isolates by tropism was not as clear a classification as by replicative capacity and cytopathology. Conflicting data existed for many years concerning macrophage tropism of HIV-1. Today's consensus is that macrophage tropism, as defined by the ability of the virus to infect and replicate in primary monocyte /macro-phage cultures, is a general characteristic of primary HIV-1 isolates[36,81-84]. The earlier described pattern[85] that macrophage tropism is a feature of those HIV-1 isolates that lack T cell line tropism might have been due to quantitative differences in replication in primary macrophage cultures. It was a too simplistic view to call viruses with NSI/R5 phenotype macrophage tropic and viruses with SI/X4 phenotype T-cell tropic. HIV-1 primary isolates are dual-tropic in the sense they are able to replicate in primary T-cells and macrophages[81,84,86,87]. It is clear today that macrophages (at least monocyte-derived macrophages from blood) express functional CCR5 and CXCR4 receptors on their surface[83,88,89], although alternative views relating to a non-functional CXCR4 have also been expressed[90]. HIV-1 isolates may use, according to their requirements, either CCR5 or CXCR4 for entry into macrophages. Dual tropism of HIV-1 can result from the utilisation of both of these receptors on macrophages and T cells like by R5X4 viruses, while efficient utilisation of CXCR4 on both macrophages and T cells (and T cell lines) by X4 viruses equally leads to dual tropism[84]. It is therefore more adequate to describe macrophage tropism as a continuous spectrum of efficiencies with which viruses infect and replicate in macrophages[91]. The replication of HIV-1 with different co-receptor use in MDM cultures is illustrated in figure 1. A panel of cloned HIV-1 variants and chimeric viruses with known co-receptor use[92,93] initiated productive infection in every case, regardless whether CCR5 or CXCR4 was used for cell entry. The quantitative differences in replication of different virus strains may be explained by other viral or host cell factors and not by receptor use alone.

One possibility is that different HIV-1 isolates have different requirements for receptor concentrations and that CD4 and co-receptor requirements are quantitatively interdependent[94]. In fact, Valentin, et al. suggested that the limiting factor for HIV-1 infection of terminally differentiated macrophages may be the low surface expression of CD4[86]. While X4 viruses are more dependent on high CD4 concentrations, R5 viruses are not[95,96]. This could also favor replication of R5 viruses in macrophages. In addition to the variation in receptor concentrations related to the differentiation process itself, different culture conditions seem to favour different receptor concentrations on the cell surface[97,98] and may therefore select for different variant viruses.

Recently, interesting new ideas have been put forward about HIV-1 entry and receptor signalling. Arthos, et al.[55] found differences among CCR5-using HIV-1 isolates in their capacity to signal through the CCR5 receptor. Efficient signal transduction was associated with efficient replication in macrophages. In contrast, envelopes obtained from isolates not able to replicate gave weak or no signal at CCR5. This indicates that signalling is essential for some post-entry steps of virus replication. Replication of viruses carrying envelopes not able to signal by themselves can be promoted if signalling is provided exogenously, for example by a chemokine.

