JVI Accepts, published online ahead of print on 2 April 2008
J. Virol. doi:10.1128/JVI.00098-08


# Alphaherpesviruses and Chemokines: Pas-de-Deux Not Yet Brought to Perfection

**Gerlinde R. Van de Walle[1], Keith W. Jarosinski[1] & Nikolaus Osterrieder[1,2]**

[1]Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA; [2]Institut für Virologie, Freie Universität Berlin, Philippstraße 13, 10115 Berlin, Germany.

Correspondence to: Nikolaus Osterrieder, Institut für Virologie, Freie Universität Berlin, Philippstraße 13, 10015 Berlin, Phone: +49-30-2093-6564; FAX: +49-30-2093-6540; e-mail: no.34@fu-berlin.de

The coexistence of viruses and their hosts implies constant and mutual evolutionary pressure. In addition to the fundamental systems necessary for viruses to replicate and spread, viruses have developed accessory systems to escape killing by the host's immune system. Herpesviruses have been co-evolving with their hosts over millions of years and are exquisitely well adapted to their respective partners. Biological criteria have long been used to subdivide the family *Herpesviridae* into three subfamilies, namely *Alpha-, Beta-* and *Gammaherpesvirinae*. Members of the *Alphaherpesvirinae* have a narrow *in vivo* host range, a short replication cycle and the capacity to establish lifelong, latent infection, primarily, but not exclusively, in neurons of sensory ganglia (1). The length of their linear, double-stranded DNA genome varies between 124 and 177 kbp and generally consists of regions of unique sequences flanked by direct or inverted repeat sequences. The subfamily includes human pathogens as well as a number of animal viruses of considerable agricultural and economical importance (Table 1). The human pathogens herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and varicella zoster virus (VZV) are the causative agents of cold sores, genital ulcerous disease, and chickenpox/shingles, respectively. Some of the animal herpesviruses can cause diseases with potentially devastating economic consequences: Infection with equine herpesvirus type 1 (EHV-1) results in respiratory disorders, abortion and neurological disorders; bovine herpesvirus type 1 (BHV-1) leads to respiratory infection and abortions in cattle; pseudorabies virus (PRV, suid herpesvirus 1) infection (Aujeszky's disease) is characterized by respiratory and neurological disorders, abortion and infertility in swine; and Marek's disease virus (MDV), an oncogenic

alphaherpesvirus, causes massive immunosuppression and invariably lethal T cell lymphomas in unvaccinated chickens.

Infection with herpesviruses, as is the case with most viruses, normally stimulates the production of cytokines and chemokines, and one of the components of the immune system for viral subversion are ligands and receptors of the cytokine and chemokine network (2;3). These secreted proteins mediate and regulate fundamental processes such as immune responses, inflammation and haematopoiesis, and play a crucial role in leukocyte migration during both innate and adaptive immune responses. Certain cytokines, such as interferons (IFN) and tumor-necrosis factor (TNF), result in intracellular signals that can lead to an antiviral state and/or apoptosis of the cell and thereby as such limit viral replication (4). Several cytokines aid in enhanced immune recognition, modulate immune responses that protect against viral infection, or can even mediate the killing of infected cells by natural killer (NK) cells or cytotoxic T lymphocytes (CTL) (5).

Chemokines are chemoattractant molecules that regulate traffic and effector functions of leukocytes, and are key regulators of inflammation and immune surveillance (6). Functionally, they can be divided in two major groups: housekeeping chemokines, which are expressed constitutively, and pro-inflammatory chemokines, which typically are inducible. The physiological activities of chemokines are mediated by the selective recognition and activation of chemokine receptors (GPCRs) belonging to the seven-membrane-domain, G-protein-coupled receptor superfamily (7). In addition, chemokines also bind to glycosaminoglycans (GAGs) through distinct binding sites. Chemokine binding to GAGs on cells, particularly endothelial cells, results in chemotactic chemokine

63 gradients that allow correct presentation of chemokines to leukocytes, and, therefore,
64 enable target cells to cross the endothelial barrier and migrate into tissues (8-10) (Figure
65 1).

