THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 4, Issue of January 25, pp. 2785–2789, 2002
Printed in U.S.A.


# Comprehensive Mapping of Poxvirus vCCI Chemokine-binding Protein

## EXPANDED RANGE OF LIGAND INTERACTIONS AND UNUSUAL DISSOCIATION KINETICS*

Received for publication, October 21, 2001
Published, JBC Papers in Press, November 5, 2001, DOI 10.1074/jbc.M109884200

**Jennifer M. Burns‡, Daniel J. Dairaghi‡, Mary Deitz§, Monica Tsang§, and Thomas J. Schall‡¶**

*From the ‡Divisions of Discovery Biology and Molecular Pharmacology, ChemoCentryx, San Carlos, California 94070 and §R&D Systems, Minneapolis, Minnesota 55413*

**In compiling a comprehensive map of the ligand binding capacity of elements within the chemokine system, we have determined the spectrum of chemokines capable of interacting with the poxvirus-encoded viral CC chemokine inhibitor, vCCI. More than 80 chemokines were tested in parallel for their ability to displace radiolabeled signature chemokines from vCCI. Of these chemokines, 26 showed potential high affinity interactions. These interactions revealed an expanded spectrum of binding capacity for vCCI to now include molecules such as human myeloid progenitor inhibitory factor-1 as ligands. In addition, high affinity viral protein-protein interactions were revealed. For example, binding between poxvirus vCCI and the herpesvirus vMIP-II from HHV8 occurs with $IC_{50}$ ~10–50 nM. Unusual dissociation kinetics were observed between certain chemokines and vCCI. Notably, many ligands displayed a precipitous displacement profile, suggesting marked positive cooperativity of binding. Finally, heterologous competition provided evidence for overlapping but distinct binding sites for the many chemokines that bind to vCCI. The determination of the binding fingerprint and unusual binding interactions of vCCI with a large number of chemokines suggest a finely honed evolutionary strategy of chemokine sequestration during viral infection.**

The ability of viruses to evade destruction by the host is achieved in part by the molecular neutralization of immune defenses by virus-encoded components. Poxviruses encode a secreted chemokine binding protein, viral CC chemokine inhibitor (vCCI),[1] which likely represents one such mechanism, allowing the virus to achieve a molecular preemption of the primary immune response. Also known as "T1" (in leporipoxviruses) or "35 kDa" (in orthopoxviruses), vCCI has been shown to bind to several human chemokines, but it possesses no close molecular similarity to any endogenous proteins (2). Because chemokines (for chemoattractant cytokines) are secreted proteins that serve to direct the trafficking of leukocytes during the body's response to infection and inflammation, the sequestration of chemokines by poxviruses may serve an anti-inflammatory function that is advantageous to the virus.

Chemokines comprise a seemingly vast array of structurally and functionally related proteins; currently the number approaches ~50 distinct gene products identified to date in humans (reviewed in Ref. 1). These are divided into four classes, CC, CXC, C, and CX3C, based on the number and spacing of the N-terminal cysteine residues in a conserved structural motif. In addition, several viruses are known to make their own versions of chemokines. These include vMIP-I, -II, and -III (3–7) made by HHV8, the proteins vCXC-1 and -2 (8) produced by human CMV and m131/139 (9, 10) made by murine CMV, the HHV6 viral chemokine U83 (11), and vMCC-1 (6, 12–14) from MCV.[2] Adding further complexity to the chemokine system is the fact that post-translational modifications of chemokines (*e.g.* by enzymatic modifications via CD26 (reviewed in Ref. 15) and other proteolytic processing steps, as well as alternative splicing events in some cases (16–19)) create an even more diverse spectrum of chemokine variants, some of which have different activities than the parental chemokine.

vCCI has been reported to bind preferentially to certain human CC chemokines including such molecules as hRANTES (20, 21), hMCP-1 (20–23), hMIP-1$\alpha$ (20–22) and hMIP-1$\beta$ (20). However, there are discrepancies in the literature both in terms of specificity and affinity of binding. For example, initially vCCI was thought to interact broadly with CC and CXC chemokines (20); however, others (22, 23) have suggested a pattern of selectivity for CC chemokines. Additionally, vastly disparate binding affinities have been reported for the same chemokines ranging from low micromolar to low nanomolar values (20, 22, 23). This may be due in part to the diverse methods of binding analyses employed. To date there have been no studies attempting a comprehensive, direct, and real-time comparison of the binding of many chemokines to vCCI under similar analytical conditions.

