Restricted species tropism of maedi–visna virus strain EV-1 is not due to limited receptor distribution

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The distribution of receptors for maedi–visna virus (MVV) was studied using co-cultivation assays for virus fusion and PCR-based assays to detect the formation of virus-specific reverse transcription products after virus entry. Receptors were present on cell lines from human, monkey, mouse, chicken, quail, hamster and ovine sources. Thus, the distribution of the receptor for MVV is more similar to that of the amphotropic type C retroviruses than to that of other lentiviruses. The receptor was sensitive to proteolysis by papain, but was resistant to trypsin. Chinese hamster ovary (CHO) and lung cells (V79 TOR) did not express functional receptors for MVV. The receptor was mapped to either chromosome 2 or 4 of the mouse using somatic cell hybrids. This allowed several candidates (e.g. MHC-II, CXCR4) that have been proposed for the MVV receptor to be excluded.

Introduction

Many different cell surface biomolecules function as cellular receptors for viruses. For example, sialyloligosaccharides (Tsvetkova et al., 1967; Markwell & Paulson, 1980; Cahan et al., 1983), phospholipids (Schlegel et al., 1983), heparan sulphate (Jackson et al., 1996), glycolipids (Brown et al., 1993) and proteins. All retroviruses investigated to date use membrane-bound glycoproteins as receptors (Weiss & Tailor, 1995). With the exception of the lentiviruses, there are no obvious relationships between these proteins. Human immunodeficiency virus (HIV) type 1, HIV-2 and simian immunodeficiency virus (SIV) all use CD4 as their primary receptor for cell binding (Klatzmann et al., 1984; Dalgleish et al., 1984; Sattentau et al., 1988) and have an obligate requirement for a second receptor component (co-receptor) to initiate virus–cell fusion (Deng et al., 1996). A growing variety of co-receptors has been identified, all of which currently belong to the 7-transmembrane G-protein-coupled receptor super-family, the principal ones being the CCR5 and CXCR4 chemokine receptors (Deng et al., 1997). The recent discovery that feline immunodeficiency virus (FIV) also uses CXCR4 as its receptor raised the possibility that a chemokine receptor constituted the archetypal primate lentivirus receptor (Willett et al., 1997) and led to speculation that other lentiviruses might also use members of this family of proteins for entry.

Maedi–visna virus (MVV) is a lentivirus that infects sheep and goats, and predominantly infects cells from the monocyte–macrophage lineage (Gendelman et al., 1986). The tropism of MVV is partly due to differential expression of cellular transactivators of the virus LTR (Gabuzda et al., 1989) but is also due to other factors that have yet to be determined. Co-receptor expression is an important determinant of macrophage tropism for HIV at the level of entry, although it is not entirely predictive (Deng et al., 1996; Dittmar et al., 1997; reviewed by Berger et al., 1999). However, the receptor for MVV is unlikely to be the major determinant of host cell tropism. Evidence from tissue culture experiments showed that MVV readily infects fibroblastic cells from a variety of ovine and caprine sources (Lee et al., 1994; Da Silva Teixeira et al., 1997). Experiments using vesicular stomatitis virus (VSV) pseudotyping and early cell culture experiments indicated that the MVV receptor was present on cell lines derived from many non-ungulate species from a variety of tissue origins (MacIntyre et al., 1972; August & Harter, 1974; Gilden et al., 1981).

Efforts to identify the receptor using virus overlay protein blotting assays led to MHC-II being proposed as a possible receptor (Dalziel et al., 1991). However, while this protein could be involved in some situations, the known distribution of the receptor is inconsistent with this hypothesis. A second candidate identified using chemical cross-linking and co-immunoprecipitation with the MVV Env protein had a molecular mass of 50 kDa (Crane et al., 1991). Despite successfully raising antibodies to this protein, it has not been
possible to identify, clone or definitively demonstrate its role in virus fusion and entry (Bruett et al., 2000).