66 Given the central role of cytokines and chemokines in antiviral defense, it is not
67 surprising that herpesviruses have evolved strategies to subdue pivotal elements of this
68 network to their service. For the *Beta*- and *Gammherpesvirinae*, several virus-encoded
69 proteins with cytokine/chemokine modulatory properties have been identified based on
70 their sequence similarities with host cytokines and chemokines (11-16). In many cases,
71 viral cytokine/chemokine modulators are derived from host genes and were originally
72 pirated during ancestral virus infections. Consequently, they have evolved as virus
73 constituents allowing their carriers, the viruses themselves, to modify or evade the
74 antiviral defense. Interestingly, when looking at *Alphaherpesvirinae*, only MDV has
75 been shown to express a viral chemokine modulator, called viral interleukin 8 (vIL-8),
76 with homology to a chicken gene (17;18). Several studies investigating evolutionary
77 relationships within the *Herpesviridae* have shown that the alphaherpesviruses are the
78 most recently evolved, and MDV has been proposed within this subfamily as the original
79 alpha class antecedent species, which later was transferred from birds into mammals
80 (19;20). These evolutionary considerations raises the possibility that mammalian
81 alphaherpesviruses may be too "young" in their co-evolutionary relationship with their
82 hosts to have hijacked genes encoding chemokines. On the other hand, and a more likely
83 scenario, it has been noted that molecular mimicry by viral proteins in fact resembles the
84 interspecies diversity of host immune pathways themselves (21). Many
85 alphaherpesviruses cause infections that are initiated through the respiratory or genital

route and are restricted to immunologically privileged sites, such as the central and peripheral nervous system, where host immune responses are more repressed (1). This would imply that alphaherpesviruses might have fashioned virus-encoded proteins, which account for immunomodulatory functions that are different from those of other subfamilies, and adapted them to their very specific and unique needs. Indeed, alphaherpesviruses are well known for the expression of the glycoprotein E-glycoprotein I complex, an Fc receptor-like molecule targeting the constant region of immunoglobulins, and the expression of glycoprotein C, which binds complement factor C3b. These viroreceptors were shown to allow viruses to avoid recognition and destruction by the complement system *in vitro* and *in vivo*. However, complement immune evasion strategies used by alphaherpesviruses have previously been reviewed extensively and are therefore beyond the scope of this review (22;23), where we will focus on more recent findings on alphaherpesviral interaction with other immunomodulatory functions. Rather, we will give an updated overview of the recent developments on chemokine interference by *Alphaherpesvirinae*, more specifically the alphaherpesviral encoded vIL-8 and gG proteins.

In spite of the absence of alphaherpesviral mimicry of cytokines and chemokines, with the notable exception of the virokine vIL-8 encoded by MDV, there are recent data indicating that alphaherpesviruses are in fact capable of effectively modulating the chemokine network to their liking and benefit. Several members of the *Alphaherpesvirinae* subfamily express glycoprotein G (gG), a viral protein shown to interfere with a broad range of chemokines, which appears to intercept chemokine networking at different levels (24;25). It is these viral factors that have garnered

attention lately and we will provide a description of their properties and putative functions.

**Virus-encoded IL-8 (vIL-8)**

MDV or gallid herpesvirus type 2 (GaHV-2) is the only alphaherpesvirus shown to encode and express a virokine, vIL-8 (17;18). Most likely, *vIL*-8 was pirated from the chicken genome early in the divergence of the members of the *Mardivirus* gene, since non-oncogenic close relatives of MDV, gallid herpesvirus type 3 (GaHV-3) and meleagrid herpesvirus type 1 (MeHV-1 or herpesvirus of turkeys, HVT), do not harbor an *IL-8*-like gene. Two copies of *vIL-8* in each of the long repeat regions are present in the MDV genome. vIL-8, which is encoded by three exons (I-III), shares significant homology with cellular CXC chemokines like IL-8, also designated CXCL8, and GRO-α. Exon I of vIL-8 is rich in hydrophobic residues and most likely serves as a signal peptide, while exons II and III contain the CXC motif and a three amino acid motif (DKR) that determines cell attraction specificity (18). Chicken IL-8, originally designated chicken chemotactic and angiogenic factor (cCAF), is the product of the 9E3/CEF4 gene and shares high amino acid similarity with human IL-8 (26). In contrast to human IL-8 however, which is chemotactic for neutrophils, chicken IL-8 predominantly targets cells of the monocyte/macrophage lineage (26). Similar to chicken IL-8, the vIL-8 encoded by MDV also functions as a chemoattractant for chicken peripheral blood mononuclear cells (PBMC) when expressed and tested in chemotaxis assays *in vitro* (18).

