HIV-1 Coreceptor Use: A Molecular Window into Viral Tropism

Robert W. Doms[1] **and John P. Moore**[2]

[1] *Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104;*
[2] *The Aaron Diamond AIDS Research Center, The Rockefeller University, 455 First Avenue, New York, NY 10016*

The identification of the chemokine receptors CCR5 and CXCR4 as the major coreceptors for HIV-1 has provided a new framework for understanding viral tropism and pathogenesis at the molecular level. It is now possible to assign molecular designations to virus isolates, based on their coreceptor use, that largely explain viral phenotype (reviewed in [Berger(1997), Broder & Collman(1997), Doms & Peiper(1997), Moore(1997)]). The need to accurately describe viral phenotype arises because HIV-1 strains can exhibit distinct cellular tropisms that have important implications for viral pathogenesis and disease progression. Generally, virus strains that are transmitted between individuals are able to infect both macrophages and primary CD4+ T-cells, but are unable to replicate in transformed T-cell lines [Connor et al.(1993), Roos et al.(1992), Schuitemaker et al.(1992), Zhu et al.(1993)]. As a result, they fail to form syncytia when grown in MT-2 cells or in certain other commonly used cell lines. Viruses with these properties have been referred to both as macrophage tropic (M-tropic) due to their ability to infect macrophages, non-syncytium inducing (NSI) due to their inability to form syncytia on T-cell lines, or slow-low (SL) in reference to their replication kinetics in culture [Fenyo et al.(1988), Schuitemaker et al.(1992)]. With time, typically about 4-5 years after infection, virus strains evolve in some individuals (about 50%) which can infect T-cell lines in addition to primary T-cells [Tersmette et al.(1988), Tersmette et al.(1989)]. While this shift in tropism can sometimes be accompanied by a loss of ability to infect macrophages, more often primary isolates retain this property and so are referred to as dual-tropic [Collman et al.(1992)]. Viruses able to infect T-cell lines have been variously referred to as T-tropic, syncytium-inducing (SI), or rapid-high (RH) according to the nomenclature schemes mentioned above. The emergence of these virus types is correlated with accelerated disease progression [Connor et al.(1993)]. Finally, viruses that are well-adapted to grow on transformed cell lines by continual passage are referred to as T-cell line adapted (TCLA). TCLA viruses exhibit greater sensitivity to neutralization by both antibodies and soluble CD4.

The above three systems for classifying viral strains are problematic for several reasons. The M-tropic designation, for example, can be taken incorrectly to mean that a virus is unable to replicate in primary T-cells. Likewise, T-tropic viruses have been described that retain the ability to infect macrophages. The NSI designation is also misleading, as it suggests that there is something inherently wrong with the env protein from these virus strains. It also implies that SI viruses are more fusogenic and cytopathic, which is not necessarily so when they are grown in primary CD4+ T-cells. It is now known that the SI/NSI designation is an artifact resulting from the fact that T-cell lines express CXCR4 but not CCR5; the env proteins from NSI viruses are perfectly capable of forming syncytia provided that target cells expressing CCR5 are used. Finally, an additional complication arising from the current classification schemes is that the terms M-tropic, NSI, and SL are often used synonymously, as are T-tropic, SI, and RH. While this is sometimes appropriate, there are many instances in which these terms are not synonymous.

Chemokine Receptors and Viral Tropism

The major determinant of viral tropism is at the level of entry, more specifically at the level of membrane fusion with CD4+ cells. This occurs efficiently only if the appropriate coreceptor is present. Thus, M-tropic, NSI viruses use CCR5 in conjunction with CD4 for fusion. Direct interactions between the env glycoprotein and CCR5 have been detected [Trkola et al.(1996), Wu et al.(1996)],