As a prelude to employing an expression cloning strategy to identify the MVV receptor, we have studied its distribution on cell types from a variety of ovine and non-ovine origins. Cell fusion assays employing recombinant vaccinia virus-expressed MVV Env and semi-quantitative PCR assays for virus reverse transcription demonstrated that the receptor was present on a wide range of cell lines from different species. It was sensitive to proteolytic digestion with papain, but was resistant to trypsin. Cell lines of Chinese hamster origin (CHO) do not express the receptor. However, somatic cell hybrid lines carrying various complements of murine chromosomes were permissive for virus entry. This demonstrated that receptor function could be complemented by transfer of genes from a permissive line to a non-permissive line, indicating that a cDNA expression cloning strategy may be feasible. The hybrid cell analysis showed that the receptor was carried on murine chromosome 2 or 4 (although involvement of chromosomes 6 and X could not be definitively excluded). Consequently, several potential candidates for the MVV receptor can be discounted, including MHC-II, Lam-1, and most currently mapped chemokine receptors.

Methods

| Cell lines. | LSCC-H32, a spontaneously transformed quail embryo cell line, and DU24, an immortal chicken cell line were maintained in Ham’s F12 medium supplemented with 10% foetal calf serum (FCS) and 4 µg/ml uridine (Morais et al., 1988). The hamster/murine somatic cell hybrid panel (Williamson et al., 1995) was maintained in minimal essential medium (MEM) supplemented with 10 mM HEPES, 10% FCS, 100 µM hygromycin, 10 µM methotrexate and 16 µM thymidine. BHK 21, COS-7, 3T3 Swiss, BSC1, CHO (ECACC nos 85011433, 87021102, 85022108, 85011422, 93020113 and 85050302, respectively) and 293T (DuBridge et al., 1987) cells were maintained in Eagle’s MEM plus 10% FCS. Ovine kidney (OK) cells and ovine skin cells (OSC) are primary ovine fibroblast cells obtained by proteolytic disaggregation of kidney or skin tissue and were maintained in MEM plus 10% FCS.

| Plasmid and viruses. | Plasmid pT7P2GFP comprises the T7 late promoter, poliovirus type 2 IRES, green fluorescence protein (GFP) gene and T7 late terminator cloned in sequence into pACYC184 (New England Biolabs). It produces high levels of GFP when transfected into mammalian cells (Boehringer Mannheim). Transfection efficiencies were estimated by the transfection of pEGFP-C1 (Clontech) and were found to be approximately 20–40%. After 24 h culture in MEM plus 10% FCS cells were infected with vascular MVV-Env at an m.o.i. of 3 and cultured for 16 h in MEM plus 25% FCS. Target cells were infected with vaccinia virus vTF7.3 at an m.o.i. of 3 and cultured for 16 h to allow expression of the T7 RNA polymerase. Donor and target cells were washed three times with PBS and dissociated using non-enzymatic dissociation buffer (Sigma). The two populations of cells were then co-cultured at a ratio of 1:1 for 6–8 h at 37°C. Fusion between donor and target cells activates the expression of GFP due to the pre-made T7 RNA polymerase in the target cell.

| Semi-quantitative PCR to detect MVV reverse transcription products. | Virus inoculum plus 10 mM MgCl₂ was treated with 100 µl/ml DNAse I for 30 min at room temperature and filtered through a 0.2 µm filter to reduce the level of contaminating viral DNA in the inoculum. Cells were treated at an m.o.i. of 0.25 TCID₅₀ in medium containing 2.5% FCS for 1 h at 37°C with 5% CO₂. They were then incubated for 16 h in medium containing 10% FCS. The cells were washed three times in PBS and lysed in 10 mM Tris–HCl pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS and 100 µg/ml proteinase K for 16 h at 37°C. The lysates were extracted with phenol–chloroform and the DNA content was quantified by the PicoGreen fluorescence assay (Molecular Probes). Serial tenfold dilutions were analysed for the presence of viral DNA by nested-PCR. Primary amplification was performed in 20 µl total volumes containing the following: diluted template DNA; 1 µM primer 5’ ACT GTC AGG RCA GAG AAC ARA TGC C 3’ (nt 9049–9071 in U1); 1 µM primer 5’ CTC TCT TAC CCT TCT ACA GG 3’ (nt 328–309 downstream of U1). The PCR products were resolved by 1% or 2% agarose gel electrophoresis and visualized by ethidium bromide fluorescence.

