Disruption of CCL21-Induced Chemotaxis In Vitro and In Vivo by M3, a Chemokine-Binding Protein Encoded by Murine Gammaherpesvirus 68

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Chemokine-binding proteins represent a novel class of antichemokine agents encoded by poxviruses and herpesviruses. One such protein is encoded by the M3 gene present in the murine gammaherpesvirus 68 (MHV-68) genome. The M3 gene encodes a secreted 44-kDa protein that binds with high affinity to certain murine and human chemokines and has been shown to block chemokine signaling in vitro. However, there has been no direct evidence that M3 blocks chemokine activity in vivo, nor has the nature of M3-chemokine interaction been defined. To better understand the ability of M3 to block chemokine activity in vivo, we examined its interaction with a specific subset of chemokines expressed in lymphoid tissues, areas where gammaherpesviruses characteristically establish latency. Here we show that M3 blocks in vitro chemotaxis induced by CCL19 and CCL21, chemokines expressed constitutively in secondary lymphoid tissues. Moreover, we provide evidence that chemokine M3 binding exhibits positive cooperativity. In vivo, the expression of M3 in the pancreas of transgenic mice inhibits recruitment of lymphocytes induced by transgenic expression of CCL21 in this organ. The ability of M3 to block the biological activity of chemokines may represent an important strategy used by MHV-68 to evade immune detection and favor viral replication in the infected host.

Chemokines and their receptors have a key role in immune homeostasis via their ability to regulate leukocyte migration, differentiation, and function (23). Disturbances in the physiological expression and function of chemokines are often associated with increased susceptibility to infections and autoimmune diseases (10).

Viruses have acquired and optimized molecules that interact with the chemokine system. These virus-encoded molecules are used to promote cell entry, facilitate dissemination of infected cells, and evade the immune response (15). So far, three classes of molecules that interact with the chemokine system have been identified: viral chemokine ligands, viral chemokine receptors, and chemokine-binding proteins (15, 18). Viral chemokines have been shown to function as agonists and/or antagonists in their interaction with mammalian chemokine receptors. Acting as agonists they facilitate viral infection and dissemination; as antagonists they inhibit recruitment of specific leukocyte populations, thus contributing to immune evasion. Viral chemokine receptors have also been described, but their role in viral pathogenesis is unclear. Recent studies have implicated virally encoded chemokine receptors in proliferation and migration of cells, as well as in the pathogenesis of Kaposi’s sarcoma (22, 26, 32). The most recently discovered family of virus-encoded molecules capable of interfering with chemokine function is composed of the chemokine-binding proteins. This class of proteins shows no significant homology to mammalian proteins, which suggests that it may have evolved independently of mammalian genomic elements. The myxomavirus, for example, encodes the protein M-T7, which binds C, CC, and CXC chemokines with submicromolar affinity by interacting with the low-affinity proteoglycan binding site conserved in many chemokines (15). Other members of the chemokine-binding protein family disrupt the interaction of chemokine ligands with their cellular receptors. Members of this subgroup include proteins encoded by many poxviruses and M3, the first chemokine-binding protein found to be encoded by a herpesvirus. M3 is a 44-kDa protein encoded by murine gamma herpesvirus 68 (MHV-68). This protein binds chemokines of the CC, CXC, CX3C, and C families with high affinity and prevents chemokine-induced signal transduction in vitro (21, 27).

MHV-68 is a natural pathogen of murid rodents which bears homology to the human pathogens Kaposi’s sarcoma-associated herpesvirus and Epstein-Barr virus (24, 31). Introduction of virus intranasally leads to a productive infection of respiratory epithelial cells, which is eventually controlled by CD8 T cells (25). The initial productive infection is followed by dissemination of the virus to secondary lymphoid tissue and establishment of latency in B cells, macrophages, and dendritic cells (8).

Studies of a mutant MHV-68 containing a lacZ insertion...
disrupting the M3 open reading frame (ORF) suggested a role for M3 in establishing and maintaining latency in secondary lymphoid tissue (2). More recently, a mutant MHV-68 in which the M3 ORF was disrupted by insertion of a translational stop codon and frameshift mutation was found to be attenuated after intracerebral inoculation but had no effect on viral latency or the induction of chronic arthritis (28). The phenotypes observed in both reports are likely to be caused by the inability of the M3-deficient viruses to block chemokine activity.