To gain a thorough understanding of the biology of vCCI and its role in viral pathogenesis, we undertook to map comprehensively its binding capacity and to investigate in detail the nature of its multipotential binding activity. Toward this end, we employed the parallel interrogation of >80 chemokines and chemokine variants from human and viral sequences as well as those from other species, assessing their ability to bind to vCCI. We found that vCCI is capable of binding with high affinity ($K_d$ ~5–55 nM) to a spectrum of ligands more wide ranging than previously reported. These include chemokines encoded by other types of viruses as well. The ligand binding profile determined here also reveals that vCCI does not bind to all CC chemokines, nor does it interact with members of the CXC, C, or CX3C groups. Finally we noted examples of unusual binding

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: ChemoCentryx, 1539 Industrial Rd., San Carlos, CA 94070. Tel.: 650-632-2900; Fax: 650-632-2910; E-mail: tschall@chemocentryx.com.

[1] The abbreviations used are: vCCI, viral CC chemokine inhibitor; HHV, human herpesvirus; CMV, cytomegalovirus; MCV, poxvirus *Molluscum contagiosum*; v, viral; h, human; m, mouse; r, rat.

[2] For chemokine abbreviations see Ref. 1.

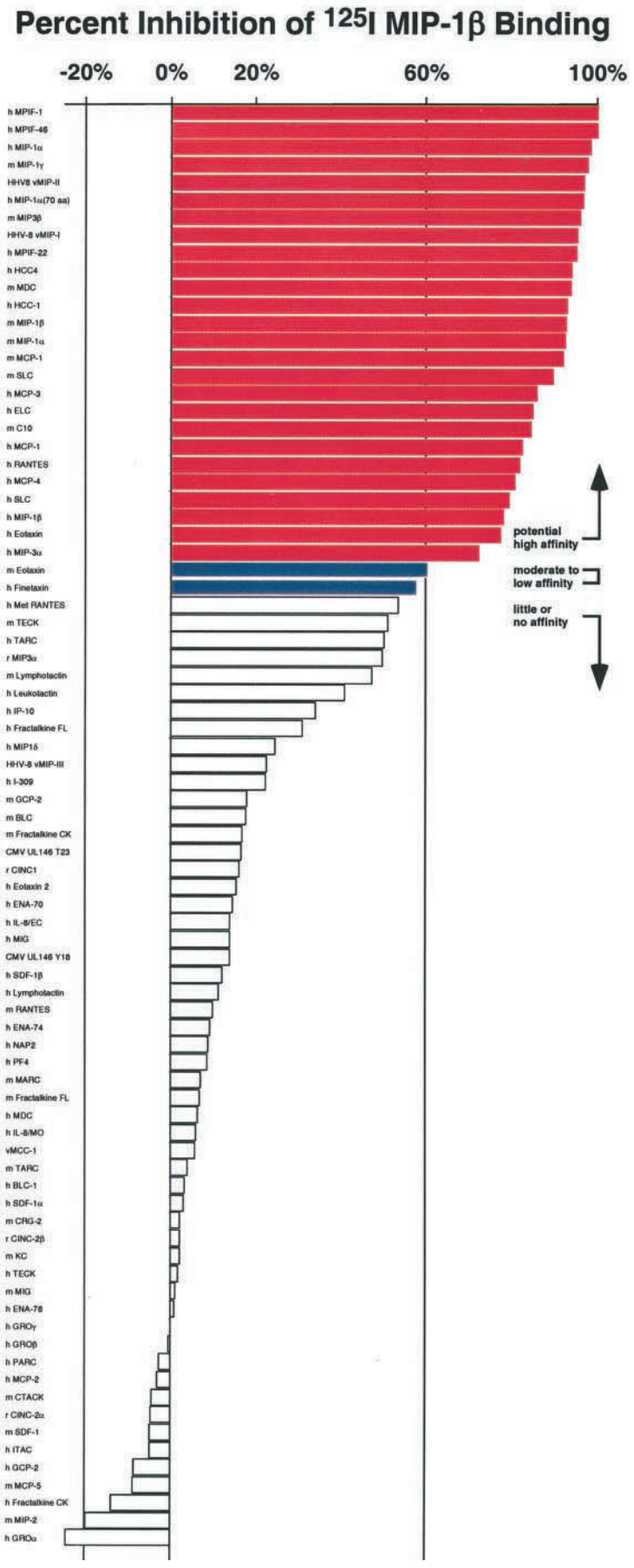



FIG. 1. **Ligand binding fingerprint of vCCI.