A HHV6 encoded CCR2 agonist

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A highly selective CCR2 chemokine agonist encoded by
human herpesvirus 6

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Abstract:

The chemokine-like, secreted protein product of the U83 gene from human herpes virus 6 – here named vCCL4 - was chemically synthesized to be characterized in a complete library of the eighteen known human chemokine receptors expressed individually in stably transfected cell lines. vCCL4 was found to cause calcium mobilization as efficiently as the endogenous chemokine ligand CCL2 through the CCR2 receptor whereas the virally encoded chemokine did not affect any of the other seventeen human chemokine receptors tested. Mutual cross desensitization between CCL2 and vCCL4 was demonstrated in the CCR2 transfected cells. The affinity of vCCL4 for the CCR2 receptor was 79 nM as determined in competition binding against radioactively labeled CCL2. In the murine pre-B lymphocyte cell line L1.2 stably transfected with the CCR2 receptor, vCCL4 acted as a relatively low potency but highly efficacious chemoattractant being equally or more efficacious in causing cell migration than CCL2 and CCL7 and considerably more efficacious than CCL8 and CCL13. It is concluded that human herpes virus 6 encodes a highly selective and efficacious CCR2 agonist, which will attract CCR2 expressing cells - for example macrophages and monocytes - conceivably for the virus to infect and establish latency in. It is suggested that vCCL4 during reactivation of the virus in for example monocyte-derived microglia could perhaps be involved in the pathogenesis of the CCR2 dependent disease, multiple sclerosis.
Introduction:

Human herpes virus 6 (HHV6) was identified in 1986(1). Subsequently, it has been shown that over 95% of people older than 2 years are seropositive for HHV6(2). The primary infection with HHV6 can give rise to exanthema subitum (ES) also known as roseola infantum or the sixth disease(2). The course is generally benign, but complications such as seizures, meningitis and encephalitis are well recognized(2). HHV6 is known to establish latency in its host and to be highly neurotropic(2). HHV6 has been suggested as an agent involved in multiple sclerosis (MS) (2), however, the ubiquitous presence of HHV6 in the human population from early age on combined with the frequent finding of HHV6 in the central nervous system of infected persons has made it difficult to determine whether re-activation of HHV6 in the CNS is in fact implicated in plaque formation in multiple sclerosis or whether the virus is just an innocent bystander(2).

Large DNA viruses such as pox- and herpes viruses encode proteins, which interfere with their host immune system. Several of these immune-evasive genes encode proteins, which target the chemokine system. Chemokines are 70-80 amino acid proteins with well-characterized three-dimensional structures, which are involved in attracting and activating distinct leukocyte subsets(3;4). The current number of endogenous human chemokine genes is approaching 50. These can be divided into four sub-families on the basis of the pattern and number of the conserved cysteine residues located near their N-terminus, which are involved in disulfide binding formation. In the CC-family the two N-terminal cysteines are adjacent, in the CXC-family and CX3C-family the two residues are separated by a single amino acid and by three amino acids respectively, whereas in the XC-family only one cysteine is present.
Chemokines act through 7TM\(^1\), G-protein coupled receptors of which we today know ten CC-chemokine receptors, six CXC-receptors, one CX\(_3\)C-receptor (CX\(_3\)CR1) and one XC-receptor (XCR1)(4;5).

Among herpesvirus CMV or HHV5(6), HHV6(7;8), HHV7(9) and HHV8(10) are all known to encode chemokine-like proteins and/or chemokine receptors. For most of these receptors the function has not yet been identified, except in the case of the ORF74 CXC-chemokine receptor from HHV8, which apparently is crucially involved in the angiogenic lesions in Kaposi’s sarcoma (11-13) and the CC/CX\(_3\)C-chemokine receptor US28 receptor from CMV which appears to be involved in the cellular transfer of virus and as a chemokine scavenger(14;15).

The virally encoded chemokine proteins have rather different pharmacological phenotypes. Thus we found that the HHV8 encoded vMIP-II functions as a broadspectrum chemokine antagonist, whereas the MC148 chemokine encoded by the poxvirus Molluscum Contagiosum is a highly selective CCR8 antagonist(16-18). Other groups have characterized the CC-chemokines vMIP-I and vMIP-III, which are also encoded by HHV8 as being CCR4- and CCR8- agonists respectively(19-21). CMV encodes two CXC-chemokines vCXC1 and vCXC2(22) of which vCXC1 has been characterized as a CXCR2 agonist(23). vMIP-II was shown to function in-vivo as an efficient immunosuppressive agent in a rat model for glomerulonephritis and rat model for spinal cord contusion (24;25).