| Co-cultivation cell fusion assays. | Preliminary assays using VV-MVV-Env were performed by infecting near-confluent monolayers of cells with an m.o.i. of 0.1 TCID₅₀ of virus and incubating overnight. The monolayers were stained with Giemsa’s or methylene blue stain prior to microscopic examination for self-fusion. Cell lines that did not show a self-fusing phenotype were co-cultivated for 8 h at a ratio of 5:1 with BHK 21 cells infected with VV-MVV-Env 16 h earlier with an m.o.i. of 3.

| Protease digestion of cell surface proteins. | Target LSCC-H32 cells were incubated in EMEM plus 10% FCS and 1 µg/ml brefeldin A for 1 h before infection. The cells were washed twice in PBS and incubated for 5 min in 0.25% papain (21 U/mg) or 0.25% trypsin 250 (Difco). The cells were washed with medium containing antipain (Sigma) and 10% FCS to stop proteolysis. Treated cells were co-cultured at a ratio of 5:1 with BHK 21 cells (infected with VV-MVV-Env, 16 h earlier) in EMEM plus 10% FCS and 1 µg/ml brefeldin A. The cultures were stained 8 h later with methylene blue and were examined for fusion.

| Fusion-activated reporter assay. | A modified version of a vaccinia-based fusion assay was developed (Nussbaum et al., 1994). Donor CHO cells were transfected with pT7P2GFP using Fugene 6 (Boehringer Mannheim). Transfection efficiencies were estimated by the transfection of pEGFP-C1 (Clontech) and were found to be approximately 20–40%. After 24 h culture in EMEM plus 10% FCS cells were infected with VV-MVV-Env at an m.o.i. of 3 and cultured for 16 h in EMEM plus 25% FCS. Target cells were infected with vaccinia virus vTF7.3 at an m.o.i. of 3 and cultured for 16 h to allow expression of the T7 RNA polymerase. Donor and target cells were washed three times with PBS and dissociated using non-enzymatic dissociation buffer (Sigma). The two populations of cells were then co-cultured at a ratio of 1:1 for 6–8 h at 37°C. Fusion between donor and target cells activates the expression of GFP due to the pre-made T7 RNA polymerase in the target cell.
Digital image manipulation. Electronic versions of photographic images were produced using a Umax Magiscan II scanner at 600 d.p.i. resolution. Composite images were produced manually or by using Adobe Photoshop 5LE.

Results

The receptor for MVV is present on a wide range of species

A recombinant vaccinia virus that expresses the full-length Env protein for the EV-1 strain of MVV was used to screen cell lines from various species. Cell lines that express a receptor for MVV and are also able to express a functional form of the recombinant Env would be expected to self-fuse and form syncytia when infected with the recombinant vaccinia virus. Using this simple assay it was shown that ovine skin fibroblasts (OSC), African Green monkey (BSC1, COS-7), human (HeLa), quail (LSCC-H32), chicken (DU24) and Syrian hamster (BHK 21) cells all show this self-fusing phenotype (Fig. 1a). No self-fusion was seen when these cells were infected with a control vaccinia virus vTF7.3 expressing T7 RNA polymerase (data not shown). Several cell lines did not show self-fusion. This could be due to the lack of a receptor or the inability to express sufficient levels of functional Env protein. To exclude this latter possibility, the cells were co-cultivated with infected BHK 21 cells. Preliminary assays had found that BHK 21 cells showed moderate levels of self-fusion, but particularly high levels of fusion when co-cultured with other putative receptor-bearing cell lines. Co-culture of OK, 293T and 3T3 Swiss cells with infected BHK 21 cells showed high levels of fusion indicating that these cells expressed the receptor (Fig. 1b). The only cell line that failed to show self-fusion or fusion in the co-culture assay was the CHO cell line.