The chemoattractant specificity of vIL-8 is an excellent example of a cellular gene that is pirated and tailored to the needs of the virus by strong and regulated expression at early times after virus uncoating. Upon entry into the chicken and passage to lymphoid

132 organs by hijacking antigen-presenting cells, MDV requires B and activated T cells for
133 efficient replication. It is in the former, where virus lytically replicates and the latter
134 where MDV establishes latency and induces transformation. It is unknown what function
135 exactly vIL-8 serves during MDV pathogenesis (Figure 2). It has been suggested that
136 secretion of vIL-8 by infected cells helps recruit lymphocytes to initially infected cells
137 that function as "virus ferries" and carry MDV from the periphery to primary lymphatic
138 organs. The recruitment of lymphocytes helps increase the efficiency of early virus
139 replication, since MDV only spreads from cell to cell *in vivo*, which requires quite
140 intimate contacts between infected and new target cells. Alternatively, vIL-8 may act as
141 a mimicry molecule, helping to evade the immune system by antagonizing host IL-8
142 responses. Still, a third possibility is that vIL-8 expression augments viral replication by
143 binding to a receptor on infected cells and activating a transcriptional/translational
144 cascade inducing MDV promoters. Based on experiments using MDV vIL-8 deletion
145 mutants, the first function is favored. Deletion of both copies of vIL-8 in the very
146 virulent RB-1B MDV strain showed that, while *in vitro* replication in tissue cultured cells
147 was unaltered, *in vivo* replication was severely impaired (18;27;28). Likewise, Cui et al.
148 (29) showed that the number of infected cells in lymphoid organs (bursa of Fabricius,
149 thymus, and spleen) were significantly lower in virus lacking vIL-8. Consistent with the
150 behavior of deletion mutants, recombinant vIL-8 strongly binds to predominantly B but
151 also T lymphocytes, as demonstrated with a baculovirus-expressed vIL-8 tagged with
152 human Fc (Kamil and Osterrieder, unpublished observation). Thus, it appears that MDV
153 maintains and utilizes vIL-8 for its replication. According to current knowledge, other
154 alphaherpesviruses have not subverted a cellular chemokine for their purposes, although

some of the mammalian species, such as VZV and EHV-1, also exhibit strong lymphotropism and would seem to have a vested interest in such a mechanism of manipulating the chemokine environment and attraction of putative targets or the exclusion of unwanted visitors.

**Glycoprotein G (gG)**

Glycoprotein G (gG) homologues have been described in several alphaherpesviruses and are expressed as non-essential membrane-anchored proteins with type I membrane topology (30;31). gG is unusual compared to other herpesvirus glycoproteins since it also gets secreted into the medium of infected cells. Generally speaking, gG can therefore exist in three isoforms: a full-length membrane-bound form, a smaller membrane-bound form and a secreted form (32). The latter two isoforms appear to be the result of a proteolytic cleavage event of the full-length membrane-bound form (32). Alphaherpesviral gG can interfere at different distinct stages of chemokine action and it therefore constitutes yet another immunoevasion tool used by alphaherpesviruses (Figure 3). Full length, membrane-anchored gG of feline herpesvirus type 1 (FeHV-1) and equine herpesvirus type 1 (EHV-1) can function as a viroreceptor and are capable of binding a broad range of chemokines (24;33). The cleaved gG protein of several alphaherpesviruses has been described to function as a viral chemokine binding protein (vCKBP) and has recently been classified as the prototype of a new subfamily, vCKBP-4 (25). By using cross-linking assays with supernatants from infected cells and recombinant chemokines, it was shown that gG of EHV-3, BHV-1, BHV-5, RanHV-1, CapHV-1 and CerHV-1 (Table 1) also bind a plethora of chemokines, with each virus, however, having its own signature of specificities (24). In addition, it has been shown for