and it is likely that this interaction triggers the final conformational changes in env that promote fusion between the viral and cellular membranes. The importance of CCR5 for HIV-1 infection in vivo was shown by the discovery that approximately 1% of Caucasians lack CCR5, and that these individuals are highly resistant but not entirely immune to virus infection [Dean et al.(1996), Liu et al.(1996), Michael et al.(1997), Samson et al.(1996)]. Thus, CCR5 is the major coreceptor for HIV-1 transmission in vivo. However, while CD4-positive cells obtained from CCR5-negative individuals are resistant to infection by viruses that require this coreceptor, they are readily infectable by viruses which use CXCR4 [Liu et al.(1996), Rana et al.(1997), Samson et al.(1996), Zhang et al.(1997)]. It is not clear why CCR5-negative individuals are only rarely infected by viruses that can use CXCR4 for cellular entry (for some examples see [Balotta et al.(1997), Theodorou et al.(1997), O'Brien et al.(1997), Biti et al.(1997)]. It appears that viruses which use CXCR4 are inefficient at establishing an infection in a naive host, for unknown reasons.

While M-tropic NSI viruses use CCR5 for cellular entry, CXCR4 is the coreceptor most commonly used by T-tropic, SI virus strains [Feng et al.(1996)]. Viruses that use CCR5 for entry can evolve to use CXCR4 through mutations in the env glycoprotein, usually but not always in the V3- loop. Despite less than 20% amino acid identity between CCR5 and CXCR4 in their extracellular domains, several viruses are known to efficiently use both coreceptors for cellular entry. Use of CXCR4 is largely dependent upon the first and second extracellular loops of this receptor, which are considerably more anionic than the corresponding domains in CCR5 [Brelot et al.(1997), Lu et al.(1997)]. It may be relevant that the V3-loops of T-tropic HIV-1 env proteins tend to be more basic than those found in viruses which use CCR5 as a coreceptor [J. et al.(1992), Fouchier et al.(1992)], suggesting a charge-charge interaction may be involved in the gp120-CXCR4 interaction.

In addition to CCR5 and CXCR4, at least nine other chemokine or orphan receptors have been shown to support the cellular entry of one or more virus strains. These include CCR2b, CCR3, CCR8, GPR1, GPR15, STRL33, US28, V28, and ChemR23 [Choe et al.(1996), Deng et al.(1997), Doranz et al.(1996), Farzan et al.(1997), Liao et al.(1997), Pleskoff et al.(1997b), Reeves et al.(1997), Rucker et al.(1997), Samson et al.(1997)]. Though the in vivo significance of these alternative coreceptors is not clear, it is possible that the use of receptors other than CCR5 and CXCR4 may be important for certain aspects of viral pathogenesis. For example, CCR3 is expressed in microglia, and use of this receptor may be correlated with neurotropism [He et al.(1997)]. A major challenge in this rapidly developing field will be to determine if use of these additional receptors has implications for viral pathogenesis.

A Classification System Based on Coreceptor Use

The importance of viral phenotype for pathogenesis and disease progression coupled with the imprecise nature of the current systems for classifying virus strains calls for the development of a more accurate classification scheme. Recently, a new system based on coreceptor use has been proposed [Berger et al.(1997)]. In this scheme, virus strains that use CCR5 as a coreceptor are designated R5, while those that use CXCR4 are designated X4. Viruses that use both coreceptors are designated R5X4. Thus, under this system the HIV-1 strains most commonly transmitted would be called R5 viruses. However, this designation makes no assumptions about the cells in which HIV-1 replication must occur (unlike the M-tropic/T-tropic designation), or the rate of virus replication (unlike the SL/RH system). Viruses that evolve the capacity to use CXCR4 in infected individuals with or without concurrent use of CCR5 would now be called R5X4 or X4 viruses, respectively. The primary advantage of this classification system is that it offers a precise molecular description of the major coreceptors used by any given virus strain without assuming that this necessarily results in the ability to efficiently replicate in a particular target cell. Furthermore, this system can be readily modified, taking into account other coreceptors (such as CCR3 by using an R3 designation) if their use proves to be a major determinant of tropism. We have compiled a table listing the coreceptors used by more than 100 HIV-1 strains, and discuss some of the nuances of co-receptor use below.

