

HIV-1 subtype and second-receptor use

SIR—The identification of the chemokine receptors fusin (now renamed CXCR4) and CCR5 as the main co-receptors for HIV-1 entry into CD4⁺ cells of the immune system will assist our understanding of HIV-1 transmission and pathogenesis¹⁻⁶. Here we report data showing that the phenotype of the virus, rather than its genetic subtype, determines receptor usage.

The phenotype of a virus is closely associated with whether it preferentially uses CXCR4 or CCR5: NSI viruses, which are macrophage-tropic and do not

is also a more divergent set of viruses known as the outlier (O) group. It is possible that different genetic subtypes might preferentially use different co-receptors, hence contributing to the uneven worldwide distribution of viral subtypes. The only recent report on this topic indicates that virus strains from subtypes A, C and E can use CCR5, although only a few viruses were tested⁵.

We therefore examined 18 viral isolates from the HIV-1 M group (at least three for each of subtypes A, B, C, D and E), and two from the O group, for second-

tion may not be at the expense of losing the ability to use CCR5. Alternatively, it is possible that most SI isolates contain a mixed population of SI and NSI quasi-species, and thus seem to be able to use both CCR5 and CXCR4.

To confirm our observations on HOS cells, we tested the ability of isolates from different subtypes to replicate in peripheral blood mononucleocytes (PBMC) from individuals homozygous or heterozygous for a recently identified defective allele of CCR5 that contains a 32-nucleotide deletion (Δ 32) in the second external loop and encodes a non-functional protein for HIV-1 entry^{7,8}. We reasoned that if isolates from non-B subtypes could use cofactors other than CCR5 for entry into PBMC, they would replicate in the cells of Δ 32 homozygotes. We therefore tested the same panel of viruses for replication in stimulated PBMC from two homozygous (Δ 32/ Δ 32), one heterozygous (wt/ Δ 32) and one wild-type (wt/wt) individuals (see table). No NSI isolate, irrespective of the genetic subtype, could replicate in PBMC from Δ 32 homozygotes, but all replicated efficiently in wild-type and heterozygote PBMC. In contrast, every SI isolate could replicate in all PBMC regardless of CCR5 genotype, presumably by using CXCR4. Thus, there is an absolute requirement for the presence of a functional CCR5 allele for replication of NSI strains of all the genetic subtypes tested.

We conclude that CCR5 and CXCR4 are the main co-receptors for HIV-1 subtypes A-E and group O. Viral phenotype is dominant over genotype in terms of co-receptor usage. Thus, the uneven worldwide distribution of HIV-1 subtypes is more likely to be the result of stochastic dissemination. However, it is conceivable that sequence polymorphisms in CCR5 and CXCR4 in different human populations could affect the efficiency of HIV-1 entry in a subtype-dependent manner. Finally, the common usage of CCR5 and CXCR4 by multiple genetic subtypes suggests that drugs targeting these receptors might not be unduly compromised by HIV-1 genetic diversity.

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SECOND-RECEPTOR USAGE BY DIFFERENT GENETIC SUBTYPES OF HIV-1

Virus	Subtype	Phenotype	p24 production in HOS.CD4 cells*				p24 production in PBMC		
			CCR1-4 [†]	CCR5	CXCR4	PB [‡]	wt/wt	wt/ Δ 32	Δ 32/ Δ 32 [§]
93KE101	A	NSI	-	+	-	-	+	+	-
93IN103	A	NSI	-	+	-	-	+	+	-
92RW009	A	SI	-	+	+	-	+	+	+
92HA593	B	SI	-	+	+	-	+	+	+
92HA594	B	SI	-	+	+	-	+	+	+
92HA596	B	SI	-	+	+	-	+	+	+
JRCSF	B	NSI	-	+	-	-	+	+	-
JRFL	B	NSI	-	+	-	-	+	+	-
NL43	B	SI	-	-	+	-	+	+	+
92ZW101	C	NSI	-	+	-	-	+	+	-
92ZW102	C	NSI	-	+	-	-	+	+	-
92ZW106	C	SI	-	-	+	-	+	+	+
94KE102	D	NSI	-	+	-	-	+	+	-
94KE103	D	NSI	-	+	-	-	+	+	-
92UG046	D	SI	-	+	+	-	+	+	+
93TH304	E	SI	-	+	+	-	+	+	+
93TH305	E	NSI	-	+	-	-	+	+	-
93TH307	E	NSI	-	+	-	-	+	+	-
CA9	O	NSI	-	+	-	-	+	+	-
MVP5180	O	SI	-	+	+	-	+	+	+

* Plus symbol, p24 concentration > 60 pg ml⁻¹; minus symbol, \leq 10 pg ml⁻¹.

[†] Identical results were obtained with CCR1, 2, 3 and 4.

[‡] Negative control cells containing vector pBABE-puro only².

[§] Identical results were obtained with PBMC from two cases.

induce syncytia, use CCR5, whereas SI strains, which induce syncytia or are adapted to T-cell lines, prefer CXCR4. There is one example of a broadly tropic HIV-1 strain able to use both CCR5 and CXCR4 for entry⁴. In addition, CCR1, CCR2b and CCR3 can function to a limited extent as HIV-1 co-receptors for some viral strains³⁻⁵. With a single exception⁵, these studies used HIV-1 strains or *env* genes derived solely from genetic subtype B, the subtype of HIV-1 that predominates in North America and Europe but not globally. Nine HIV-1 genetic subtypes (A to I) have now been designated within the major (M) group, and there

receptor usage. These 20 HIV-1 isolates, originating from 8 different countries, had previously been genetically subtyped and their NSI or SI phenotypes determined in MT-2 cells. To assess second-receptor use, we added 500 TCID₅₀ (half-maximal tissue-culture infectious dose) of each isolate to HOS cells engineered to express human CD4 and CCR1, CCR2b, CCR3, CCR4, CCR5 or CXCR4 (ref. 2). We assessed virus replication using supernatant p24 antigen concentration on day 6 after infection.

We found that each NSI isolate could utilize CCR5 for entry, irrespective of its genetic subtype (see table). However, SI isolates could use both CCR5 and CXCR4, except for one subtype B TCLA virus (NL43) and one subtype C SI primary isolate (92ZW106), which could only utilize CXCR4. The finding that dual-receptor usage is more common among SI strains suggests that adaptation of HIV-1 to use CXCR4 during phenotypic evolu-

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