178 EHV-1, BHV-1 and FeHV-1 that gG-chemokine interaction prevents the binding of
179 chemokines to GPCRs, thereby neutralizing chemokine activity (24;33). Moreover, gG
180 can inhibit chemokine activity by blocking the interaction of chemokines with heparin,
181 although gG does not appear to bind heparin directly, but rather indirectly through the
182 crosstalk of chemokines with GAGs (24). By preventing chemokine-GAG interactions,
183 gG specifically disrupts pre-established chemokine gradients, and, in combination with
184 preventing chemokine-receptor binding, efficiently controls the local microenvironment
185 of infected tissues. We will now discuss what is known on the general roles of gG of the
186 different alphaherpesviruses, and how they interfere with the chemokine network.

187 ***HSV-1 and HSV-2 gG***

188 No chemokine binding of HSV-1 and HSV-2 gG has been reported to date, based on the
189 observation that supernatants from HSV-1- or HSV-2-infected cells are unable to cross-
190 link chemokines of murine or human origin (24). For HSV-1, this might simply be
191 related to the fact that its gG is not secreted into the medium of infected cells (34). An
192 HSV-1 gG deletion mutant has been evaluated *in vivo* and displayed only marginal
193 attenuation in the mouse ear model, suggesting that the role of gG during HSV-1
194 pathogenesis might be limited (30). In contrast to HSV-1 gG, the HSV-2 gG homologue
195 is secreted into the medium, as a 34kDa moiety representing the ectodomain of the
196 protein (35;36). Although no specific function has been ascribed to HSV-2 gG as a
197 whole, peptides derived from gG have been shown to possess pro-inflammatory
198 properties. These gG-derived peptides are not only chemoattractants for monocytes and
199 neutrophils, but also have profound downregulatory effects on NK cells (37-39). Still, it
200 remains unclear if the native HSV-2 gG protein has the same pro-inflammatory properties

as gG-derived peptides, and whether (regulated) proteolytic degradation of HSV-2 gG would release peptides with such activities. In addition, gG of both simplex viruses have been described to display additional functions, which are unrelated to chemokine-binding or any other immunomodulatory function: HSV-1 gG appears to be required for infection of polarized epithelial cells through apical surfaces (40). More recently, it has been suggested that HSV-2 gG is directly involved in HSV-2 attachment to cells, since gG present in the viral envelope was shown to interact with sulphated polysaccharides including cell surface GAGs (41).

***BHV-1 and BHV-5 gG***

BHV-1 and BHV-5 gG are non-structural proteins that are present on the plasma membrane of infected cells and are secreted as 65 kDa polypeptides. In addition, secreted gG can also be found as a protein species ranging from 90 to 240 kDa when linked to GAGs (42;43). BHV-1 gG is non-essential for viral growth, but essential for cell-to-cell spread in bovine kidney cells (44;45). Moreover, BHV-1 gG has been proposed to be important for maintenance of intact cell-to-cell junctions (46). Binding of BHV-1 and BHV-5 gG to chemokines was demonstrated using cross-linking assays with both supernatants of infected cells and baculovirus-expressed gG (24). In addition, recombinant BHV-1 and BHV-5 gG inhibited migration of human neutrophils induced by CXCL1 or of IFN-α-treated human lymphoma cells mediated by CCL-3 (24). *In vivo* studies using BHV-1 mutants devoid of gG, showed significant attenuation and increased immunogenicity in cattle (47). However, since no rescuant virus was used in this particular study, nor the expression of adjacent genes investigated, it is difficult to

conclusively determine whether BHV-1 gG plays an important role in pathogenicity, let alone which function, if any, can be attributed to gG-chemokine interaction.