HHV6 encodes two putative chemokine receptors and a chemokine-like protein, which based on its primary structure, is predicted to be either a CC- or a CX\(_3\)C- chemokine (fig 1). In the present study we show that the HHV6 U83 gene encodes a CC chemokine, which functions

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\(^1\)Abbreviations used in this paper: CHO, chinese hamster ovary; MCP, monocyte chemoattrcctant protein; MIP, macrophage inflammatory protein; 7TM receptors, seven transmembrane receptors;
as a highly selective and efficacious agonist for the human CCR2 receptor both in respect of signal transduction and ability to induce chemotaxis. In analogy with the two CMV encoded chemokines vCXC1 and vCXC2 we suggest that the U83 gene product be named vCCL4 as it is the fourth human herpesvirus encoded CC-chemokine to be characterized and this name is used throughout the present paper.
Materials and Methods:

U-83 constructs:

U83 was cloned from HHV6B-DNA bought from Autogen Bioclear England into the pTej8-vector using standard techniques. The sequence of the gene was similar to GenBank accession number 405187. In order to knock-out two reported splice-sites another construct was made with two silent mutations (167-TACCTG-172 to 167-TGCCAG-172 and 244-TACCTG-249 to 244-TATTTG-249).

Recombinant protein;

In order to purify vCCL4 the same method which had been successfully used for MC148 was applied with minor modifications. In brief COS-7 cells and CHO-cells were transiently transfected with the two U83 constructs by a calcium phosphate precipitate method with addition of chloroquine. Serum- and phenolred-free medium was collected 24 hours after transfection. The medium was centrifuged at 1500 g for 20 min, the supernatant was adjusted to pH 9.5 and filtered through 0.22 um filters (Nalgene, Rochester, NY). The medium was diluted with water 1:1 to decrease ionic strength and loaded on anion exchange SP-sepharose columns (Pharmacia Biotech, Uppsala, Sweden). The columns were washed with 20 mM ethanolamin buffer pH 9.5 and protein was eluted with 2 M NaCl in the same buffer. The eluate was made 0.2 % in trifluoroacetic acid (TFA), filtered and loaded on a C8 (Vydac, Hesperia, CA) column for reverse-phase HPLC from where bound material was eluted with 0.1 % TFA in water on a gradient of CH$_3$CN. We tried to identify the elution position of vCCL4 by mass-spectroscopi of different protein fractions.

Chemokines;
vCCL4 (21-113 from Genbank NC 000898), CCL2, CCL7, CCL8, CCL13 and CCL27 were chemically synthesized as described previously(26). Mass-spectroscopy and HPLC of vCCL4 revealed a monomeric protein with the predicted molecular mass of 9957 Da. CXCL8 was expressed in Escherichia Coli and purified in-house. CCL1, CCL5, CCL11, CCL16, CXCL12, CXCL16, CX3CL1 and murine XCL1 were bought from R&D (Minneapolis, MN), whereas CCL25 and CXCL11 were bought from PeproTech (Rocky Hill, NJ). CCL20 was kindly provided by Tim Wells (Serono, Geneve, Schwitzerland). CCL21 and CCL22 were kindly provided by Thomas Schall (ChemoCentryx, CA). CXCL13 was kindly provided by Bernhard Moser (Thedor-Kocher Institute, Bern, Schwitzerland).

Stable cell lines;

CCR2b and CCR8 were transfected into the murine pre-B cell line 300.19 by electroporation, and stable transfectants were obtained after limiting dilution and chemical selection with G418 and subsequently functional selection by testing the clones for calcium response to CCL2 and CCL1 respectively. Kuldeep Neote (Pfizer, Groton, CT) kindly provided 300.19 cells expressing the CXCR3 receptor and Bernhard Moser (Thedor-Kocher Institute, Bern, Schwitzerland) kindly provided 300.19 cells expressing the CXCR5 receptor.

CCR10 and CXCR6 were transfected into the murine pre-B cell line L1.2 by electroporation, and stable transfectants were obtained after limiting dilution and chemical selection with G418 and subsequently functional selection by testing the clones for calcium response to CCL27 and CXCL16, respectively. The L1.2-cell lines expressing the CCR2b, CCR3, and CCR7 receptors were established at ICOS (Seattle, WA). The L12-cells stably
expressing CCR4, CCR6, XCR1, and CX3CR1 were a kind gift from Osamu Yoshie (Kinki University, Japan).

The CHO-cells stably expressing CCR1, CCR2b, CCR5, CXCR1, CXCR2, and CXCR4 were generously provided by Tim Wells (Serono, Geneva, Switzerland).

Gabriel Marquez (Autonomous University, Madrid, Spain) kindly provided HEK293 stably expressing the CCR9 receptor.