** Competitive binding profile of vCCI binding to $^{125}$I-hMIP-1 against a comprehensive array of purified viral, human, and murine chemokines. Chemokines are grouped into classes as follows: "potential high affinity" (*red bars*), demonstrating >75% inhibition; "potential moderate to low affinity" (*blue bars*), showing 75–60% inhibition; "little or no affinity" (*white bars*), possessing <60% inhibition. Percent inhibition of binding is shown and represents the mean of three determinations (~29,000 cpm input; ~8000 cpm total bound; ~1000 cpm nonspecific bound). The standard deviation values for potential high affinity ligand interactions are <10% in all cases; however, with the little-to-no affinity interactions, the standard deviations are ~40%; error bars were omitted for clarity. Intra-assay variability was ~40% for lower affinity interactions, strongly suggesting that binding values to the left of the zero meridian are not likely to be significant.

interactions between vCCI and selected chemokines, suggesting that vCCI interacts with different chemokines through similar yet distinct binding sites. Such findings illuminate not only the biochemical characteristics of this unusual virally encoded binding protein but also may provide key insights into the virus's ability to evade the host immune response after infection.

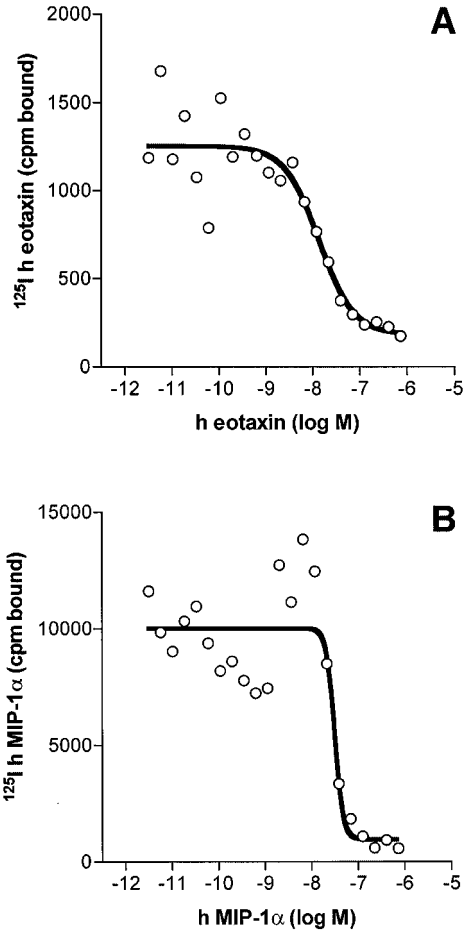



FIG. 2. **Homologous binding competition.** Profile of vCCI binding to $^{125}$I-human eotaxin (~32,000 cpm input; ~3000 cpm total bound; ~200 cpm nonspecific bound) (*A*) and $^{125}$I-hMIP-1a (~29,000 cpm input; ~9500 cpm total bound; ~250 cpm nonspecific bound) (*B*) in competition with the identical unlabeled ligands. Data points represent the mean of quadruplicate wells. Calculated $IC_{50}$ and Hill coefficients are shown in Table I.