***PRV gG***

PRV secretes a non-structural viral glycoprotein of approximately 99 kDa, which was formerly referred to as gX, but more recently renamed to gG for its similarity with the gG homologues of other alphaherpesviruses (48). Since PRV gG is not required for efficient growth *in vitro* and *in vivo*, gG mutants have been suggested as useful marker vaccines to distinguish between vaccinated and infected pigs, mostly in combination with attenuating mutations in other glycoprotein genes (49). Most gG deletion mutants did not exhibit altered virulence in pigs (50;51), but one gG mutant, based on the PRV Bartha strain, did show impaired cell-to-cell spread *in vitro* and reduced virulence *in vivo*. This effect, however, was later explained by reduced expression of the upstream US3 gene, which encodes a serine/threonine protein kinase (52). Therefore, in the models employed in the PRV system, gG was shown not to play a major role in PRV pathogenesis and experiments on the potential role of PRV gG as a vCKBP - to our knowledge - have not been done yet.

***FeHV-1 gG***

Recently, gG encoded by FeHV-1, an alphaherpesvirus of cats, has been evaluated for its possible chemokine binding properties. It was first shown that FeHV-1 secretes gG into the culture medium and that secreted gG not only displays high-affinity binding to a broad range of chemokines, but is also capable of blocking chemokine activity by preventing chemokine interaction with GPCRs (33). In addition, it has been demonstrated that the membrane-bound form of gG, expressed on the surface of infected

246 cells also binds to a number of chemokines with high affinity (33). It is possible that
247 membrane-bound gG acts as a *bona fide* viroreceptor, providing a decoy that prevents the
248 interaction of chemokines with cellular chemokine receptors and inhibits the biological
249 activity of chemokines. In addition, FeHV-1 gG is a structural protein and present on the
250 surface of virus particles (19). This observation begs the speculation that membrane-
251 bound gG, besides functioning as a viroreceptor, might also play a role in virus
252 attachment to cells, which present chemokines bound to GAGs. The FeHV-1 gG
253 homologue may as such be a determinant for cell and tissue tropism *in vivo* and/or aid in
254 virus entry. Although it has been shown that FeHV-1 gG can act as a vCKBP when
255 present on the virion surface, pre-incubation of virions with chemokines including
256 CXCL1, CCL3 or XCL1, did not alter the infectivity of FeHV-1, and these data would,
257 therefore, not be in support of a role of gG in cell and tissue tropism in the chosen *in vitro*
258 system (53). However, a cell-type specific interaction between FeHV-1 gG and GAG-
259 bound chemokines on target cells is easily conceivable and the experiments would need
260 to be repeated with feline lymphocytes or other target cells under different conditions.

261 ***ILTV gG***

262 ILTV causes acute respiratory disease in poultry and its gG has been identified as a
263 secreted, glycosylated protein of 32 kDa in size (54). Although no experiments have
264 been performed to evaluate the role of ILTV gG as a vCKBP, some interesting
265 observations have been made using a gG deletion mutant in the natural host, the chicken.
266 It was shown that gG-deficient ILTV was significantly attenuated in chickens with
267 respect to clinical signs, weight loss and mortality. The wild-type phenotype was
268 completely restored upon reinsertion of gG, and expression of the adjacent genes was not

altered by the genetic manipulations (55). In addition, it was observed that the degree of inflammatory cell infiltration in the trachea of chickens was increased in the absence of gG, strongly suggesting that ILTV gG may have an immunomodulatory role and act as a vCKBP *in vivo* (55). In a follow-up study, the same gG deletion mutant was shown to protect SPF chickens against clinical signs subsequent to challenge with virulent ILTV, demonstrating the mutant's potential to serve a new modified live vaccine candidate (56) against this poultry disease affecting the upper and lower airways.