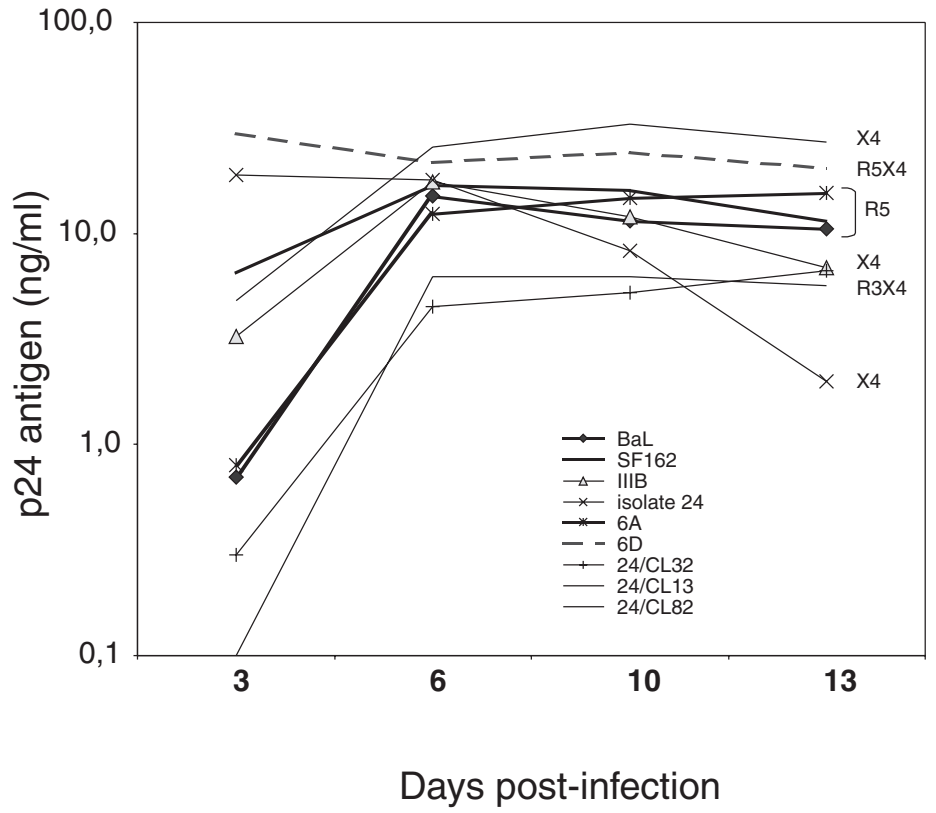

Figure 1. *Kinetics of HIV-1 p24 antigen production in monocyte-derived macrophage (MDM) cultures following virus infection (1.4-2.2 ng p24). MDM cultures were prepared according to a previously established protocol[81]. All virus stocks were prepared in PBMC. MDM cultures were infected overnight, then rinsed twice to remove added virus, fresh medium added and the cultures were further incubated. Samples were collected from duplicate cultures at time points indicated. Biological characterisation of the different viruses was described in[18,93,124]. Data from[124].*

Taken together, HIV-1 signalling capacities and the chemokine concentration in the microenvironment of tissues may determine efficient replication of HIV-1 in a cell-type specific manner. We are just beginning to appreciate the complexity of HIV-1 macrophage tropism.

HIV-2, the promiscuous relative

HIV-2 is more closely related by nucleotide sequence to SIV of sooty mangabey origin than to HIV-1. HIV-2 does not show the same explosive epidemiology than HIV-1 but is confined mainly to West Africa. The time to onset of symptomatic HIV-2 infection has been estimated to be at least 10 years longer than for HIV-1 infection and HIV-2 appears to be less transmissible (1-2% from mother to child)[99-101]. This lower pathogenic potential of HIV-2 makes it an excellent model for comparative studies of HIV-1 pathogenesis. Is HIV-2 less virulent than HIV-1? How is HIV-2 phenotype, in particular co-receptor use, related to that of HIV-1?

The first studies of HIV-1 co-receptor use indicated that use of CXCR4 is indicative of a virus with increased virulence. In contrast to R5 viruses, CXCR4 using viruses could often use several co-receptors and it was an attractive idea to explain virulence with the broader range of cells infected and damaged by these viruses. It was therefore surprising to see that HIV-2 can enter cells using many different co-receptors[25,102]. Our earlier work showed that HIV-2 biological phenotype is similar to HIV-1[103,104]. This has been confirmed in more recent experiments involving tests of co-receptor use of 10 HIV-2 isolates. Accordingly, nearly all viruses used CCR5, and two isolates (out of six) obtained from AIDS patients used CXCR4[25]. The similarities to HIV-1 end here because none of the HIV-2 isolates were R5 monotropic, but used in addition to CCR5, at least the orphan receptor BOB/GPR15. Due to use of BOB as co-receptor all HIV-2 isolates could initiate productive infection in MT-2 cells, even if this appeared with a delay and was not associated with syncytia formation like the infection with CXCR4-using viruses. All but two HIV-2 isolates used CCR1, four CCR2b, six CCR3 and nine used Bonzo/STRL33 very inefficiently. Co-receptor usage pattern showed no correlation to the severity of HIV-2 infection.

It was then of interest to see whether HIV-2 can indeed use alternative receptors when entering PBMC. Comparison of infectious dose-50 (ID-50) titres in PBMC lacking functional CCR5 receptor due to the Δ32 deletion in the ccr5 gene, homozy-

gote (–/–) and wild type (+/+) PBMC showed an occasionally > 2 $\log_{10}$ difference in this 7-day assay[105]. This led the authors to conclude that HIV-2, like HIV-1, uses CCR5 and CXCR4 as the main co-receptors. Analysing the kinetics of replication in the two kinds of PBMC showed, however, that the four HIV-2 isolates tested, all replicate in CCR5 –/– PBMC. Replication was delayed with approximately 10 days but peaked higher in three out of four cases as compared to CCR5 +/+ PBMC. This indicates that HIV-2 can use a co-receptor(s) other than CCR5 (and CXCR4) to enter PBMC. The delay in replication in these experiments may be due to the delayed expression of the particular receptor during *in vitro* culture of PBMC. It is tempting to speculate that use of different receptors by HIV-2 *in vivo* may provide a signal(s) counteracting immune destruction caused by virus replication.

What can we learn from the SIV model?