Binding;

Whole cell binding (2 x 10^5 cells per well) was performed at 4°C for 3 hours in 0.5 ml of 25 mM Hepes buffer containing 1 mM CaCl2 and 5 mM MgCl2 at pH 7.2, supplemented with 0.5 % bovine serum albumin (BSA) on CHO-cells stably transfected with CCR2. The incubation was stopped by washing four times with 0.5 ml of ice-cold binding buffer made 0.5 mM in NaCl. Cell-associated radioactivity was determined after extraction of the cells with 8 M urea in 3 M acetic acid supplemented with 1 % NP-40. Non-specific binding, determined in the presence of the relevant chemokine peptide (0.1 µM), was subtracted. 125I-CCL2 (IM280) was obtained from Amersham.

Calcium flux;

Cells stably transfected with various chemokine receptors were loaded with Fura-2AM (Molecular Probes, Eugene, OR) in RPMI with 1 % FCS for 20-30 min., aliquots were made of 1x 10^6 cells, each aliquot was pelleted and resuspended in RPMI with 10 mM EGTA. Fluorescence was measured on a Jobin Yvon FlouroMax-2 (Jobin Yvon Spex, Edison, NJ) as the ratio of emission at 490 nm when excited at 340 nm and 380 nm respectively.
Chemotaxis;

Chemotaxis was measured using 24 Transwells Polycarbonate 3 μm Membranes (Corning Costar, Cambridge MA). Chemokines were diluted in 0.6 ml chemotaxis buffer (RPMI medium containing 0.5 % BSA) and added to the lower chemotaxis chamber. 1x10^6 CCR2/L1.2 cells were resuspended in 0.1 ml chemotaxis buffer and added to the top chamber insert. Chemotaxis plates were then incubated for 4 hours at 37°C in a 5% CO₂-humidified incubator. After the incubation the cells from the bottom well were collected and counted by a fluorescence-activated cell sorter (Becton Dickinson, Franklin Lakes, NJ).
Results:

Initially we tried to express the U83 gene in transfected COS-7- and CHO cells and purify the protein on an anion-exchange column and HPLC as described previously(17), but we were unable to recover the protein. One possibility could be that the transcript was spliced in uninfected cells (splicing is normally silenced under viral replication) resulting in a protein truncated 8 amino acids after the CC-chemokine motif(27). Therefore two internal splice-sites were removed with silent mutations. This construct was transfected into CHO-cells, but again no protein could be recovered. Using prediction servers the precursor protein of the U83 gene product was predicted to have one cleavage site for a signal peptide between residues 20 and 21 and the supposed secreted form of the viral chemokine was produced synthetically. This predicted N-terminus was identical to the one reported from a group, who expressed a fusion construct of the U83 and a IgG gene in mammalian cells and did aminoacid sequence analysis of the purified protein(28). The synthetic vCCL4 protein was used for receptor activation studies, receptor binding studies and chemotaxis assays.

To screen for a possible interaction of vCCL4 with the known chemokine receptors, vCCL4 was probed in calcium mobilization assays on a panel of cell lines individually expressing the eighteen known human chemokine receptors. Initially, one micromolar of vCCL4 was used in these assays. vCCL4 was unable to elicit a calcium response through seventeen of the eighteen human chemokine receptors. However, in 300.19 cells stably transfected with CCR2, vCCL4 gave a robust calcium response similar in magnitude to the response elicited by CCL2 - the major endogenous CCR2 ligand. The same concentration of vCCL4 was for example unable to induce a calcium response in CX3CR1 L12 transfected cells indicating that the U83 gene product is indeed a CC- chemokine. In contrast, vCCL4 was not able to induce a calcium
response in 300.19 cells stably transfected with for example CXCR3 and CXCR5 (Fig 2) showing that the effect was mediated through CCR2 and not an endogenous receptor in 300.19 cells. vCCL4 did not act as an antagonist on any of the tested human chemokine receptors as pretreatment of the cell-lines with vCCL4 was unable to inhibit or block the response to the relevant endogenous chemokine through any of the human chemokine receptors tested as seen in (Fig 2). However, in the CCR2 transfected 300.19 cells a diminished response to CCL2 was observed after pretreatment with one micromolar of vCCL4 (Fig 2). This inhibitory effect is due to specific desensitization (see below).

In order to characterize the affinity of vCCL4 and compare this affinity with known endogenous human CCR2 ligands we did binding studies on CHO cells stably transfected with the CCR2 receptor using $^{125}$I-CCL2 (Fig 3). vCCL4 displaced $^{125}$I-CCL2 from the CCR2 receptor with an affinity of 79 nM. In contrast, the endogenous human CCR2 ligands all belonging to the proteins in the previously designated MCP family of chemokines bound the receptor with higher affinity. Thus, the IC$_{50}$ for CCL2 was 0.08 nM, for CCL7 3.1 nM, for CCL8 2.7 nM and for CCL13 2.2 nM. CCL11 was found to have a comparable affinity of 72 nM, whereas the affinity of CCL16 was higher >100 nM (data not shown).