***EHV-1 and EHV-4 gG***

Both viruses are economically important pathogens of horses, and each encodes gG as membrane-associated and secreted forms, the latter representing moieties of approximately 55-60 kDa in size (32;57;58). The full-length, membrane-anchored form of EHV-1 gG has vCKBP properties, since recombinant gG expressed on the surface of insect cells was capable of binding human CXCL1 and CXCL8 (24). Secreted EHV-1 gG has also been shown to bind a broad range of chemokines with high affinity and in a species-independent manner (24). The potential role of EHV-1 gG in chemotaxis and cell trafficking has since then been extensively studied, both *in vitro* and *in vivo*. In line with what has been described for other alphaherpesviruses, gG of EHV-1 was found to be dispensable for virus replication in cultured cells (59). No significant differences in virulence were detected in the murine Balb/c model of EHV-1 infection between a gG deletion mutant and its revertant virus when high doses of infectious virus was used. A clear phenotype was observed, however, when the gG deletion mutant was applied to mice at lower doses of infection. Intriguingly, at these lower doses of infection ($1x10^3$ to $1x10^4$ infectious units/animal), the gG deletion mutant induced more severe clinical signs

292 and a more pronounced inflammatory response in the lungs of infected mice when
293 compared with wild-type or revertant viruses (59).
294 The vCKBP activity of gG was also studied in more detail using chemotaxis assays *in*
295 *vitro*. First, it was demonstrated that baculovirus-expressed full length EHV-1 gG was
296 capable of inhibiting CXCL8-induced chemotaxis of human neutrophils (24). In a
297 following study, this observation was extended to equine cells and equine chemokines
298 and it was shown that secreted EHV-1 gG (both from supernatant as well as baculovirus-
299 expressed) was capable of interfering with chemotaxis of equine neutrophils induced by
300 equine CXCL8 (58). In contrast, gG was unable to interfere with CCL2-induced
301 chemotaxis of equine monocytes (58). Other studies demonstrated a functional
302 interference of EHV-1 gG with chemotaxis of murine neutrophils and macrophages
303 induced by the CXCL-8 relative KC and the proinflammatory chemokine CCL3
304 respectively (58;60). Moreover, gG was shown to have a significant effect on the
305 migration of immune cells into murine airways *in vivo* (58;60). Interestingly, a re-
306 infection experiment in which mice were inoculated with a gG deletion mutant and
307 subsequently challenged with wild-type virus revealed that the presence of gG-specific
308 antibodies not only had a protective effect, but were able to control vCKBP activity of gG
309 (60). This observation was supported by *in vitro* data showing that the presence of gG-
310 specific antibodies could restore chemokine-induced chemotaxis (60). This seems to
311 suggest that gG-specific antibodies can control gG's vCKBP function and might be
312 important in preventing EHV-1 from evading the immune system. These findings also
313 put into question the use of gG deletion mutants as modified-live virus marker vaccines

for protection against EHV-1 infections in particular, and possibly alphaherpesvirus infections in general.

Whereas EHV-1 gG clearly has vCKBP activities both *in vitro* and *in vivo*, no such role was found for its EHV-4 counterpart (24;58). EHV-4 is a close relative of EHV-1 and the gG amino acid sequences share 72% homology, although approximately 100 amino acids of the ectodomains are highly divergent and harbor type-specific epitopes (61). In general, the structural features of gG important for binding to chemokines remain undetermined to date, but preliminary data with baculovirus-expressed EHV-1/EHV-4 gG chimeric proteins indicate that the binding epitope for chemokine binding is located in the extracellular and hypervariable region of EHV-1 gG (Van de Walle and Osterrieder, unpublished observation). The observation that EHV-1 gG is a vCKBP, whereas gG of the closely related EHV-4 does not show chemokine binding properties is very interesting, especially when one takes into account the different pathogenetic patterns of these two equine herpesviruses. An infection with EHV-1 can lead to multi-organ clinical signs, whereas EHV-4 infection is predominantly associated with highly localized and mild upper respiratory disease (62;63). This directs us to hypothesize that the ability of gG to interfere with the chemokine network might contribute to dissemination and virulence of EHV-1. In turn, the inability of EHV-4 gG to stop or modulate the host's first line of defense may help restrict EHV-4 to the upper airways. However, we cannot formally exclude that EHV-4 gG posseses (restricted) chemokine-binding properties, since not all EHV-4 gG-chemokine interactions have been fully explored to date.