Issues Pertaining to Coreceptor Use

As shown in the Table, the major coreceptors used by greater than 100 HIV-1 strains have been determined in little more than a year. All strains listed in the Table have been examined for the ability to use CCR5 or CXCR4, though not necessarily for other coreceptors such as CCR3. From the studies published to date, it is clear that CCR5 and CXCR4 are the major HIV-1 coreceptors since all HIV-1 strains examined thus far use one or both of these receptors. In addition, coreceptor use is phenotype but not genotype dependent. All NSI viruses (irrespective of genetic subtype) studied to date use CCR5 while all SI viruses use CXCR4 (though many also use CCR5). Further, our review of the literature has shown that determining which of these receptors is used by a given virus strain is straightforward and independent of technique or assay. We have found no discrepancies between various virus infection, virus-cell fusion, and cell-cell fusion assays that have been used to determine if virus strains use CCR5 or CXCR4. The only differences concern the relative efficiencies with which R5X4 viruses use CCR5 and CXCR4, but these are relatively insignificant in terms of magnitude. For the purposes of the Table, we have arbitrarily chosen 10% efficiency as the cut-off for relevant coreceptor use. Thus, a virus that uses CCR5 as its most efficient coreceptor is listed as a R5X4 virus if it uses CXCR4 to levels > 10% of that observed for CCR5. The efficiency with which different co-receptors are used by some strains is likely to vary between assay systems, and low-level entry via several co-receptors may not be uncommon. Whether this low level of relative efficiency can support virus infection in vivo is not known. As a result, the threshold efficiency for assigning relevance to coreceptor use may have to be raised or lowered in the future.

While many virus strains have been examined for the ability to use CCR5 and CXCR4, only a small number have been tested for use of most of the other viral coreceptors (see Table). Thus, we have not listed coreceptors that are NOT used by a given virus strain. Unlike studies in which use of CCR5 and CXCR4 was examined, we occasionally found discrepancies between different studies with regards to the use of additional coreceptors by some virus strains. The reasons for this are not clear, but may well be due to the fact that use of coreceptors other than CCR5 and CXCR4 tends to be inefficient, thus magnifying assay dependent differences. Perhaps the most significant variable in determining whether a given chemokine or orphan receptor can function as a coreceptor is the level of its expression. Very high levels of CCR3 expression support env-mediated membrane fusion by the majority of HIV-1 strains tested, while at lower levels only a few virus strains could use CCR3 [Rucker et al.(1997)]. Thus, expression levels can strongly influence coreceptor use. Another important variable is the expression level of the coreceptor relative to CD4 [Kozak et al.(1997)]. When CD4 is present on the cell surface at high levels, surprisingly low levels of CCR5 or CXCR4 are needed to support virus entry. However, if CD4 is expressed at low levels, higher expression levels of the coreceptors are needed [Kozak et al.(1997)]. Whether this will hold true for other coreceptors is not known, but clearly both CD4 and coreceptor cell surface expression levels can influence the efficiency of viral entry. As antibodies to additional chemokine and orphan receptors are identified, it will be possible to determine expression levels on relevant target cells. This will make it possible to assess the significance of coreceptor use determined from in vitro assays using cell lines or transient expression systems.

Determining the levels at which various receptors are expressed is but one step in determining their significance in vivo, since it is also important to determine if they are expressed in relevant target cells. In general, this is likely to be limited to CD4-positive cells. However, HIV-1 has been detected in a variety of CD4-negative cells, and several HIV-2 and many SIV strains have been identified that can utilize either CXCR4 or CCR5 for cellular entry in the absence of CD4 [Edinger et al.(1997), Endres et al.(1996), Reeves et al.(1997)]. In the case of SIV, a number of neurotropic SIV isolates have been shown to infect brain capillary endothelial cells, the principal component of the blood-brain barrier, in a CD4-independent, CCR5-dependent manner [Edinger et al.(1997)]. Thus, expression of an HIV-1 coreceptor in some CD4 negative cells may still have implications for viral pathogenesis. In fact, for many receptors, expression in cells other than T-cells and macrophages may be the only place that they support virus infection, at least during the early stages of disease - it is becoming increasingly clear that the R5 viruses which predominate during the asymptomatic period of the disease fail to infect stimulated PBMCs from individuals who lack CCR5, indicating that receptors such as CCR2b and CCR3 do not play a significant role in supporting virus entry into the primary targets of HIV-1 in vivo

[Zhang et al.(1997)]. However, this may not be the case for viruses which emerge later in the course of disease.