As shown in Fig. 4 one micromolar of vCCL4 induced a maximal calcium response through CCR2 in stably transfected 300.19 cells. In these cells CCL2 was found to have an EC$_{50}$ of 0.7 nM, whereas EC$_{50}$ values for CCL7 was 2.2 nM, for CCL8 15.4 nM, for CCL13 8.2 nM and for the HHV6 encoded vCCL4 the EC$_{50}$ was 156 nM, which correlated well with the affinities found in binding experiments in the CCR2 transfected CHO-cells. As shown in Fig 4, vCCL4 was able to cross-desensitize the response of one nanomolar of CCL2 and vice-versa indicating that the effect of vCCL4 is indeed mediated through the common CCR2 receptor.
The chemotactic activity of vCCL4 was compared to that of the endogenous human chemokines from the MCP-family in the murine pre-B lymphocyte cell line L1.2 stably transfected with the human CCR2 receptor (Fig 5). The endogenous chemokines demonstrated different patterns with respect to both efficacy and potency. Thus, CCL2 and to a certain degree also CCL7 both showed high potency and high efficacy, whereas CCL13 had a poor potency and a rather poor efficacy and CCL8 although being of rather high potency showed a very limited efficacy. All of the endogenous chemokines demonstrated the classical bell-shaped dose-response curve in the chemotactic experiments (Fig 5). Surprisingly the efficacy of vCCL4 was very high and in fact it is unclear whether the maximal response was even reached with the available amount of the virally encoded protein (Fig. 5). The chemotactic response to vCCL4 was at least as high as that of CCL2 and CCL7 and much higher than that of CCL13 and CCL8.
Discussion:

In the present study we find that the chemokine-like protein product of the U83 gene from HHV6B vCCL4 acts as a highly efficacious but relatively low potency agonist for the human CCR2 receptor. Thus, the virally encoded chemokine could serve a role initially in the infectious life cycle of the virus by attracting, for example macrophages and monocytes, which express the CCR2 receptor, in order to infect and establish latency in these. During re-activation of latent virus in monocyte derived microglia in the CNS, vCCL4 could be involved in the pathogenesis of MS, which at least in experimental animals is known to be totally dependent on the presence of a functional CCR2 receptor(29;30).

The vCCL4 protein has very limited homology with the known human chemokines, however it is clearly a chemokine protein as it has the required disulfide pattern (Fig. 1). The notion that vCCL4 has a very long N-terminal extension of 27 amino acid residues is particularly interesting since often important structural epitopes related to receptor activation are located in the N-terminal extension of chemokines. Thus, the N-terminal of vCCL4 lacks the glutamine in the first position that is conserved among all human endogenous CCR2 ligands and when missing has been found to reduce the biological activity of for example CCL2 100-1000 fold (31;32). Moreover, a potential glycosaminoglycan (GAG) binding epitope, BBXB (B being a basic residue)(33), is found right before the third Cys residue, which very likely forms a disulfide bridge back to the first Cys of the protein. This location would place the GAG binding epitope in a loop region conceivably optimally exposed for interaction with GAG’s in the tissue. It is in fact very likely that vCCL4 is a good GAG binding chemokine since it has an additional potential basic pair located in the opposite end of the canonical 3D structure of the molecule.
Endogenous CCR2 ligands - Most studies with CCR2 ligands have been done on primary cells especially monocytes. So when comparing data it is important to bear in mind that primary cells express multiple chemokine receptors and that the CCR2 ligands CCL7, CCL8 and CCL13 can activate CCR1, CCR3 and CCR5 besides CCR2. Other groups using CCR2 transfected cells have found binding affinities of the four members of the MCP-family in the nanomolar range, which are in agreement with our results(34;35) except that we found CCL2 to have an affinity in the subnanomolar range which probably is due to cell line and assay differences. The finding that CCL8 and CCL13 were unable displace all bound $^{125}$I-CCL2 from CCR2 are known from other binding studies on monocytes and CCR2 transfected cell lines(35-38), where one group even reported increasing binding of $^{125}$I-CCL2 with increasing concentrations of CCL8(36) a phenomenon known from other chemokines in binding assays and which probably is due to fact that certain chemokines tend to oligomerize(39)The potencies in chemotaxis for CCL2, CCL7 and CCL13 were in agreement with another study using a CCR2 transfected 300.19 murine pre-B cell line(38).