SIV from sooty mangabey origin (SIVsm) is closely related to HIV-2 by nucleotide sequence (85% homology), but like HIV-2, a more distant relative of HIV-1. SIVsm infection is pathogenic in macaques causing immunodeficiency and death in a few months to years[106,107]. Since simian AIDS (SAIDS) has many similarities to AIDS in humans, SIVsm infection in macaques has been used as a model for HIV-1 infection in humans (reviewed in[108,109]). We have examined evolution of co-receptor usage of SIVsm during pathogenic infection of cynomolgus macaques[29]. Experimental infection was performed with virus stocks that had slightly different passage history: low passage in a human T-lymphoid cell line (A), prolonged passage in human PBMC (B) or isolated directly from a diseased macaque (C). Sequential virus samples, isolated on human PBMC, were available from eight monkeys[110] and were tested for co-receptor use in the GHOST(3) indicator cell system. The results showed that all virus stocks and re-isolates used CCR5 with high efficiency. CCR5 use remained stable over time. BOB and Bonzo use were less efficient and, particularly the ability to use Bonzo, declined over time. CXCR4-using virus could not be detected at the time of overt immunodeficiency, even if such virus was present early in infection. The results thus show that evolution of SIVsm co-receptor use during pathogenesis involves a narrowing down from initial multi-tropism to CCR5 use. This is a very different result from that obtained in HIV-1 infection, where evolution, whenever detected, proceeds from R5 to a CXCR4-using mono- or multi-tropic virus (Table IV). However, in a few cases disappearance of CXCR4-using virus has been described also in HIV-1 infected individuals[34,39]. Fine dissection of functional T-cell subsets involved may give a clue to this phenomenon.

In spite of these differences, the macaque model allows studies of the very early time of infection. We found that early re-isolates obtained 12 days post-infection use a receptor spontaneously expressed on GHOST (3) cells. Virus isolated a few weeks later no longer used this receptor. Likewise, there was a decline in the ability of the isolated virus to use CXCR4 as well. We suggest that in the initial phase of infection the availability of target cells governs the replication pattern of the virus. The initial target cells may subsequently decrease in number as a consequence of virus replication. Veazey, et al.[111] found that activated memory T-lymphocytes in the lamina propria of the intestinal epithelium are the initial target cells for SIVmac (from macaque origin) replication. Profound and selective depletion of these cells could be observed during the first two weeks of infection. Loss of these preferred target cells was permanent, and replacement occurred by *naïve* lymphocytes and macrophages. We know today that different types of T-cells express different chemokine receptors[20,95] thus providing a different set of target cells at various times of SIV/HIV infection.

Future directions

We have to learn more about the interaction between the viral envelope and co-receptors. The crystal structure of the interaction of an envelope core structure with CD4 and a neutralising monoclonal antibody has already provided a model that shaped our thinking for the last three years[112-115]. We know that the co-receptor binding site is formed by non-continuous regions that come together in the native envelope structure. Other parts of the viral envelope –absent from the crystal structure due to their disturbing high mobility– also seem to participate in the receptor binding process. Increased

Table IV. *Comparison of HIV-1, HIV-2 and SIV co-receptor use*

	Monotropic R5	Multitropic		Change over time	
		Non-CXCR4 user	CXCR4 user		
HIV-1	Majority	Few*	In AIDS	Yes	R5 → (R5X4) →X4
HIV-2	None	All	In AIDS	Unknown	?
SIVsm	None	All**	Early in infection	Yes	Narrowing tropism R5BonzoBOB(X4) → R5BOB → R5

*Mainly subtype A: R5Bonzo[116].
**Exception: one SIVsm strain after prolonged passage in human PBMC used CXCR4.

envelope charge may facilitate binding of virus particles to the cell surface[116-119]. How exactly does the virus choose its co-receptor? Viruses with widely different envelope sequence, such as HIV and SIV, can use the same receptor, CCR5. This is possible if envelope conformation is conserved and the basic shapes recognised are provided by the folding envelope protein. What part of the 7-transmembrane receptor is important in binding the viral envelope? This question has been addressed in several studies[120-122], still the complexity is astounding. Different virus isolates may use different parts of CCR5 and CXCR4, the N-terminal and 3 extra-cellular loops and their combinations. This may be still another trick played by HIV variability and capacity to adapt, but the way of ligand binding may be important in signalling by these G-protein coupled receptor molecules[123]. We are just beginning to see that receptor signalling may be part of the viral life cycle, promoting virus replication in one cell but not in another. In this respect the issue of macrophage tropism will have much to disclose. Similarly, interaction between different receptors in supporting (or not) HIV-1 entry and replication in cells will have to be dissected. Different organs in the body may provide very different microenvironments for virus replication and HIV-1 with its high plasticity may use the different opportunities in various ways. By learning its ways, we may master HIV one day.

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