In this report, we used a multifaceted approach to further investigate the chemokine blocking potential of M3. We report that M3 blocks chemotaxis induced in vitro by CCL19 and CCL21, chemokines constitutively expressed in lymphoid tissues and in lymphatic vessels in the periphery. Furthermore, we provide direct evidence for the ability of M3 to block chemokine function in vivo.

MATERIALS AND METHODS

Transgenic construction and microinjection. A plasmid containing a segment of the rat insulin promoter 2 (RIP) and the rabbit β-globin poly(A) signal was generated by replacing the tumor necrosis factor alpha (TNF-α) fragment in RIP-TNF-α-pBS (12) with the rabbit β-globin poly(A) DNA segment from a plasmid containing the CMV-EGFP transgene (20). The rabbit β-globin poly(A) signal was PCR amplified using the oligonucleotides 5'-ACAGAGGATATCTCAATGA-3' and 5'-ACAGAGGATATCTCAATGA-3', inserting an EcoRI site, and 5'-TGGTCTC TCTAGAGGTCGAGGATGCTAA-3', inserting an XhoI site. TNF-α was released from RIP-TNF-α-pBS by EcoRV/SalI digestion and replaced by the rabbit β-globin poly(A) PCR-amplified fragment.

The cloning of the M3 ORF from infected-cell DNA into a baculovirus expression vector has been described previously (21). The M3 coding sequence was PCR amplified from this vector using the oligonucleotides 5'-ACAGAGGATTAGTCGGCCACAGTGGCTCTCTCTACTCTGAG-3', inserting an EcoRI site and a consensus Kozak sequence, and 5'-ACAGAGGATATCTCTAATAGTA TCCCTAAAATACTCCAG-3', inserting an EcoRV site. This PCR fragment was subcloned into the EcoRI/EcoRV site of the RIP-poly(A) vector described above, creating pRIPM3. The transgene (RMS) was released from pRIPM3 by SacII/PmlI digestion. The construction of the RCCL21 transgene has been described elsewhere (4). In this study, we used animals expressing CCL21a (line 21).

Separation of the RM3 transgene from vector DNA was accomplished by zonal sucrose gradient centrifugation as described (32). Fractions containing the transgene were pooled, microcentrifuged through Microcon-100 filters (Amicon, Beverly, Mass.) and washed five times with microinjection buffer (5 mM Tris-HCl [pH 7.4], 5 mM NaCl, 0.1 mM EDTA).

Generation of transgenic mice. The RM3 transgene was resuspended in microinjection buffer (5 mM Tris-HCl [pH 7.4], 5 mM NaCl, 0.1 mM EDTA) to a final concentration of 1 to 5 μg/ml, microinjected into [C57BL/6 × DBA/2]F1 (The Jackson Laboratory, Bar Harbor, Maine) eggs, and transferred into oviducts of ICR foster mothers (Charles River Laboratories, Wilmington, Mass.), according to published procedures (14). At 10 days after birth, a piece of tail from the resulting animals was clipped for DNA analysis. Identification of the transgenic mice was accomplished by PCR amplification of mouse tail DNA using specific primer sets. Specifically, the primers used for detection of the RM3 transgene were 5'-AGTGTGAGGCTGCTTCTAGAATG-3' and 5'-TTCTGGATTATTAAATGATTGTGGCCCTCCC-3', which are specific for a region in the rabbit β-globin poly(A) sequence. The endogenous ZP3 gene, used as an internal control, was amplified with the following primers: 5'-CAAGCTATCACTACATGGCCAA-3' and 5'-CACTGGGAAAGACACTCAGAC-3'. PCR conditions were 94°C, 30 s; 60°C, 30 s; and 72°C, 60 s. The resulting transgenic mice were kept under pathogen-free conditions. All experiments involving animals were performed following the guidelines of the Schering-Plough Animal Care and Use Committee.