vCCL4 and the HHV6 lifecycle - Why does HHV6 specifically target the CCR2 receptor? In this connection it should be noted that a herpesvirus beside the capacity to generate a productive infection should be able to establish latency in certain cell-types, where it can hide from the immune system waiting for the moment, when it will be reactivated. HHV6 can replicate in several cell types, for example T-cells and macrophages/monocytes (2), and HHV6 can be recovered from macrophages from normal adult human beings indicating that HHV6 establishes latency in this cell type(40). CCR2 is known to be expressed on macrophages and lymphocytes(4). Thus, it could be speculated that HHV6 has optimized vCCL4 to attract T-cells and macrophages/monocytes in order to infect them and to establish latency at least in the
monocyte/macrophage lineage. It is interesting that HIV seems to have chosen the same strategy to exploit the chemokine system as the HIV encoded Tat-protein acts as a CCR2 agonist capable of attracting monocytes and thereby favour rapid spread of infection(41).

**vCCL4 as a CCR2 agonist** – vCCL4 appear to be a highly efficacious but relatively low potency agonist for CCR2. Obviously, the potency of a ligand should be compared to the actual available concentrations of the ligand, which for vCCL4 at present is unknown, but could be considerable locally around a HHV6 infected cell due to potential highly efficient expression and the potential efficient GAG binding - discussed above. In a previous study Zou and coworkers found that a protein consisting of the U83 chemokine domain fused to an IgG molecule was biologically active as this molecule could induce chemotaxis and calcium mobilization in the monocytic THP-1 cell line(28). The THP-1 cells are known to express at least the CCR1, CCR2, CCR5 and CX3CR1 chemokine receptors and no attempt was done in that study to identify the specific target receptor for the U83 gene product (42;43). The present study indicates that the fusion protein conceivably acted through CCR2 receptors on the THP-1 cells.

Despite its high efficacy it is still puzzling that the HHV6 encoded chemokine has 25- to 1000-fold less affinity compared with the endogenous human ligands. In general it is not uncommon that viral homologues of cytokines have less affinity than their endogenous mammalian counterparts. This is the case for example for the HHV8 encoded viral chemokine vMIP-III which has been characterized to be a low affinity CCR4 agonist (19). Also the EBV encoded IL-10 homologue has a 1000 fold less affinity to the human IL-10 receptor than endogenous Il-10(44) and the HHV8 encoded IL-6 homologue has a 10 fold less affinity to the human IL-6 receptor than endogenous IL-6(45). Similarly, poxvirus homologues of the epidermal growth factor (EGF) have 10- to 1000 fold lower affinity to the EGF receptor than
endogenous EGF(46). Importantly, in the latter study the viral EGF homologues were in respect of mitogenic activity even more potent than their mammalian counterparts due to attenuation of receptor degradation leading to a sustained signal transduction. These results underline the point that viral homologues of human gene products can be significantly different from their human counterparts not just quantitatively but also qualitatively.

**vCCL4 and possible HHV6 associated diseases** – MS is regarded as an autoimmune disease with a likely environmental trigger(47). Many viral candidates have been suggested during the years as being involved in MS. In recent years HHV6 has attracted much attention as the virus is neurotropic and in a number of studies and reports has been linked to MS. For example, strong IgM responses to HHV6 have been detected in MS patients(48;49), HHV6 has been detected in oligodendrocytes around MS-plaques(49;50) and clinical cases have been reported where active HHV6 infection was closely associated with demyelinative disease(51-53). Importantly, experiments have shown that mice deleted for CCR2 - in contrast to mice deleted for CCR5 - are resistant to experimental autoimmune encephalomyelitis, EAE(29;30;54). Thus, it could be speculated that when HHV6, residing latently in the CNS, is reactivated, vCCL4 is produced whereby CCR2 expressing lymphocytes and macrophages are recruited. In this way HHV6 could be one factor among others in the generation of the autoimmune response seen in MS. The identification of vCCL4 in active plaques with immunohistochemistry and the development of vCCL4 transgenic mice could help to clarify these questions.
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Reference List

1. Salahuddin, S. Z., Ablashi, D. V., Markham, P. D., Josephs, S. F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Staal, F., Kramarsky, B., and . (1986) *Science* **234**, 596-601

2. Braun, D. K., Dominguez, G., and Pellett, P. E. (1997) *Clin.Microbiol.Rev.* **10**, 521-567

3. Rollins, B. J. (1997) *Blood* **90**, 909-928

4. Murphy, P. M., Baggiolini, M., Charo, I. F., Hebert, C. A., Horuk, R., Matsushima, K., Miller, L. H., Oppenheim, J. J., and Power, C. A. (2000) *Pharmacol.Rev.* **52**, 145-176