Another important consideration in determining the in vivo relevance of an alternative coreceptor is the number and type of virus strains than can use the coreceptor, and if there is any correlation between viral phenotype and its use. Also, studies of sequential virus isolates may reveal correlations between the acquisition of the ability to use a given coreceptor and clinical aspects of HIV-1 disease, such as the development of neurological symptoms. Ultimately, identification of the ligands for orphan receptors and the development of specific antibodies should make it possible to identify the coreceptors that can be used by HIV-1 to enter various cell types.

The expression of a given coreceptor in conjunction with CD4 is not necessarily sufficient for virus infection. For example, CXCR4 is expressed on the surface of macrophages, which are resistant to infection by virus strains which use this receptor. Interestingly, infection of macrophages obtained from CCR5-negative individuals by a dual-tropic virus isolate, 89.6, is inhibited by the CXCR4 ligand SDF-1, suggesting that CXCR4 can be used as a coreceptor on the surface of macrophages, albeit rarely [Yi et al.(1998)]. There are a variety of reasons why CXCR4 may not generally support entry of HIV-1 into macrophages, including differential posttranslational processing, surface expression levels relative to CD4 (as discussed above), and the way in which CXCR4 is presented in relation to CD4. At present, very little is known about the architecture of the fusion site - the number of molecules of env, CD4, and coreceptor that are required for a productive membrane fusion reaction, and their spatial relationships relative to one another.

Summary

The discovery of the HIV-1 coreceptors has important implications for understanding viral tropism and pathogenesis. Identifying which coreceptors are used by particular virus strains, and then determining whether their use correlates with particular aspects of viral pathogenesis, will be of general interest. Thus, a list of coreceptors used by virus strains will be maintained and updated yearly in this database. In addition, this will provide a forum where recent advances in the field can be discussed, particularly with regards as to how coreceptor usage patterns can be determined and our understanding of the significance of receptors other than CCR5 and CXCR4. If other receptors are found to be important for viral pathogenesis, then the classification scheme that we have proposed can be modified to take these new findings into account.