Histological analysis. Tissues for light microscopic examination were fixed in 10% phosphate-buffered formalin and processed for paraffin sections. Routinely, 5-μm-thick sections were cut and stained with hematoxylin and eosin (H&E). For immunohistochemical studies, fresh frozen sections were first fixed with 4% ice-cold acetone for 10 min and then dried and stored at −20°C. Slides were stained and analyzed as previously described (32). Purified primary antibodies were incu-
Cell membrane preparation. Ba/F3-hCCR7 membranes were prepared as previously described (13). Cells were pelleted by centrifugation, incubated in homogenization buffer (10 mM Tris-HCl, 5 mM EDTA, 3 mM EGTA [pH 7.6]) and 1 mM phenylmethylsulfonyl fluoride for 30 min on ice. The cells were then lysed with a Dounce homogenizer using steril type RZR3 polytron homogenizer (CFSramo, WaIarton, Ontario, Canada) with 20 strokes at 900 rpm. The intact cells and nuclei were removed by centrifugation at 500 × g for 5 min. The cell membranes in the supernatant were then pelleted by centrifugation at 10,000 × g for 30 min. The membranes were then resuspended in glycy buffer (20 mM HEPES, 1 mM CaCl2, 5 mM MgCl2, 125 mM NaCl, 0.002% glycglycine, 1 mM MgCl2, 250 mM sucrose [pH 7.2]), aliquoted, quick frozen, and stored at −80°C. Protein concentration in membrane preparations was determined using the method of Bradford (1).

[^35S]GTPγS binding. Guanosine 5′-[γ-35S]triphosphate ([35S]GTPγS) exchange was measured using a scintillation proximity assay (SPA) as previously described (5). For each assay point, membranes (4 μg/well in triplicate) were preincubated for 30 min at room temperature with 300 μg of wheat germ agglutinin-coated SPA beads (Amersham, Arlington Heights, Ill.) in SPA binding buffer (50 mM HEPES, 1 mM CaCl2, 5 mM MgCl2, 125 mM NaCl, 0.002% NaNO3, 1.0% bovine serum albumin). The beads and membranes were transferred to a 96-well Isoplate (Wallac, Gaithersburg, Md.) and incubated for 60 min at room temperature with 10 μM GDP, 10 nM of either recombinant mouse 6Ckine (CCL21) or MIP-3β (CCL19) (R&D Systems), and the indicated concentrations of M3 protein. The reactions were then incubated for a further 60 min in the presence of 0.01 nM guanosine 5′-[γ-35S]triphosphate ([35S]GTPγS, triethyl ammonium salt; specific activity = 1,250 Ci/mmol; NEN, Boston, Mass.). Membrane-bound [5[S]GTPγS was then measured using a Wallac Trilux 1450 microscintillation counter.

Statistical analysis. Graphical analyses, statistical analysis and nonlinear regression analysis of the data and calculation of functional IC50 were performed using Prism 2.0c (GraphPad Software, San Diego, Calif.). Data in the text are given as means ± standard deviations unless otherwise stated.

RESULTS

M3 blocks CCL19- and CCL21-induced receptor activation. M3 has been shown to block chemokine-induced calcium flux in vitro (21, 27), but it is unknown whether it can block chemokine-induced chemotaxis. To investigate this, we tested the effect of M3 on migration of transfected cells in an in vitro chemotaxis assay. We used the CC chemokines CCL19 (also known as MIP-3β, EBl1-ligand chemokine, chemokine β-11, and Exodus-3) and CCL21 (also known as 6Ckine, secondary lymphoid chemokine, Exodus-2, and thymus-derived chemotactic agent 4) and Ba/F3 cells stably transfected with human CCR7. Expression of hCCR7 in this cell line at the cell surface was confirmed by binding assay (not shown) and flow cytometry as shown in Fig. 1A.

Recombinant CCL19- and CCL21-induced chemotaxis of Ba/F3-hCCR7 cells in a dose-dependent manner (Fig. 1B). However, as shown in Fig. 1C, preincubation of either chemokine with purified rM3 efficiently blocked chemotaxis of cells. M3 blocked with equal potency the chemotactic activity of CCL19 and CCL21. The functional 50% inhibitory concentration (IC50) of M3 for blocking chemotaxis induced by 2.0 nM CCL19 was 2.5 ± 1.0 nM (n = 3), whereas the IC50 of M3 for blocking chemotaxis induced by 2.0 nM CCL19 was 2.6 ± 0.9 nM (n = 3) (means ± standard deviations). Interestingly, the slope of the inhibition curve had a very steep profile. We expanded on this observation using [35S]GTPγS exchange as a more biochemically defined functional assay to investigate the interaction between M3 and CCL19 and CCL21. Membranes from the Ba/F3-hCCR7 cells were incubated with [35S]GTPγS in the absence or presence of 10 nM CCL19 or CCL21 and the indicated concentrations of M3 protein (Fig. 2). As was the case in the chemotaxis experiments, receptor activation by CCL19 and CCL21 decreased in a concentration-dependent manner with increased M3 concentrations (IC50 = 19.2 ± 2.3 and 16.9 ± 2.6 nM, respectively [n = 3]). The slope of the inhibition curve was found to have Hill coefficients of >3. Hill coefficients reflect the biophysical nature of receptor-ligand interactions, where a Hill number of ~1 implies a single binding site, Hill values of >1 indicate positive cooperative binding.
and Hill values of <1 suggest negative cooperativity. Thus, our data indicate that the interaction between M3 and CCL19 and CCL21 is positively cooperative.