![Species distribution of the MVV receptor](image-url)

**Fig. 1.** (a) Cell fusion induced by MVV Env protein. Top left of each panel shows uninfected cells; bottom right shows cells 16 h p.i. with recombinant vaccinia virus (VV-MVV-Env) expressing the MVV Env protein. (b) Top left of each panel shows cells co-cultured with uninfected BHK 21 cells. Bottom right of each panel shows cells co-cultured for 8 h with VV-MVV-Env-infected BHK 21 cells at a ratio of 5:1.
Sensitivity of the MVV receptor to protease treatment. LSCC-H32 cells were co-cultured with VV-MVV-Env-infected BHK 21 cells at a ratio of 5:1 for 8 h. (a) Untreated co-culture; (b) co-culture of LSCC-H32 cells pre-treated with brefeldin A alone for 1 h and maintained in the presence of brefeldin A; (c) co-culture using LSCC-H32 cells previously treated with brefeldin A and trypsin; (d) co-culture using LSCC-H32 cells previously treated with brefeldin A and papain.

The MVV receptor is sensitive to proteolysis

The co-culture assay was used to test whether fusion was dependent on a protein component of the target cells. LSCC-H32 cells were pre-treated with trypsin or papain to digest the receptor from the cell surface. After treatment the cells were washed, treated with serum and antipain and washed again to ensure removal of the protease prior to use in the co-culture assay. A fivefold excess of treated LSCC-H32 cells was cocultured for 8 h with infected BHK 21 cells in the presence of brefeldin A to inhibit re-expression of the receptor at the cell surface during the course of the assay. Extensive fusion occurred in the cultures that were not exposed to protease (Fig. 2a, b). Trypsin treatment did not effect fusion. However, papain treatment severely inhibited fusion (Fig. 2c, d). This provides evidence for a cell surface protein component of the receptor on the target cell.

Demonstration of virus entry by PCR

The co-culture assay described above provided a simple, but non-quantitative measure of receptor expression on different cells. A subset of the cell lines tested above was examined to see if they would be permissive for the early stages of MVV entry and reverse transcription. Semi-confluent monolayers of cells were infected with 0.25 TCID_{50} of DNase treated, filtered virus per cell. Total DNA was then harvested 16 h post-infection (p.i.) and accurately quantified using a DNA-specific fluorescence assay. Tenfold serial dilutions were prepared and subjected to a sensitive nested-PCR assay that detects late stage-specific reverse transcription products (Fig. 3). The presence of viral DNA is indicated by the synthesis of a 203 bp PCR product. The dilution end-point indicates the amount of viral DNA in the starting material. Viral DNA could be detected at the 10^{-4} dilution equivalent to 2 pg total cellular DNA. This is slightly less than one cellular genome-equivalent of DNA. This suggests that the TCID_{50} value significantly underestimates the amount of virus in the inoculum that can enter the cell and undergo reverse transcription. All of the cell lines that were positive in the fusion assay were also positive in the virus entry/PCR assay. CHO cells did not contain any detectable viral DNA, consistent with the absence of the virus receptor. Occasionally DNA was detected in the undiluted CHO DNA samples. This may indicate very low levels of virus entry into these cells, or more likely represents residual DNA present in the virus inoculum. This is supported by the finding that such low levels of DNA could be detected in lysates prepared from CHO cells immediately after adsorption of the inoculum (data not shown). The qualitative differences in fusion for the various cell lines correlated with the amount of viral DNA determined by PCR. LSCC-H32, BSC1 and OK cells showed high levels of fusion and large amounts of viral DNA. HeLa and BHK 21 cells showed smaller syncytia involving...
fewer cells and less viral DNA. LSCC-H32 cells and BSC1 cells underwent ‘fusion from without’ approximately 6–8 h p.i. with MVV when an m.o.i. of > 3 was used. This has been reported previously for BSC1 cells (August & Harter, 1974).