## EXPERIMENTAL PROCEDURES

*Binding Assays*—We employed a variation of the recently developed DisplaceMax™ technique (6, 24) to interrogate an array of chemokine interactions with vCCI and to examine specific binding characteristics. These assays exploit the observation that vCCI binds to the filter in radioligand filter binding assays. By including a radiolabeled chemokine that interacts with vCCI it is then possible to detect vCCI binding to the filter. Chemokines that compete for this binding then prevent vCCI-$^{125}$I chemokine complexes from forming, and thus the decreasing cpm on the filter indicates competitor chemokine bound to vCCI displacing the $^{125}$I chemokine. Eighty-three distinct chemokine elements were incubated with 25 nM vCCI (R&D Systems) followed by the addition of radiolabeled chemokine ($^{125}$I-labeled human eotaxin, $^{125}$I-hMIP-1$\alpha$, and $^{125}$I-hMIP-1$\beta$, Amersham Biosciences, Inc.) for 3 h at 4 °C in the following binding medium (25 mM HEPES, 140 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$ and 0.2% bovine serum albumin, adjusted to pH 7.1). Where indicated, the detergents Tween 20 (Mallinckrodt Baker, Inc.) and Triton X-100 (Bio-Rad) were included in the reaction at the indicated final concentrations. (Because of the addition of high levels of detergent in the binding medium, the ability to detect input cpm and total cpm bound differs for this adaptation of the binding assay slightly as follows: Tween 20 1%: ~49,000 cpm input, ~14,000 cpm total bound, ~775 cpm nonspecific bound; Tween 20 0.1%: ~63,000 cpm input, ~14,000 cpm total bound, ~550 cpm nonspecific bound; Tween 20 0.01%: ~60,000 cpm input, ~16,000 cpm total bound, ~765 cpm nonspecific bound; Triton X-100 1%: ~54,000 cpm input, ~19,000 cpm total bound, ~650 cpm nonspecific bound; Triton X-100 0.1%: ~62,000 cpm input, ~11,000 cpm total bound, ~600 cpm nonspecific bound; Triton X-100 0.01%: ~59,000 cpm input, ~9,600 cpm total bound, ~650 cpm nonspecific bound; binding medium only: ~51,000 cpm input, ~18,000 cpm total bound, ~1000 cpm nonspecific bound.) Following incubation in all binding assays, reactions were aspirated onto polyethyleneimine-treated GF/B glass filters (Packard) using a cell harvester (Packard) and washed twice (25 mM HEPES, 500 mM NaCl, 1 mM $CaCl_2$, 5 mM $MgCl_2$, adjusted to pH 7.1). Scintillant (MicroScint 10, Packard) was added to the wells, and the filters were counted in a Packard Topcount scintillation counter. Data were analyzed and plotted using Prism (GraphPad Prism, version 3.0a, for Macintosh, GraphPad Software).

## RESULTS AND DISCUSSION

*Comprehensive Binding "Fingerprint" of vCCI*—A more thorough understanding of the chemokine binding profile of vCCI could ultimately provide insight into the biologic function of this protein in the *in vivo* setting. As a first approximation, we examined the chemokine binding profile of the poxvirus-encoded vCCI in an assumption-blind manner. We tested a starting panel of >80 purified chemokine elements and compared their ability to displace selected "signature" chemokines, such as $^{125}$I-hMIP-1$\beta$, from vCCI in a filtration-based assay. As many as 26 chemokines, when used as cold competitors (at 200 nM final concentration), were found to inhibit >60% of the specific binding of the radiolabeled signature chemokine to vCCI (Fig. 1). The spectrum of ligands that bound to vCCI included many interactions not reported previously. For exam-

TABLE I
*Summary of vCCI-chemokine binding interactions*

| | | $IC_{50}$ | Hill coefficient |
|---|---|---|---|
| | | *nM* | |
| Homologous competition | $^{125}$I-hMIP-1$\alpha$/hMIP-1$\alpha$ | 32 | >4.0 |
| | $^{125}$I-human eotaxin/human eotaxin | 32 | 1.2 |
| Heterologous competition | | | |
| High affinity | $^{125}$I-hMIP-1$\alpha$/vMIP-II | 26 | >4.0 |
| | $^{125}$I-hMIP-1$\beta$/hMIP-1$\alpha$ | 23 | >4.0 |
| | $^{125}$I-hMIP-1$\beta$/hMPIF | 40 | >4.0 |
| | $^{125}$I-hMIP-1$\beta$/mMCP-1/JE | 32 | >4.0 |
| | $^{125}$I-human eotaxin/hMIP-1$\alpha$ | 8 | >4.0 |
| | $^{125}$I-human eotaxin/vMIP-II | 15 | 2.8 |
| | $^{125}$I-human eotaxin/rMIP-3$\alpha$ | 46 | 1.7 |
| | $^{125}$I-human eotaxin/hSLC | 17 | 1.4 |
| Lower affinity | $^{125}$I-hMIP-1$\alpha$/hMPIF | 75 | 3.3 |
| | $^{125}$I-hMIP-1$\beta$/vMIP-II | 50 | 2.8 |
| | $^{125}$I-hMIP-1$\beta$/rMIP-3$\alpha$ | 130 | 1.5 |
| | $^{125}$I-human eotaxin/hMCP-1 | 147 | 0.4 |