Acknowledgements

Strain	Proposed Accession[a]	Primary Clade	Other Tropism[b]	Designation[c]	Receptor[d]	Receptors[e]	References
DJ258	L22939	A	NSI	R5	CCR5		[Trkola et al.(1997)]
92RW026	NA	A	NSI	R5	CCR5		[Trkola et al.(1997)]
93KE101	NA	A	NSI	R5	CCR5		[Zhang et al.(1996)]
93IN103	NA	A	NSI	R5	CCR5		[Zhang et al.(1996)]
92UG037-8	U51190	A	NSI	R5	CCR5	CCR8*	[Bjorndal et al.(1997), Rucker et al.(1997)]
92RW020-5	U08794	A	NSI	R5	CCR5		[Rucker et al.(1997)]
92UG31	L34667	A	NSI	R5	CCR5		[Dittmar et al.(1997)]
92RW20	U08794	A	NSI	R5	CCR5		[Dittmar et al.(1997)]
92UG029	NA	A	SI	X4	CXCR4		[Trkola et al.(1997)]
92RW009	U88823	A	SI	R5X4	CXCR4, CCR5		[Zhang et al.(1996)]
JR-FL	U63632	B	NSI	R5	CCR5	CCR3	[Deng et al.(1997), Farzan et al.(1997), Rucker et al.(1997)]
JR-CSF	M38429	B	NSI	R5	CCR5		[Simmons et al.(1996), Trkola et al.(1997), Zhang et al.(1996)]
SF162	M65024	B	NSI	R5	CCR5	STRL33*	[Liao et al.(1997), Rucker et al.(1997)]
YU2	M93258	B	NSI	R5	CCR5	CCR3, GPR15	[Choe et al.(1996), Farzan et al.(1997)]
ADA	AF004394	B	NSI	R5	CCR5	CCR3, GPR15, STRL33*, CCR8	[Choe et al.(1996), Farzan et al.(1997), Rucker et al.(1997)]
Ba-L	M68893	B	NSI	R5	CCR5	CCR3, STRL33*	[Deng et al.(1997), Dragic et al.(1996), Liao et al.(1997), Rucker et al.(1997)]
92US657	U04908	B	NSI	R5	CCR5		[Trkola et al.(1997)]
92US715.6	U08451	B	NSI	R5	CCR5		[Bjorndal et al.(1997)]
92Br20-4	U08797	B	NSI	R5	CCR5		[Choe et al.(1996), Rucker et al.(1997)]
91US005.11	U27434	B	NSI	R5	CCR5		[Bjorndal et al.(1997), Rucker et al.(1997)]
SL-2	NA	B	NSI	R5	CCR5		[Simmons et al.(1996)]
92TH014.12	U08801	B	NSI	R5	CCR5		[Bjorndal et al.(1997)]
CM243	NA	B	NSI	R5	CCR5	GPR15, STRL33	[Rucker et al.(1997)]
M23	NA	B	NSI	R5	CCR5		[Dittmar et al.(1997)]
E80	NA	B	NSI	R5	CCR5		[Dittmar et al.(1997)]
BR92	NA	B	NSI	R5	CCR5		[Dittmar et al.(1997)]
BR49	NA	B	NSI	R5	CCR5		[Dittmar et al.(1997)]
BR53	NA	B	NSI	R5	CCR5		[Dittmar et al.(1997)]
BR90	NA	B	NSI	R5	CCR5		[Dittmar et al.(1997)]
92HA593[g]	U08444	B	SI	R5X4	CXCR4, CCR5		[Zhang et al.(1996)]
92HT593.1[g]	U08444	B	NSI	R5X4	CXCR4, CCR5		[Bjorndal et al.(1997)]

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Strain	Proposed Accession[a]	Primary Clade	Other Tropism[b]	Designation[c]	Receptor[d]	Receptors[e]	References
2028	NA	B	SI	R5X4	CXCR4, CCR5	CCR3	[Dittmar et al.(1997), Simmons et al.(1996)]
2076	NA	B	SI	R5X4	CXCR4, CCR5		[Dittmar et al.(1997), Simmons et al.(1996), Trkola et al.(1997)]
89.6	U39362	B	SI	R5X4	CXCR4, CCR5	CCR3, CCR2b, CCR8, V28	[Farzan et al.(1997), Rucker et al.(1997)]
DH123	NA	B	SI	R5X4	CXCR4, CCR5		[Trkola et al.(1997)]
Isolate C 7/86	NA	B	SI	R5X4	CXCR4, CCR5		[Trkola et al.(1997)]
92HA594	U08445	B	SI	R5X4	CXCR4, CCR5		[Zhang et al.(1996)]
92HA596	U08446	B	SI	R5X4	CXCR4, CCR5		[Zhang et al.(1996)]
M13	NA	B	SI	R5X4	CXCR4, CCR5		[Simmons et al.(1996)]
2006	NA	B	SI	R5X4	CXCR4, CCR5		[Simmons et al.(1996)]
2044	NA	B	SI	R5X4	CXCR4, CCR5		[Simmons et al.(1996)]
2036	NA	B	SI	R5X4	CXCR4, CCR5		[Simmons et al.(1996)]
2005	NA	B	SI	R5X4	CXCR4, CCR5		[Simmons et al.(1996)]
92HT599.24	U08447	B	SI	X4	CXCR4		[Bjorndal et al.(1997)]
BK132	L03697	B	SI	X4	CXCR4	CCR3*, CCR8*	[Rucker et al.(1997)]
BR65	NA	B	SI	X4	CXCR4		[Dittmar et al.(1997)]
HC4	NA	B	SI	X4	CXCR4		[Trkola et al.(1997)]
SF2	K02007	B	SI/TCLA	R5X4	CXCR4, CCR5		[Trkola et al.(1997)]
RF	M17451	B	SI/TCLA	R5X4	CXCR4, CCR5		[Alkhatib et al.(1996), Deng et al.(1997), Rucker et al.(1997)]
NL 4-3	M19921	B	SI/TCLA	X4	CXCR4		[Trkola et al.(1997), Zhang et al.(1996)]
LAI	X01762	B	SI/TCLA	X4	CXCR4		[Trkola et al.(1997)]
HXBc2	K03455	B	SI/TCLA	X4	CXCR4		[Choe et al.(1996)]
GUN-1	D34590	B	SI/TCLA	R5X4	CXCR4, CCR5		[Simmons et al.(1996)]
BH8	K02011	B	SI/TCLA	X4	CXCR4	CCR3*, STRL33*	[Rucker et al.(1997)]
92ZW101	NA	C	NSI	R5	CCR5		[Zhang et al.(1996)]
92BR025.9	U52953	C	NSI	R5	CCR5		[Bjorndal et al.(1997), Dittmar et al.(1997)]
BR28	U16217	C	NSI	R5	CCR5		[Dittmar et al.(1997)]
93MW965.26	U08455	C	NSI	R5	CCR5		[Bjorndal et al.(1997)]
BR70	NA	C	NSI	R5	CCR5		[Dittmar et al.(1997)]
JW1	NA	C	NSI	R5	CCR5		[Dittmar et al.(1997)]
JW4	NA	C	NSI	R5	CCR5		[Dittmar et al.(1997)]
92ZW102	NA	C	NSI	R5	CCR5		[Zhang et al.(1996)]