**Generation of transgenic mice expressing M3.** To study the biological effects of M3 in vivo, we generated transgenic mice expressing M3 in the pancreas. To this end, we constructed a transgene where the M3 gene was placed downstream of the RIP (Fig. 3A). This RIP has previously been shown to target transgene expression predominately to the pancreatic islets and the kidney (12). Eleven founders were generated from microinjection of this transgene into fertilized mouse eggs. Ten transgenic lines were established from these founders. These transgenic mice are referred to as RM3 mice. Pancreata from control and RM3 transgenic mice were initially analyzed for transgene expression by immunohistochemistry, using an anti-M3 polyclonal antibody. Seven transgenic lines showed expression of M3, and one of these was selected for further experiments (line 31). As shown in Fig. 3B, strong immunostaining could be detected in the islets of Langerhans from RM3 mice, whereas no M3 immunostaining was detected in islets from control mice. The expression of M3 in the islets of RM3 mice did not appear to compromise pancreatic function. Blood glucose levels were normal in mice from all transgenic lines indicating normal insulin production and secretion.

To examine whether constitutive expression of M3 in the pancreas had affected the development of lymphoid or non-lymphoid tissue, we examined H&E-stained sections from RM3 transgenic mice. All major organs of the RM3 mice, including pancreas, kidney, thymus, spleen, and peripheral lymph nodes, appeared normal by light microscopy (data not shown).

**M3 from islets is secreted and prevents CCL21-induced chemotaxis in vitro.** M3 is abundantly secreted from infected cells in vitro (29). To examine whether M3 produced by the RM3 islets was secreted and biologically active, pancreatic islets were isolated by collagenase digestion and cultured for 24 h. As shown in Fig. 4A, Western blot analysis of media from RM3 islets showed an immunoreactive band of 44 kDa. No immunoreactivity was found in media from control islets.

![FIG. 2. M3 inhibition of CCL19- and CCL21-induced [35S]GTPγS exchange in Ba/F3-hCCR7 membranes. Ba/F3-hCCR7 membranes were incubated for 60 min at room temperature in GTPγS binding buffer (as described in Materials and Methods) containing 10 μM GDP in the presence or absence of 10 nM CCL19 or CCL21 and the indicated concentrations of rM3. Following the addition of 0.3 nM [35S]GTPγS, the incubation continued for a further 60 min. Membrane-associated radioactivity was measured using SPA technology. Data represent the mean total binding and standard errors of the means (error bars) of triplicate determinations from a representative experiment (n = 3).](http://jvi.asm.org/)

![FIG. 3. Generation of transgenic mice expressing M3 in pancreatic islets. (A) M3 expression is under control of the RIP; p(A) represents the rabbit β-globin polyadenylation signal. (B) Expression of M3 in the islets of RM3 transgenic mice detected by immunohistochemistry.](http://jvi.asm.org/)
These results indicate that M3 is secreted from the cultured islets into the media. The conditioned media from transgenic and control islets were incubated with recombinant CCL21. Media from control islets did not alter the chemotaxis induced by recombinant CCL21. However, in accordance to the findings using RM3, the conditioned medium from cultured RM3 islets blocked in a concentration-dependent manner the chemotaxis of Ba/F3-hCCR7 cells (Fig. 4B). Together these results show that M3 is secreted from the RM3 islets in vitro and that M3 produced by the islets is biologically active.