**Hamster/mouse hybrid cell lines support virus infection**

The absence of the receptor on Chinese hamster cells and its presence on mouse cells provided a means to determine whether receptor-deficient cells could be complemented by genes from a permissive cell line. A panel of Chinese hamster/mouse somatic cell hybrid lines containing known complements of mouse chromosomes (Williamson et al., 1995) in a Chinese hamster lung cell background (V79 TOR) was tested using the virus entry/PCR assay (Fig. 4). The amount of viral DNA detected by PCR varied between the different cell lines. Viral DNA was absent from V79 TOR cells indicating that these cells did not allow virus entry and that the receptor deficiency was common to more than one Chinese hamster line. Cell lines CV1 (not to be confused with the monkey kidney line of the same name) and CV3 also did not permit virus entry. CV8, CV4/2/1/1 and MOV11/3/1/2 showed the highest levels of signal, although these were substantially lower than those that would normally be seen for the highly permissive cell lines (including mouse 3T3). CV4 and CV7 showed borderline levels of viral DNA, but were consistently positive.

To confirm that the phenotype being complemented by the mouse chromosomes was receptor-mediated fusion rather than a defect in uncoating or reverse transcription, the cell lines were tested using a cell fusion co-cultivation assay. The morphology of some of the hybrids made it difficult to assess fusion microscopically because a significant proportion of the cells were already polykaryons. To overcome this, a modified fusion assay (Nussbaum et al., 1994) was developed. CHO cells containing the reporter plasmid pT7P2GFP and expressing MVV-Env on their surface (donor cells) were co-cultured with target cells containing T7 RNA polymerase. The presence of the virus receptor on the target cell surface was indicated by fusion of the two cell populations and activation of GFP expression. Fusion was shown to be dependent on MVV Env expression by performing the assay in the presence of Env-specific antiserum or normal rabbit serum. GFP expression and syncytium formation were detected when donor cells were co-cultivated with MOV11/3/1/2 cells or positive control LSCC-H32 cells (Fig. 5e, f, i and j). Fusion was dependent on the
Table 1. Chromosome content and MVV entry phenotype of Chinese hamster/mouse somatic cell hybrid lines

MVV entry phenotype of Chinese hamster/mouse somatic cell hybrid lines and the mouse chromosome content (Williamson et al., 1995). Chromosome elimination is implied where the chromosome is found to be absent from a permissive line (\); present in a non-permissive line (+); or by both criteria (X). Chromosomes not eliminated (O) are also indicated. The presence (+) or absence (−) of the chromosome in the cell line is indicated. A weak signal (+) that has been observed for a probe known to map to the chromosome indicated by Southern blot analysis suggests that the chromosome is present in only a proportion of the cells.

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* Chromosomes previously believed to be absent from this cell line.

Discussion

A characteristic feature of MVV is its highly restricted pattern of infection. The virus has a narrow species tropism, restricted to sheep, goats and mouflon. Within these species, the virus is limited primarily to cells of the monocyte/macrophage and myeloid dendritic cell lineages, and virus replication is almost exclusively associated with mature macrophages. There is some evidence for natural infection of mammary epithelial tissue (Lerondelle et al., 1999) and experimental evidence for infection of bronchiolar epithelium that is consistent with the primary routes of transmission. By analogy with HIV, it might be anticipated that cell type-specific expression of the virus receptor would be an important factor in determining what cell types can be infected. However, significant and often overlooked data suggest that the distribution of receptors for MVV is in fact remarkably broad (August & Harter, 1974; Gilden et al., 1981; Harter et al., 1968). Tropism for specific sheep tissues can be accounted for by the differential activity of the virus LTR (Gendelman et al., 1986; Clements et al., 1990), although the LTR is active in lymphocytes that are not permissive for productive infection (Small et al., 1989). Species tropism is dependent on other factors that have yet to be determined. This may have negative consequences for the future development of retrovirus vectors based on MVV. It is important to know the identity of the virus receptor in order to determine its contribution to the pathogenesis of MVV infection.