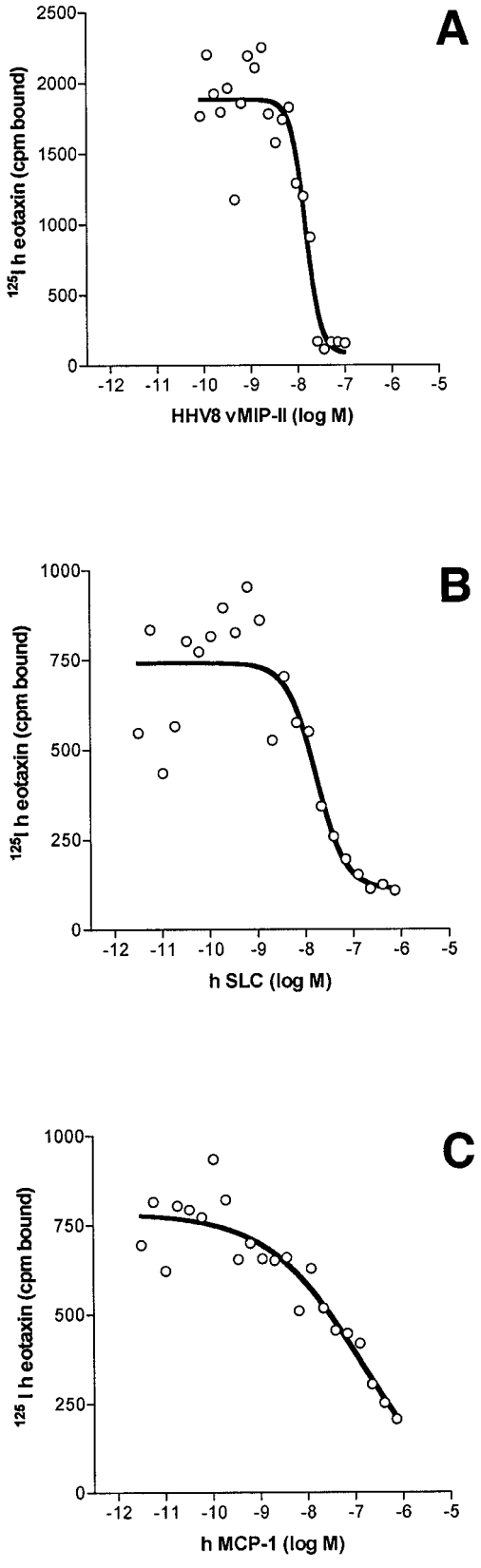
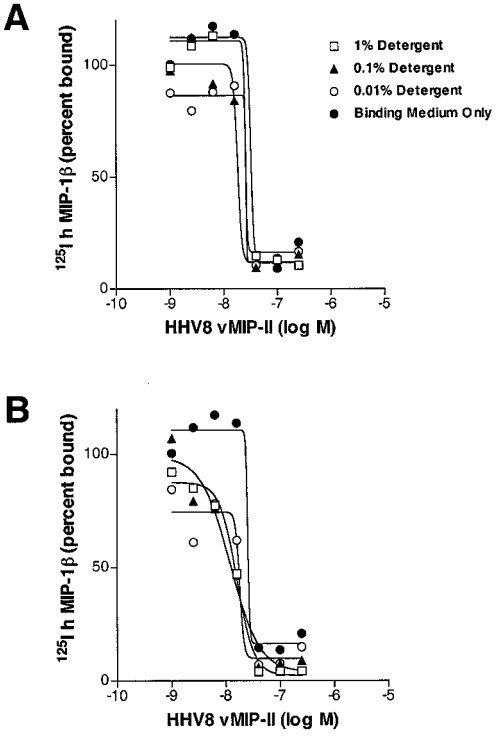