**M3 blocks lymphoid cell recruitment induced by CCL21 in vivo.** Next we tested if expression of M3 could block lymphocyte recruitment induced by CCL21 in vivo. RM3 mice were crossed with mice expressing CCL21 in pancreatic islets (RCCL21 transgenic mice). Double-transgenic mice are referred to here as CCL21/M3 mice. As described before (4, 7), ectopic expression of CCL21 in pancreatic islets leads to the development of lymphoid aggregates that resemble lymph nodes. These aggregates are composed primarily of T and B cells and few dendritic cells (4, 7). To evaluate whether expression of M3 could disrupt CCL21-induced recruitment of mononuclear cells into the pancreas, we examined H&E-stained paraffin sections of pancreata from control, single-transgenic mice and CCL21/M3 mice. Pancreata from both mature (10 weeks old) and aged mice (older than 26 weeks) were examined (40 to 170 islets were examined in each case). No infiltrates were found in islets of control or RM3 transgenic mice regardless of age (Fig. 5A). As expected, mononuclear infiltrates of various sizes were found in the pancreatic islets of RCCL21 transgenic mice. In 10-week-old RCCL21 mice, 18% ± 3% (n = 5) of the islets had infiltrates (means ± standard deviations) (Fig. 5D). These infiltrates typically contained large clusters of mononuclear cells with >50 cells per islet. A representative islet with an infiltrate is shown in Fig. 5B. In striking contrast, only 2% ± 1% of islets (n = 4) from 10-week-old mice expressing both M3 and CCL21 had infiltrates (Fig. 5D). Moreover, the infiltrates detected in the pancreas from these mice were composed of few scattered mononuclear cells, in contrast to the large infiltrates found in the RCCL21 transgenic mice (Fig. 5C). CD45 staining of pancreata from WT, RCCL21 transgenic mice and CCL21/M3 transgenic mice supported these findings (not shown). Interestingly, the proportion of infiltrated islets in both RCCL21 transgenic mice and CCL21/M3 transgenic mice increased in mice older than 26 weeks. In this age group, 34% ± 18% of the islets from RCCL21 mice had infiltrates (n = 4), whereas 14% ± 10% (n = 5) of the islets from CCL21/M3 transgenic mice had infiltrates (Fig. 5D). Interestingly, there was a very clear difference in the location of the infiltrates in the double transgenic mice compared to RCCL21 transgenic mice. Mononuclear cells occupied the center of the majority (60%) of the infiltrated RCCL21 islets (Fig. 5C). Islets from CCL21/M3 transgenic mice showed a strikingly different pattern. In 90% of the CCL21/M3 infiltrated islets the mononuclear cells accumulated near ducts or in the periphery of the islets (Fig. 5B). These results indicate that expression of M3 in pancreatic islets significantly reduce the accumulation of mononuclear cells induced by the ectopic expression of CCL21.

**DISCUSSION**

The M3 protein encoded by MHV-68 has been shown to bind certain chemokines with high affinity and to block chemokine-induced signal transduction in vitro (21, 27). However, there has been no definitive evidence to date that M3 blocks chemokine activity in vivo, nor has the nature of M3-chemokine interaction been defined. In this report we show for the first time that M3 can block chemotaxis induced by CCL19 and CCL21, key regulators of lymphocyte trafficking. Moreover, we show that CCL19/CCL21-M3 binding exhibits positive cooperativity.

The ability of M3 to block CCL19 and CCL21 activity is likely to have implications for MHV-68 pathogenesis. In the course of infection, MHV-68 replicates transiently at the site of infection and spreads to lymphoid tissue, where latency is established. Analysis of a recombinant MHV-68 containing a disrupted M3 gene has provided insight into a possible role of M3 in the pathogenesis of this virus (2). The M3-deficient virus replicated normally during the productive infection in the lung, and there was little difference in the initial seeding to the

![FIG. 4. Characterization of islet-derived M3. Islets were isolated by collagenase digestion and cultured in vitro. (A) Western blot showing the presence of M3 in media from cultured transgenic islets. No reactivity was detected in media from WT islets. Reactivity against rM3 is also shown. (B) Inhibition of CCL21-induced chemotaxis by islet-derived M3. Data represent the means and standard deviations (error bars) of triplicates. The results shown are representative of two independent experiments.](http://jvi.asm.org/Downloadedfrom)
draining mediastinal lymph node when compared to WT virus. However, amplification of latent M3-deficient virus from splenocytes and virus-driven B-cell activation were grossly impaired. These effects of M3 deficiency were partially reversed by CD8⁺/H11001-T-cell depletion, suggesting that M3 chemokine blockage protects MHV-68-infected cells from elimination by CD8⁺/H11001 T cells in secondary lymphoid tissues.

