The simian immunodeficiency virus mnd(GB-1) strain uses CXCR4, not CCR5, as coreceptor for entry in human cells

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The simian immunodeficiency virus (SIV) mnd(GB-1) strain, isolated from a mandrill, replicates in a human T cell line, CEM cells, and is inhibited by the CXC-chemokines, stromal cell-derived factor 1α and 1β (SDF-1α/SDF-1β), the natural ligands for CXCR4. The IC₅₀ was around 70–80 ng/ml, which corresponds to the IC₅₀ of SDF-1α/SDF-1β for T-tropic human immunodeficiency virus type 1 (HIV-1) and HIV-2. The specific anti-CXCR4 MAb 12G5 inhibited replication of SIVmnd at an IC₅₀ of 1 µg/ml. Also, the IC₅₀ of 8 ng/ml for SIVmnd of the bicyclam AMD3100, a specific CXCR4 antagonist, is comparable with its IC₅₀ for T-tropic HIV-1 and HIV-2 strains. Two other SIV strains, SIVagm3 and SIVmac251, were insensitive to SDF-1α/SDF-1β, anti-CXCR4 MAb and AMD3100. SIVmnd replicates only in HOS.CD4 cells expressing CXCR4 and not in HOS.CD4 transfectants expressing CCR1, CCR2b, CCR3, CCR4 or CCR5. This is, to our knowledge, the first SIV strain found to use CXCR4 and not CCR5 as a main coreceptor for entering human cells.

It has been demonstrated clearly that simian immunodeficiency virus (SIV) and human immunodeficiency virus type 1 (HIV-1) macrophage-tropic (M-tropic) viruses use CCR5 as a coreceptor for entry in human cells (Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Dragic et al., 1996; Lu et al., 1997; Marcon et al., 1997) and that CXCR4 (previously called fusin) is the main coreceptor for T-cell-line-tropic (T-tropic) HIV-1 and HIV-2 strains (Berson et al., 1996; Feng et al., 1996). Recently, the mechanism of coreceptor use by immunodeficiency viruses has been found to be more complex. For example, certain M-tropic HIV-1 strains not only use CCR5 but also CCR2b and CCR3 (Choe et al., 1996). Also, some dual-tropic (M/T-tropic) HIV-1 strains seem to use CCR2b, CCR3 and CCR5 as well as CXCR4 (Choe et al., 1996; other workers (Lu et al., 1997; Edinger et al., 1997; McKnight et al., 1997) have shown the differential utilization of CCR5 by SIV and M-tropic HIV strains and also of CXCR4 by different T-tropic virus strains. In addition, several M- and T-tropic HIV strains (and SIV) are capable of using newly described chemokine receptors such as Bonzo or STRL33 (Alkhatib et al., 1997; Deng et al., 1997; Liao et al., 1997), BOB or gpr15 (Deng et al., 1997; Farzan et al., 1997), gpr1 (Farzan et al., 1997) or US28 (Pleskoff et al., 1997). For SIV, coreceptor experiments have been mostly done with SIVmac (macaque), and occasionally also with other SIV strains such as SIVagm (African green monkey) (Deng et al., 1997), or SIVcpz (chimpanzee) and SIVsm (sooty mangabey) (Chen et al., 1997). From all these studies, it was concluded that SIV uses CCR5 (or other coreceptors), but not CXCR4. However, in none of the studies was the SIVmnd (mandrill) strain tested.

Here, we demonstrate that SIVmnd(GB-1) is the first SIV strain described to use CXCR4 to enter human cells. As shown in Table 1, SIVmnd replicates in CEM cells and is inhibited by the CXC-chemokine stromal cell-derived factor 1α (SDF-1α) of the natural ligand for CXCR4 (Bleul et al., 1996; Oberlin et al., 1996). The IC₅₀ was 75 ng/ml, which corresponds to the IC₅₀ of SDF-1β for T-tropic HIV-1 viruses such as strain NL4.3 (Schols et al., 1997a, b). SDF-1α does not bind to other known chemokine receptors (Bleul et al., 1996; Oberlin et al., 1996). SDF-1β, which differs from SDF-1α only in four carboxy-terminal amino acids (Bleul et al., 1996), was also active against SIVmnd (IC₅₀ 80 ng/ml). We also examined the effect of the CXCR4 MAb 12G5 (McKnight et al., 1997) on replication of SIVmnd. MAb 12G5 inhibited replication of SIVmnd by 50% at 1 µg/ml. The anti-CXCR4 MAb had no effect whatsoever on the replication of the two other SIV strains at concentrations up to 20 µg/ml (Table 1).