We were searching for a cell line that lacked the MVV receptor that would be suitable for a strategy to identify the receptor by expression cloning. The EV-1 strain of MVV was isolated by co-culture of primary ovine skin fibroblasts with PBMCs from a sheep with acute maedi–virus disease. Skin cells are not normally productively infected in the natural host but are highly permissive for field isolates and laboratory strains of...
MVV in culture. The tissue culture grown EV-1 has been studied extensively both in vitro and in vivo. It maintains its narrow tropism for macrophages and dendritic cells, and causes disease in sheep that is indistinguishable from natural infection (Eriksson et al., 1999). Hence, it does not appear that the virus has undergone a major adaptation in its receptor tropism during its short passage history in OSCs. Our primary screens for the presence of receptor were based on a vaccinia virus recombinant that expresses the cloned MVV EV-1 Env protein. Nine, non-ovine, cell lines were tested. We confirmed that the distribution of the receptor was very broad, including cells of avian origin and thus more similar to that of the amphotropic type C retroviruses than to the other members of the lentiviruses. Only Chinese hamster cells (CHO, V79 TOR) were found to lack the receptor. This was confirmed using a sensitive virus-infection assay where virus entry was determined by the appearance of viral DNA. This also demonstrated that all cell types that bore the virus receptor were permissive for reverse transcription.

Having identified a cell line lacking the receptor, it was necessary to determine whether the receptor would be amenable to an expression cloning strategy. To this end, it was important to demonstrate that the receptor was a protein, and that transfer of genes from a receptor-bearing cell line into Chinese hamster cells would confer susceptibility to virus entry or at least Env-mediated fusion.

The case for the virus receptor being a protein was supported by its sensitivity to proteolysis. Fusion was resistant to trypsin but was sensitive to papain. This is consistent with the involvement of one or more proteins on the target cell membrane with the process of fusion but does not rule out the possibility of other non-protein components.

A panel of somatic cell hybrid Chinese hamster cells containing different complements of murine chromosomes was used to demonstrate that Chinese hamster cells could be made susceptible to virus entry. Because the chromosome complement of the hybrids was known, it also allowed the chromosome carrying the receptor to be tentatively identified. Based upon the presence of a particular chromosome in permissive hybrids, and the absence in non-permissive hybrids, the murine homologue of the MVV receptor is carried by chromosome 2 or 4. This conclusion is subject to three caveats: (i) more than one protein on different chromosomes may serve as the receptor; (ii) as in the case of HIV, there may be a requirement for a co-receptor on a different chromosome; and (iii) cell lines may be non-permissive because of transcriptional silencing or DNA rearrangement rather than because they lack the chromosome or gene concerned. Taken together it is apparent that the hybrid lines available cannot provide an absolutely certain location for the murine homologue of the MVV receptor. Chromosome 6, for example, is excluded by only a single negative data point and could still be implicated if there was a requirement for a co-receptor, as could the X chromosome. The experiments presented here do not allow us to address whether there is a requirement for a co-receptor for MVV entry. However, if one does exist, it/they too must be widely distributed across species.