Here we show that M3 can block the chemotactic properties of CCL19 and CCL21 in vitro. These chemokines and their receptor CCR7 are highly expressed in secondary lymphoid tissue and are thought to be essential for migration of lymphocytes and dendritic cells into lymphoid organs and their subsequent compartmentalization into specific microenvironments (6, 17). CCL21 is constitutively expressed by high endothelial venules of lymph nodes and Peyer’s patches, stromal cells in the T-cell zone of secondary lymphoid organs, and lymphatic vessels (33). Lack of CCR7, CCL19, or CCL21 expression in mice is associated with defective migration of T cells, B cells, and dendritic cells into lymphoid organs (9, 16, 19, 30). Based on the findings reported here, we suggest that M3 produced by infected cells may alter migration of CCR7-expressing T, B, or dendritic cells toward local gradients of CCL19 and CCL21 and thereby disrupt immune responses.

The blockage or attenuation of these responses could take place within lymphoid tissue or in the periphery. Indeed, a critical event in the host response to viral infection is the migration of antigen-loaded dendritic cells from the periphery to draining lymph nodes. Upon activation in the periphery, dendritic cells upregulate expression of CCR7 and become responsive to CCL21 (6). CCL21 is expressed by lymphatic endothelium and is thought to favor the entry of CCR7-expressing dendritic cells into the lymphatic vessels (9). By blocking the CCL21/CCR7-dependent migration of dendritic cells to the lymph node, M3 could potentially delay initiation of a specific immune response against MHV-68.

Data supporting a role for M3 in the inhibition of lymphocyte trafficking has been provided by recent studies by van Berkel and colleagues (28). In this report, it is shown that expression of M3 during MHV-68 infection in the central nervous system (CNS) reduces the relative number of lymphocytes and macrophages in the infiltrates associated with MHV-68 infection. Although a direct correlation between M3 expression and inhibition of lymphocyte and macrophage trafficking was not established, the authors demonstrated that chemokines that control trafficking of lymphocyte and macrophage are upregulated in the CNS during MHV-68 infection. Our demonstration that M3 efficiently blocks the activity of chemokines in vivo supports the contention that M3 inhibition of lymphocyte and macrophage specific chemokines is an important mechanism for immune evasion during MHV-68 infection in the CNS.

Here we have shown that when coexpressed with CCL21 in pancreatic islets, M3 effectively inhibits CCL21-induced accumulation of mononuclear cells. The number of islets infiltrated was significantly reduced in animals expressing both proteins, and more importantly, in these infiltrated islets, the mononuclear cells tended to accumulate in the periphery or in the ducts. We suggest that the ability of CCL21 to induce the formation of these infiltrates is dependent on a given effective concentration or threshold. Once this threshold is established,
cells will infiltrate the islets, starting from its periphery. M3 most likely increases this threshold and thereby reduces the number of islets being infiltrated and the number of cells per infiltrate. This hypothesis is supported by the observation that the infiltrating cells tend to accumulate in the periphery of the islets in older mice expressing both proteins.

Using both a chemokine-induced [35S]GTPγS exchange assay and a chemotaxis assay, we demonstrate that the CCL19/CCL21-M3 binding exhibits positive cooperativity, which is reminiscent of that recently reported by Burns et al. (3). These authors have shown that the binding of the poxvirus-encoded chemokine-binding protein vCCI (35-kDa/T1 protein) and certain chemokines is positively cooperative. The functional implication(s) of this observation in regard to the activities of M3 in vivo is unclear, but it suggests that its chemokine antagonism could occur within a very narrow dose range. These findings have implications for the understanding of the mechanisms associated with MHV-68 pathogenesis, and should be taken into consideration when evaluating the therapeutic potential of M3.

In conclusion: our results demonstrate that M3 blocks the biological activities of CCL19 and CCL21 in vitro and of CCL21 in vivo. The precipitous nature of the inhibition of CCR7 activation suggests that there is positive cooperativity in the M3-chemokine binding. Taken together, these data suggest that M3 may affect local trafficking in lymphoid tissues and/or in the periphery may represent important strategies used by MHV-68 to evade immune responses.

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