FIG. 3. **Heterologous binding competition.** Selected chemokine competitions with vCCI and $^{125}$I-human eotaxin demonstrate potential high, medium, and low affinity interactions. HHV8 vMIP-II represents a potential high affinity ligand for vCCI (~32,000 cpm input; ~1500 cpm total bound; ~150 cpm nonspecific bound) (*A*). By contrast, hSLC (*B*) and hMCP-1 (*C*) represent potential medium and low affinity ligands, respectively (~26,000 cpm input; ~750 cpm total bound; ~100 cpm nonspecific bound). Data points represent the mean of quadruplicate wells. Calculated $IC_{50}$ and Hill coefficients are shown in Table I.




C

| Detergent Treatment | $IC_{50}$(nM) | Hill Coefficient |
|---|---|---|
| Tween-20 1% | 35 | >4.0 |
| Tween-20 0.1% | 18 | >4.0 |
| Tween-20 0.01% | 26 | >4.0 |
| Triton X-100 1% | 16 | 2.8 |
| Triton X-100 0.1% | 12 | 1.6 |
| Triton X-100 0.01% | 17 | >4.0 |
| Binding Medium Only | 21 | >4.0 |

FIG. 4. **Addition of detergents to binding assay.** Addition of the non-ionic detergents Tween 20 (*A*) and Triton X-100 (*B*) to binding medium at indicated concentrations to attempt to disrupt the extreme cooperative binding indicated by the Hill coefficient. $^{125}$I-hMIP-1b competition with vMIP-II for binding to vCCI is shown here. The condition of buffer alone is included in both panels for clarity. Data points represent the mean of quadruplicate wells. The calculated $IC_{50}$ and Hill coefficients are indicated in the accompanying table (*C*).

ple, multiple forms of the CC chemokine hMPIF-1 (25, 26), including splice variants that possess an additional coded exon (17), bound to vCCI. Also, two herpesvirus-encoded chemokines, HHV8 vMIP-I and HHV8 vMIP-II, were very effective in displacing the signature chemokine from vCCI. Although most CC chemokines exhibited binding to vCCI, a few CC chemokines, such as hMDC, hTARC, and hTECK, did not. Qualitatively similar results were seen with another signature chemokine, $^{125}$I-hMIP-1$\alpha$. In no case have we detected evidence of vCCI binding to CXC, C, or CX3C chemokines.

*Homologous Competition with Different Chemokines Suggests Distinct Binding Interactions*—We wished to investigate in greater detail the nature of the binding interactions of vCCI with the potential high affinity ligands suggested from the displacement binding fingerprint. Initial investigations employed homologous competition binding experiments with selected radiolabeled chemokines. Representative experiments tested whether radiolabeled human eotaxin or radiolabeled hMIP-1$\alpha$ would be displaced from vCCI over a concentration range of cold homologous competitor to assess: (i) the relative binding affinities (as approximated by $IC_{50}$ values) and (ii) the

slope (Hill coefficient) of the binding displacement curves. It has been established that the Hill coefficients reveal something of the biophysical nature of receptor-ligand interactions (24), where a Hill number of ~1 implies a single binding site with a simple mass action displacement. Hill values >1 are indicative of cooperative binding, and Hill values <1 suggest negative cooperativity. Homologous displacement with the chemokines tested revealed a range of distinct interactions. For example, homologous displacement of $^{125}$I-labeled human eotaxin from vCCI with cold human eotaxin (Fig. 2*A*) revealed an $IC_{50}$ of ~32 nM and a Hill coefficient of 1.2 (see Table I). This was in marked contrast to the interactions of $^{125}$I-hMIP-1$\alpha$; whereas competition for vCCI binding with cold hMIP-1$\alpha$ showed a nearly identical $IC_{50}$ (~32 nM), the binding displacement curve had a precipitous slope, with a very large Hill coefficient (Fig. 2*B* and Table I). Thus, although these two chemokines seem to bind to vCCI with similar affinities, eotaxin seems to associate with simple one-site kinetics, whereas hMIP-1$\alpha$ displayed potentially marked positive cooperativity.