5. Murphy, P. M. (2002) *Pharmacol.Rev.* **54**, 227-229

6. Chee, M. S., Satchwell, S. C., Preddie, E., Weston, K. M., and Barrell, B. G. (1990) *Nature* **344**, 774-777

7. Milne, R. S., Mattick, C., Nicholson, L., Devaraj, P., Alcamì, A., and Gompels, U. A. (2000) *J.Immunol.* **164**, 2396-2404

8. Isegawa, Y., Ping, Z., Nakano, K., Sugimoto, N., and Yamanishi, K. (1998) *J.Virol.* **72**, 6104-6112

9. Nicholas, J. (1996) *J.Virol.* **70**, 5975-5989

10. Arvanitakis, L., Geras-Raaka, E., Varma, A., Gershengorn, M. C., and Cesarian, E. (1997) *Nature* **385**, 347-350
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11. Bais, C., Santomasso, B., Coso, O., Arvanitakis, L., Raaka, E. G., Gutkind, J. S., Asch, A.
    S., Cesarman, E., Gershengorn, M. C., Mesri, E. A., and Ghengorn, M. C. (1998) Nature 
    391, 86-89

12. Rosenkilde, M. M., Kledal, T. N., Brauner-Osborne, H., and Schwartz, T. W. (1999)
    J.Biol.Chem. 274, 956-961

13. Yang, T. Y., Chen, S. C., Leach, M. W., Manfra, D., Homey, B., Wiekowski, M., Sullivan,
    L., Jenh, C. H., Narula, S. K., Chensue, S. W., and Lira, S. A. (2000) J.Exp.Med. 191, 445-
    454

14. Kledal, T. N., Rosenkilde, M. M., and Schwartz, T. W. (1998) FEBS Lett. 441, 209-214

15. Bodaghi, B., Jones, T. R., Zipeto, D., Vita, C., Sun, L., Laurent, L., Arenzana-Seisdedos, 
    F., Virelizier, J. L., and Michelson, S. (1998) J.Exp.Med. 188, 855-866

16. Kledal, T. N., Rosenkilde, M. M., Coulin, F., Simmons, G., Johnsen, A. H., Alouani, S.,
    Power, C. A., Luttichau, H. R., Gerstoft, J., Clapham, P. R., Clark-Lewis, I., Wells, T. N.,
    and Schwartz, T. W. (1997) Science 277, 1656-1659

17. Luttichau, H. R., Stine, J., Boesen, T. P., Johnsen, A. H., Chantry, D., Gerstoft, J., and
    Schwartz, T. W. (2000) J.Exp.Med. 191, 171-180

18. Luttichau, H. R., Lewis, I. C., Gerstoft, J., and Schwartz, T. W. (2001) Eur.J.Immunol. 31,
    1217-1220
19. Stine, J. T., Wood, C., Hill, M., Epp, A., Raport, C. J., Schweickart, V. L., Endo, Y.,
Sasaki, T., Simmons, G., Boshoff, C., Clapham, P., Chang, Y., Moore, P., Gray, P. W., and
Chantry, D. (2000) *Blood* **95**, 1151-1157

20. Endres, M. J., Garlisi, C. G., Xiao, H., Shan, L., and Hedrick, J. A. (1999) *J.Exp.Med.* **189**, 1993-1998

21. Dairaghi, D. J., Fan, R. A., McMaster, B. E., Hanley, M. R., and Schall, T. J. (1999)
*J.Biol.Chem.* **274**, 21569-21574

22. Cha, T. A., Tom, E., Kemble, G. W., Duke, G. M., Mocarski, E. S., and Spaete, R. R.
(1996) *J.Virol.* **70**, 78-83

23. Penfold, M. E., Dairaghi, D. J., Duke, G. M., Saederup, N., Mocarski, E. S., Kemble, G.
W., and Schall, T. J. (1999) *Proc.Natl.Acad.Sci.U.S.A* **96**, 9839-9844

24. Chen, S., Bacon, K. B., Li, L., Garcia, G. E., Xia, Y., Lo, D., Thompson, D. A., Siani, M.
A., Yamamoto, T., Harrison, J. K., and Feng, L. (1998) *J.Exp.Med.* **188**, 193-198

25. Ghirnikar, R. S., Lee, Y. L., and Eng, L. F. (2001) *J.Neurosci.Res.* **64**, 582-589

26. Clark-Lewis, I., Vo, L., Owen, P., and Anderson, J. (1997) *Methods Enzymol.* **287**, 233-250

27. French, C., Menegazzi, P., Nicholson, L., Macaulay, H., DiLuca, D., and Gompels, U. A.
(1999) *Virology* **262**, 139-151

28. Zou, P., Isegawa, Y., Nakano, K., Haque, M., Horiguchi, Y., and Yamanishi, K. (1999)
*J.Virol.* **73**, 5926-5933
29. Fife, B. T., Huffnagle, G. B., Kuziel, W. A., and Karpus, W. J. (2000) *J.Exp.Med.* **192**, 899-905