Strain	Proposed Accession[a]	Primary Clade	Other Tropism[b]	Designation[c]	Receptor[d]	Receptors[e]	References
DJ259	L22940	C	NSI	R5	CCR5		[Trkola et al.(1997)]
94ZW103	NA	C	NSI	R5	CCR5		[Trkola et al.(1997)]
94ZW109	NA	C	NSI	R5	CCR5		[Trkola et al.(1997)]
92ZW106	NA	C	SI	X4	CXCR4		[Zhang et al.(1996)]
ZAM20	L22956	C	SI	X4	CXCR4		[Trkola et al.(1997)]
94ZW106	NA	C	SI	X4	CXCR4		[Trkola et al.(1997)]
94KE102	NA	D	NSI	R5	CCR5		[Trkola et al.(1997), Zhang et al.(1996)]
94KE103	NA	D	NSI	R5	CCR5		[Trkola et al.(1997), Zhang et al.(1996)]
92UG046	U08737	D	SI	X4	CXCR4		[Trkola et al.(1997)]
UG270	NA	D	SI	X4	CXCR4		[Trkola et al.(1997)]
92UG024.2	U08726	D	SI	X4	CXCR4	CCR8*, V28*, CCR3[f]	[Bjorndal et al.(1997), Rucker et al.(1997), Trkola et al.(1997)]
92UG021.16	U27399	D	SI	X4	CXCR4		[Bjorndal et al.(1997)]
JW5	NA	D	SI	X4	CXCR4		[Dittmar et al.(1997)]
NDK	M27323	D	SI	X4	CXCR4	GPR15	[Deng et al.(1997), Pleskoff et al.(1997a)]
93ZR001.3	U27419	D	NA	X4	CXCR4	V28*	[Rucker et al.(1997)]
CM235	NA	E	NSI	R5	CCR5		[Trkola et al.(1997)]
92TH001	NA	E	NSI	R5	CCR5		[Trkola et al.(1997)]
M53	NA	E	NSI	R5	CCR5		[Dittmar et al.(1997)]
92TH22	U09131	E	NSI	R5	CCR5		[Dittmar et al.(1997)]
92TH23	NA	E	NSI	R5	CCR5		[Dittmar et al.(1997)]
C2	NA	E	NSI	R5	CCR5		[Dittmar et al.(1997)]
93TH305	NA	E	NSI	R5	CCR5		[Zhang et al.(1996)]
93TH307	NA	E	NSI	R5	CCR5		[Zhang et al.(1996)]
93TH966.8	U08456	E	NSI	R5	CCR5		[Bjorndal et al.(1997)]
93TH976.17	U08458	E	NA	R5	CCR5		[Bjorndal et al.(1997)]
93TH304	NA	E	SI	R5X4	CXCR4, CCR5		[Zhang et al.(1996)]
SL6	NA	E	SI	X4	CXCR4		[Dittmar et al.(1997)]
SL7	NA	E	SI	X4	CXCR4		[Dittmar et al.(1997)]
SL8	NA	E	SI	X4	CXCR4		[Dittmar et al.(1997)]
94TH304	NA	E	SI	X4	CXCR4		[Trkola et al.(1997)]
BR58	NA	F	SI	R5X4	CXCR4, CCR5	CCR3	[Dittmar et al.(1997)]
BZ162	L22084	F	NSI	R5	CCR5		[Trkola et al.(1997)]
R1	NA	F	NSI	R5	CCR5		[Trkola et al.(1997)]
93BR029.2	U27413	F	NA	R5	CCR5		[Rucker et al.(1997)]
92UG975.10	U27426	G	NSI	R5	CCR5		[Bjorndal et al.(1997)]
CA9	NA	O	NSI	R5	CCR5		[Zhang et al.(1996)]
MVP5180	L20571	O	SI	R5X4	CXCR4, CCR5		[Zhang et al.(1996)]