*Heterologous Competition*—In addition to examining homologous binding competition, heterologous binding interactions were examined. For example, we assessed the susceptibility of $^{125}$I-human eotaxin to displacement by several other chemokines that appeared to be interacting with vCCI at high affinity (as suggested from the displacement fingerprint (Fig. 1)). Cold heterologous competitors tested included HHV8 vMIP-II (Fig. 3*A*), hSLC (Fig. 3*B*), and hMCP-1 (Fig. 3*C*). Strikingly, despite the fact that in each case $^{125}$I-human eotaxin is displaced by the cold competitors, the slope of the dissociation curve is markedly different depending upon the competitor used. Indeed some ligands demonstrate a "catastrophic" dissociation profile. For example, the displacement of $^{125}$I-human eotaxin by HHV8 vMIP-II (Fig. 3*A*) reveals a very sharp dissociation profile, with all of the human eotaxin displaced over a very small concentration range of HHV8 vMIP-II. In contrast, the competition of $^{125}$I-human eotaxin by hMCP-1 or hSLC reveals a substantially different slope of the resultant binding curves. Table I summarizes both $IC_{50}$ values and approximate Hill coefficients for both homologous and heterologous competition experiments. In particular the wide range of Hill coefficients over the course of the various experiments suggest that vCCI bound to its broad spectrum of chemokines with overlapping but distinct characteristics.

*Effects of Detergents on Binding Interactions*—To test whether the high Hill coefficients seen in some of the displacement experiments reflect the formation of vCCI aggregates or micelle-like structures, different detergent conditions were assessed in the binding reaction. The addition of non-ionic detergents such as Tween 20 (1–0.01%) had no effect on the slope of the binding curve (Fig. 4, *A* and *C*). Triton X-100 did have some effect in altering the slope of the curve when added to the binding buffer at concentrations of 1 and 0.1%; however, the slopes remain steep with Hill coefficients > 1.5 (Fig. 4, *B* and *C*). These data suggest that vCCI exhibits very strong cooperative binding with a select group of chemokines, and this cooperative binding is not disrupted by the addition of detergents.

In conclusion, we have developed a broad and comprehensive vCCI binding profile allowing us to characterize vCCI-chemokine interactions. Unlike other binding analyses, we endeavored to use a more native form of vCCI (*e.g.* not tethered through an epitopic tag), and we employed a filtration assay that allowed the proteins to interact without interference by other cellular factors. The binding fingerprint developed from our studies suggests that vCCI is promiscuous, but not ubiquitous, in its binding of chemokines. Twenty-six of the >80 chemokine elements tested were identified as potential high affinity vCCI ligands. Further investigation of these binding interactions suggests that vCCI binds chemokines with overlapping but distinct sites. The finding that several but not all chemokines tested had unusually steep binding slopes supports this concept. The combination of such unusual binding and displacement characteristics suggests that the virus has developed an adaptation to capture and sequester many chemokines in the frame of limited protein topography. The selectivity for certain CC chemokines by vCCI and the distinct binding interactions with a subset of these proteins indicate that the virus has adapted a function to alter its environment to the specific needs of the virus. Collectively, these data suggest that vCCI may serve not so much as a sink for certain chemokines in the local environment but as a dynamic and selective molecular switch that has evolved different strategies to regulate the local concentration of a diverse spectrum of chemokines.