30. Izikson, L., Klein, R. S., Charo, I. F., Weiner, H. L., and Luster, A. D. (2000) *J.Exp.Med.* **192**, 1075-1080

31. Zhang, Y. J., Rutledge, B. J., and Rollins, B. J. (1994) *J.Biol.Chem.* **269**, 15918-15924

32. Gong, J. H. and Clark-Lewis, I. (1995) *J.Exp.Med.* **181**, 631-640

33. Hileman, R. E., Fromm, J. R., Weiler, J. M., and Linhardt, R. J. (1998) *Bioessays* **20**, 156-167

34. Berkhout, T. A., Sarau, H. M., Moores, K., White, J. R., Elshourbagy, N., Appelbaum, E., Reape, R. J., Brawner, M., Makwana, J., Foley, J. J., Schmidt, D. B., Imburgia, C., McNulty, D., Matthews, J., O'Donnell, K., O'Shanessy, D., Scott, M., Groot, P. H., and Macphee, C. (1997) *J.Biol.Chem.* **272**, 16404-16413

35. Gong, X., Gong, W., Kuhns, D. B., Ben Baruch, A., Howard, O. M., and Wang, J. M. (1997) *J.Biol.Chem.* **272**, 11682-11685

36. Yamagami, S., Tanaka, H., and Endo, N. (1997) *FEBS Lett.* **400**, 329-332

37. Sozzani, S., Zhou, D., Locati, M., Rieppi, M., Proost, P., Magazin, M., Vita, N., van Damme, J., and Mantovani, A. (1994) *J.Immunol.* **152**, 3615-3622

38. Stellato, C., Collins, P., Ponath, P. D., Soler, D., Newman, W., La Rosa, G., Li, H., White, J., Schwiebert, L. M., Bickel, C., Liu, M., Bochner, B. S., Williams, T., and Schleimer, R. P. (1997) *J.Clin.Invest* **99**, 926-936
39. Neote, K., DiGregorio, D., Mak, J. Y., Horuk, R., and Schall, T. J. (1993) Cell 72, 415-425

40. Kondo, K., Kondo, T., Okuno, T., Takahashi, M., and Yamanishi, K. (1991) J. Gen. Virol. 72 (Pt 6), 1401-1408

41. Albini, A., Ferrini, S., Benelli, R., Sforzini, S., Giunciuglio, D., Aluigi, M. G., Proudfoot, A. E., Alouani, S., Wells, T. N., Mariani, G., Rabin, R. L., Farber, J. M., and Noonan, D. M. (1998) Proc. Natl. Acad. Sci. U.S.A 95, 13153-13158

42. Xu, L., Rahimpour, R., Ran, L., Kong, C., Biragyn, A., Andrews, J., Devries, M., Wang, J. M., and Kelvin, D. J. (1997) J. Leukoc. Biol. 62, 653-660

43. Raport, C. J., Schweickart, V. L., Eddy, R. L., Jr., Shows, T. B., and Gray, P. W. (1995) Gene 163, 295-299

44. Liu, Y., de Waal, M. R., Briere, F., Parham, C., Bridon, J. M., Banchereau, J., Moore, K. W., and Xu, J. (1997) J. Immunol. 158, 604-613

45. Osborne, J., Moore, P. S., and Chang, Y. (1999) Hum. Immunol. 60, 921-927

46. Tzahar, E., Moyer, J. D., Waterman, H., Barbacci, E. G., Bao, J., Levkowitz, G., Shelly, M., Strano, S., Pinkas-Kramarski, R., Pierce, J. H., Andrews, G. C., and Yarden, Y. (1998) EMBO J. 17, 5948-5963

47. Compston, A. and Coles, A. (2002) Lancet 359, 1221-1231

48. Soldan, S. S., Berti, R., Salem, N., Secchiero, P., Flamand, L., Calabresi, P. A., Brennan, M. B., Maloni, H. W., McFarland, H. F., Lin, H. C., Patnaik, M., and Jacobson, S. (1997) Nat Med. 3, 1394-1397
49. Friedman, J. E., Lyons, M. J., Cu, G., Ablashl, D. V., Whitman, J. E., Edgar, M., Koskiniemi, M., Vaheri, A., and Zabriskie, J. B. (1999) *Mult. Scler.* 5, 355-362

50. Challoner, P. B., Smith, K. T., Parker, J. D., MacLeod, D. L., Coulter, S. N., Rose, T. M., Schultz, E. R., Bennett, J. L., Garber, R. L., Chang, M., and . (1995) *Proc. Natl. Acad. Sci. U.S.A* 92, 7440-7444