Footnotes:

[a] Accession numbers refer to sequences from the described isolate that contain the longest available sequence including Env (full length genome when avaiable, down to a minimum of the V3 region), but may not pertain directly to the sample used to determine the isolate's phenotype. Accession numbers have been deemed "NA" if there are no related locus names in the HIV Database, or if there is insufficient information to conclude that a similarly named locus in the database is referring to the same isolate.

[b] The tropism for each virus strain is indicated. SI = syncytium inducing; NSI = non syncytium inducing; SI/TCLA = syncytium inducing T-cell line adapted; NA = not available.

[c] As described in the text, we have proposed that viruses which use CCR5 be termed R5 viruses, viruses that use CXCR4 be termed X4 viruses, and viruses that use both be termed R5X4 viruses. For a virus to be termed an R5X4 virus, CXCR4 must be used at $> 10\%$ of the efficiency of CCR5 for viruses that use CCR5 as their primary receptor, or CCR5 must be used at $> 10\%$ of the efficiency of CXCR4 for viruses that use CXCR4 as their primary receptor.

[d] All virus strains in the Table have been tested for the ability to use CCR5 and CXCR4. The receptors used by each strain are indicated. For both receptors to be listed, the least efficiently used receptor must support virus entry by $>10\%$ of the levels supported by the most efficiently used receptor. For viruses that use both receptors, CXCR4 is listed first; this does not mean that CXCR4 is used more efficiently than CCR5.

[e] Receptors other than CCR5 or CXCR4 that are used by the indicated strains are listed. Since most viruses have not yet been tested for the ability to use receptors other than CCR5 and CXCR4, the absence of other receptors used in the Table should not be taken to mean that a virus uses only CCR5 or CXCR4. For example, if CCR3 is not listed as being used by a given virus strain, it may be because it has not yet been tested. Receptors that have been shown by experiments not to be used by a given virus strain are not indicated. Occasionally there are discrepancies in the literature concerning the use of one or more receptors. In these cases, we opted for 'majority rules'. If two papers report a positive result and one a negative result, the receptor is shown as being used.

[f] The virus isolate used CCR3 whereas the cloned env from this isolate did not [Bjorndal et al.(1997)].

*Indicates that coreceptor use was determined only by fusion assays rather than by virus infection.

[g] 92HT593.1 (cloned env gene) and 92HA593 (whole virus) come from the same isolate and gave slightly different results in references [Dittmar et al.(1997)] and [Zhang et al.(1996)].

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