Despite the caveats listed above, the hybrid data excludes several important potential candidates for the MVV receptor (Table 2). Ram-1 is the receptor for amphotropic murine leukaemia viruses (Miller et al., 1994). It has a widespread distribution across different species that very closely matches that observed for MVV. CHO cells contain a homologue of Ram-1, but it is non-functional because it is blocked by a specific inhibitor secreted by CHO cells (Miller & Miller, 1992). Expression of this inhibitor is prevented by treatment with tunicamycin, with concomitant restoration of susceptibility to infection for CHO cells by amphotropic viruses. Tunicamycin has no effect on MVV entry into CHO cells (data not shown). GLVR-1 a close relative of Ram-1 is located on chromosome 2 (Kaelbling et al., 1991). Initially this protein was excluded because cell hybrid CV4/2/1/1 was believed to lack chromosome 2. However, FISH analysis using mouse chromosome-specific paints (Rabbits et al., 1995) revealed that chromosome 2 was present in this line (data not shown). Chromosome 2 is present in only a small proportion of MOV11/3/1/2 cells. Since this line was consistently the most permissive for MVV entry (see Fig. 4) it would seem unlikely that chromosome 2 carries the MVV receptor, but it cannot definitely be excluded at this stage. Thus, GLVR-1 is still a potential candidate for the MVV receptor although, it too would be expected to respond to tunicamycin treatment in the same way as Ram-1. MHC-II has been proposed previously as a candidate for the MVV receptor (Dalziel et al., 1991). While it is possible that this protein could be involved in entry into macrophages, it is extremely unlikely that it is responsible for entry into the cell lines used in this study. This is reinforced by the cell hybrid data that excludes this protein based on

### Table 2. Exclusion of candidate genes resulting from the mapping of the MVV receptor to mouse chromosomes 2 or 4

<table>
<thead>
<tr>
<th>Candidate receptor</th>
<th>Mouse chromosome location</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC-II</td>
<td>17 (H2)</td>
</tr>
<tr>
<td>Ram-1</td>
<td>8 (Slc20a2)</td>
</tr>
<tr>
<td>CXCR-2</td>
<td>1 (Cmkar2)</td>
</tr>
<tr>
<td>CXCR-3</td>
<td>X (Cmkar3)</td>
</tr>
<tr>
<td>CXCR-4</td>
<td>1 (Cmkar4)</td>
</tr>
<tr>
<td>CCR1</td>
<td>9</td>
</tr>
<tr>
<td>CCR2</td>
<td>9</td>
</tr>
<tr>
<td>CCR4</td>
<td>9</td>
</tr>
<tr>
<td>CCR5</td>
<td>9</td>
</tr>
<tr>
<td>CCR8</td>
<td>9 (Ckr-L1)</td>
</tr>
<tr>
<td>Cmkr1</td>
<td>1</td>
</tr>
</tbody>
</table>

Species distribution of the MVV receptor
chromosome location. The majority of chemokine receptors that are currently mapped in the mouse lie on chromosome 1 (CXCR) and chromosome 9 (CCR) and are excluded by this analysis. The chemokine receptors belong to a very large super-family of 7-transmembrane domain proteins. It is possible that the receptor for MVV is a member of this group of proteins, but currently there is no evidence supporting this and no a priori reason why this should be so.

In conclusion, the receptor for MVV is widespread among cell lines from many different species with the exception of Chinese hamster cells. Thus, receptor distribution is not the primary factor governing the tropism of MVV. The receptor maps to either chromosome 2 or chromosome 4 of the mouse, and consequently is not MHC-II and is unlikely to be one of the currently mapped chemokine receptors involved in HIV entry.

We are grateful to Dr J.R. Rodriguez (Centro Nacional de Biotecnologia, Madrid, Spain) for providing the VV-MVV-Env virus; Dr Y. Boyd (MRC, Harwell, UK) for the hamster/murine somatic cell hybrid panel; and Professor G. Smith (University of Oxford, Oxford, UK) for the vTF7.3 virus. This research was supported by a grant from the Wellcome Trust (grant no. 047359).

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Received 15 May 2000; Accepted 24 August 2000