REFERENCES


1. Zlotnik, A., and Yoshie, O. (2000) *Immunity* **12,** 121–127
2. Carfi, A., Smith, C. A., Smolak, P. J., McGrew, J., and Wiley, D. C. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 12379–12383
3. Boshoff, C., Endo, Y., Collins, P. D., Takeuchi, Y., Reeves, J. D., Schweickart, V. L., Siani, M. A., Sasaki, T., Williams, T. J., Gray, P. W., Moore, P. S., Chang, Y., and Weiss, R. A. (1997) *Science* **278,** 290–294
4. 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. C., and Schwartz, T. W. (1997) *Science* **277,** 1656–1659
5. Sozzani, S., Luini, W., Bianchi, G., Allavena, P., Wells, T. N., Napolitano, M., Bernardini, G., Vecchi, A., D'Ambrosio, D., Mazzeo, D., Sinigaglia, F., Santoni, A., Maggi, E., Romagnani, S., and Mantovani, A. (1998) *Blood* **92,** 4036–4039
6. Dairaghi, D. J., Fan, R. A., McMaster, B. E., Hanley, M. R., and Schall, T. J. (1999) *J. Biol. Chem.* **274,** 21569–21574
7. Endres, M. J., Garlisi, C. G., Xiao, H., Shan, L., and Hedrick, J. A. (1999) *J. Exp. Med.* **189,** 1993–1998
8. 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
9. Fleming, P., Davis-Poynter, N., Degli-Esposti, M., Densley, E., Papadimitriou, J., Shellam, G., and Farrell, H. (1999) *J. Virol.* **73,** 6800–6809
10. Saederup, N., Lin, Y. C., Dairaghi, D. J., Schall, T. J., and Mocarski, E. S. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 10881–10886
11. Zou, P., Isegawa, Y., Nakano, K., Haque, M., Horiguchi, Y., and Yamanishi, K. (1999) *J. Virol.* **73,** 5926–5933
12. Krathwohl, M. D., Hromas, R., Brown, D. R., Broxmeyer, H. E., and Fife, K. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 9875–9880
13. Damon, I., Murphy, P. M., and Moss, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 6403–6407
14. 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
15. Van Damme, J., Struyf, S., Wuyts, A., Van Coillie, E., Menten, P., Schols, D., Sozzani, S., De Meester, I., and Proost, P. (1999) *Chem. Immunol.* **72,** 42–56
16. Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinohara, T., and Honjo, T. (1995) *Genomics* **28,** 495–500
17. Youn, B. S., Zhang, S. M., Broxmeyer, H. E., Cooper, S., Antol, K., Fraser, M., Jr., and Kwon, B. S. (1998) *Blood* **91,** 3118–3126
18. Tanaka, Y., Imai, T., Baba, M., Ishikawa, I., Uehira, M., Nomiyama, H., and Yoshie, O. (1999) *Eur. J. Immunol.* **29,** 633–642
19. Baird, J. W., Nibbs, R. J., Komai-Koma, M., Connolly, J. A., Ottersbach, K., Clark-Lewis, I., Liew, F. Y., and Graham, G. J. (1999) *J. Biol. Chem.* **274,** 33496–33503
20. Graham, K. A., Lalani, A. S., Macen, J. L., Ness, T. L., Barry, M., Liu, L. Y., Lucas, A., Clark-Lewis, I., Moyer, R. W., and McFadden, G. (1997) *Virology* **229,** 12–24
21. Smith, C. A., Smith, T. D., Smolak, P. J., Friend, D., Hagen, H., Gerhart, M., Park, L., Pickup, D. J., Torrance, D., Mohler, K., Schooley, K., and Goodwin, R. G. (1997) *Virology* **236,** 316–327
22. Lalani, A. S., Ness, T. L., Singh, R., Harrison, J. K., Seet, B. T., Kelvin, D. J., McFadden, G., and Moyer, R. W. (1998) *Virology* **250,** 173–184
23. Alcami, A., Symons, J. A., Collins, P. D., Williams, T. J., and Smith, G. L. (1998) *J. Immunol.* **160,** 624–633
24. Gosling, J., Dairaghi, D. J., Wang, Y., Hanley, M., Talbot, D., Miao, Z., and Schall, T. J. (2000) *J. Immunol.* **164,** 2851–2856
25. Macphee, C. H., Appelbaum, E. R., Johanson, K., Moores, K. E., Imburgia, C. S., Fornwald, J., Berkhout, T., Brawner, M., Groot, P. H., O'Donnell, K., O'Shannessy, D., Scott, G., and White, J. R. (1998) *J. Immunol.* **161,** 6273–6279
26. Berkhout, T. A., Gohil, J., Gonzalez, P., Nicols, C. L., Moores, K. E., Macphee, C. H., White, J. R., and Groot, P. H. (2000) *Biochem. Pharmacol.* **59,** 591–596