51. Carrigan, D. R., Harrington, D., and Knox, K. K. (1996) *Neurology* 47, 145-148

52. Kamei, A., Ichinohe, S., Onuma, R., Hiraga, S., and Fujiwara, T. (1997) *Eur. J. Pediatr.* 156, 709-712

53. Balashov, K. E., Rottman, J. B., Weiner, H. L., and Hancock, W. W. (1999) *Proc. Natl. Acad. Sci. U.S.A* 96, 6873-6878

54. Tran, E. H., Kuziel, W. A., and Owens, T. (2000) *Eur. J. Immunol.* 30, 1410-1415

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1 For example 300.19 cells are known to express an endogenous CXCR4 receptor.
Legends to figures:

**Figure 1: Alignment of selected CCR2 ligands to vCCL4.** CCL2, CCL7, CCL8, CCL11, CCL13, CCL16 and vCCL4 were aligned using the ClustalW 1.8. Identical amino acids are shown white on black, whereas similar amino acids are shown white on grey. Asteriks indicate Cys residues. The region in vCCL4 with the CC-/CX3C- chemokine motif is shown magnified in box.

**Figure 2: Effect of synthetic vCCL4 on calcium mobilization on a panel of cell lines stably transfected with humane chemokine receptors.** $10^{-6}$ M of vCCL4 or vehicle were first added to the cells followed by a sub-maximal dose of an appropriate endogenous human chemokine. The height of the response with the endogenous ligand only, the height of the response with vCCL4 only and height of the endogenous ligand after addition of vCCL4 was measured. Left box diagram shows the inhibition with vCCL4 of the response of endogenous ligand compared to response with endogenous ligand only with SEM as indicated. Right box diagram shows the response of vCCL4 compared to the response of endogenous ligand with SEM as indicated. N=2. The chemokine concentrations used were (CCR1; $10^{-8}$ M CCL5), (CCR2; $10^{-9}$ M CCL2), (CCR3; $10^{-8}$ M CCL7), (CCR4; $10^{-8}$ M CCL22), (CCR5; $10^{-8}$ M CCL5), (CCR6; $10^{-8}$ M CCL20), (CCR7; $10^{-9}$ M CCL21), (CCR8; $10^{-8}$ M CCL1), (CCR9; $10^{-8}$ M CCL25), (CCR10; $10^{-8}$ M CCL27), (CXCR1; $10^{-8}$ M CXCL8), (CXCR2; $10^{-8}$ M CXCL8), (CXCR3; $10^{-9}$ M CXCL11), (CXCR4; $10^{-8}$ M CXCL12), (CXCR5; $10^{-8}$ M CXCL13), (CXCR6; $10^{-8}$ M CXCL16), (XCR1; $10^{-7}$ M mXCL1), (CX3CR1; $10^{-8}$ M CX3CL1).
A HHV6 encoded CCR2 agonist

Figure 3. Competition binding experiments with synthetic vCCL4 and selected endogenous CCR2- ligands on CHO-cells stably transfected with CCR2. CCL2 (■), CCL7 (▼), CCL8 (▲), CCL13 (◆) and vCCL4 (●). N=3.

Figure 4. Dose-response and cross-desensitization calcium mobilization experiments using synthetic vCCL4 and selected endogenous CCR2- ligands for 300.19 cells stably transfected with CCR2. A) Dose-response experiments with vCCL4, CCL2, CCL7, CCL8 and CCL13 at concentrations of $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$ M or vehicle. Ligand was added to the cells at 50 s. One representative assay out of three is shown for CCL2, CCL7, CCL13 and vCCL4 and out of two for CCL8. B) Cross-desensitization experiments with vCCL4 and CCL2. Vehicle, vCCL4 or CCL2 were added at 50 s followed by either vCCL4 or CCL2 at 250 s. $10^{-6}$ M of vCCL4 or $10^{-9}$ M of CCL2 were used. One representative assay out of two is shown.

Figure 5. Effect of synthetic vCCL4 and selected endogenous CCR2-ligands on chemotactic activity for L12 cells stably transfected with CCR2. CCL2 (■), CCL7 (▼), CCL8 (▲), CCL13 (◆) and vCCL4 (●). Top diagram illustrates relative efficacy of CCR2 ligands, whereas the bottom five diagrams shows individual chemotatic curves for the five CCR2 ligands. Average cell count for all assays for each individual ligand is shown.
% Response of endogenous ligand with pretreatment of vCCL4

% Response of vCCL4 compared to endogenous ligand
% of max. bound
125I-CCL2

log [chemokine]

vCCL4

0 -12 -11 -10 -9 -8 -7 -6

CCL2

CCL8

CCL13

CCL7
A highly selective CCR2 chemokine agonist encoded by human herpesvirus 6
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