SIVmnd was also sensitive to the bicyclam derivative AMD3100, a compound with potent anti-HIV activity (De Clercq et al., 1992, 1994; De Vreese et al., 1996) that has recently been described to act as a specific CXCR4 antagonist (Schols et al., 1997a, b). Also, the IC₅₀ (8 ng/ml) of AMD3100 for SIVmnd was comparable with its IC₅₀ for T-tropic HIV-1 and HIV-2 strains (De Clercq et al., 1992, 1994). SIVagm3 and SIVmac251 were, as expected, not sensitive to SDF-1α/SDF-1β and to AMD3100 at a concentration up to 1 µg/ml. The CC-chemokine, regulated on activation normal T cell expressed and secreted (RANTES), had no activity against any of the SIV
four separate experiments are shown.

Table 1. Effect of MAb 12G5, SDF-1(α and β), RANTES and AMD3100 on SIV replication in CEM cells

<table>
<thead>
<tr>
<th></th>
<th>SIVgag3</th>
<th>SIVmac251</th>
<th>SIVmnd(GB-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAb 12G5</td>
<td>&gt; 20</td>
<td>&gt; 20</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td>SDF-1α</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td>0.075 ± 0.010</td>
</tr>
<tr>
<td>SDF-1β</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td>0.08 ± 0.005</td>
</tr>
<tr>
<td>RANTES</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td>0.008 ± 0.005</td>
</tr>
<tr>
<td>AMD3100</td>
<td>&gt; 1</td>
<td>&gt; 1</td>
<td>0.008 ± 0.005</td>
</tr>
</tbody>
</table>

strains tested in CEM cells, although RANTES has been reported to inhibit SIVmac in PHA-stimulated peripheral blood mononuclear cells (Cocchi et al., 1995). However, CEM cells are CD4+, CXCR4+, but CCR5− and thus the SIV strains agm3 and mac251 can enter these cells through other coreceptors such as Bonzo, which is present on these cells, whereas BOB is absent (Deng et al., 1997).

To confirm the coreceptor use by SIVmnd, experiments with HOS.CD4 transfectants were performed (Connor et al., 1997). SIVmac251 replicated only in HOS.CD4 cells expressing CCR5, and not in HOS.CD4 transfectants expressing CCR1, CCR2b, CCR3, CCR4 or CXCR4 (Table 2). This preference of SIVmac for CCR5 has also been shown in other studies using the same or different types of transfectant cell lines (Marcon et al., 1997; Edinger et al., 1997; Chen et al., 1997). In accord with our results obtained with CEM cells, SIVmnd replicated only in HOS.CD4 cells expressing CXCR4; it did not replicate in the other HOS.CD4.CCR5 cells. SIVgag3 was capable of using several coreceptors (including an unknown one in HOS.CD4.p-BABE cells), although the highest p27 Ag levels were consistently found in HOS.CD4.CCR5 cells (Table 2).

The homology of the SIVmnd Env protein with that of other known HIV and SIV strains is low and this virus represents a fifth distinct group of the primate lentiviruses (Tsujimoto et al., 1989; Hirsch et al., 1993). The other four groups are: HIV-1 and SIVcpz, SIVsm and HIV-2, SIVgag and the more recently discovered SIVsyk strain isolates from Sykes’ monkeys. For the SIVsyk strain, coreceptor use has yet to be studied; it is thus possible that other SIV strains can also use CXCR4 to enter the target cells.

It was even surprising that the SIV strains tested so far were unable to use CXCR4 (Edinger et al., 1997), because CXCR4 is the major cofactor for human T-tropic HIV strains. Although we only tested the SIVmnd(GB-1) strain as representative of SIV isolated from a mandrill, further SIVmnd isolates should be evaluated before it can be generalized that this group of lentiviruses only use CXCR4. Therefore, we can conclude that the specific coreceptor(s) used to enter the target cells may become a marker for each separate virus strain. It will be important to determine the coreceptor use of primary SIV isolates that have not been passaged in transformed human cell lines. To our knowledge, this is the first SIV strain described so far to use CXCR4, and not CCR5, as main coreceptor for entering human cells.

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References

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