## Concluding remarks

In this review, we have discussed recent developments in the area of immunomodulatory proteins encoded by alphaherpesviruses, specifically those targeting chemokine signaling. To date, MDV expressing vIL-8, the viral counterpart of cellular IL-8, appears to be the only alphaherpesvirus modulating the chemokine network by molecular mimicry of a host protein. This implies that the more recent mammalian alphaherpesviruses use other strategies to manipulate the action of chemokines, as seems to be the case with gG, a vCKBP that not only interferes with a broad range of chemokines, but can also intercept chemokine networking on other levels. Still, one cannot exclude the possibility that the mammalian alphaherpesviruses might actually encode viral proteins with similarity to host cytokines or chemokines, which are not yet identified, as new host molecules involved in immunity are discovered on a regular basis. This growing knowledge about host genes and the ever more comprehensive annotation of host genomes sequenced in their entirety urges the virologist to constantly follow new developments and discoveries in genomics and immunology, as findings there might give them new insights into genes with possible immune evasion properties encoded by viruses. As such, every new discovery will not only aid in a better understanding of the viruses' "anti-immune" system, but will also aid in unraveling the complexity of the host immune systems with which viruses have established close relationships.

## Acknowledgments

We thank our colleagues, especially Drs. Carol Hartley and Michael Studdert, for sharing reagents and for fruitful discussions. Work in our laboratory on MDV has

360 received support through PHS grant AI063048 as well as USDA-NRI grants 2003-02234
361 and 2005-01806. Studies on EHV-1 immune evasion have been supported by the Harry
362 M. Zweig Memorial Fund for Equine Research at Cornell University, the Morris Animal
363 Foundation, and by a grant from the Deutsche Forschungsgemeinschaft (OS 143/4-1).
364

365 ## Figure legends

366 **Figure 1. Chemokine functions.** Chemokines are produced at sites of infection and
367 form chemotactic gradients by interacting with glycosaminoglycans at the surface of
368 endothelial cells. Leukocytes, expressing the appropriate chemokine receptors (seven-
369 transmembrane cell-surface G-protein-coupled receptors) respond to the chemokines and
370 migrate to sites of infected tissue. The presentation of chemokines to leukocytes by
371 chemotactic gradients is required for correct presentation of chemokines *in vivo* and
372 leukocyte migration through the vascular endothelium into infected or damaged tissue.

373 **Figure 2. Potential functions of MDV vIL-8.** There are three proposed functions of
374 vIL-8. The first and most likely possible function of vIL-8 is the attraction of B and T
375 lymphocytes to infected cells by secretion of vIL-8 and migration of uninfected cells by a
376 chemoattractant gradient **(A)**. Another possibility is the secretion of vIL-8 from infected
377 cells which antagonizes chicken IL-8 binding to the IL-8 receptor, thus blocking the
378 function of the chicken chemokine **(B)**. A third possibility is vIL-8 binding to chemokine
379 receptors, and the subsequent activation of transcriptional and translational cascades that
380 lead to enhanced viral replication and/or migration of infected cells **(C)**.

381 **Figure 3. Potential functions of gG.** Alphaherpesviral gG functions as a secreted
382 vCKBP, which prevents chemokines from interacting with both chemokine receptors and

glycosaminoglycans. As a result, chemokine gradients are neutralized and chemotaxis of leukocytes into virus-infected tissues is inhibited **(A)**. In addition, gG expressed on infected cells might also function as a viroreceptor, thereby sequestering chemokines from the extracellular milieu around infected tissue **(B),** or promoting infected-cell proliferation or migration **(C)**.

## References


1. **Adamiak, B., M. Ekblad, T. Bergstrom, V. Ferro, and E. Trybala**. 2007. Herpes simplex virus type 2 glycoprotein G is targeted by the sulfated oligo- and polysaccharide inhibitors of virus attachment to cells. J.Virol. **81**:13424-13434.
2. **Alcami, A.** 2003. Viral mimicry of cytokines, chemokines and their receptors. Nat.Rev.Immunol. **3**:36-50.
3. **Alcami, A.** 2007. New insights into the subversion of the chemokine system by poxviruses. Eur.J.Immunol. **37**:880-883.
4. **Alcami, A. and U. H. Koszinowski**. 2000. Viral mechanisms of immune evasion. Trends Microbiol. **8**:410-418.
5. **Baggiolini, M.** 1998. Chemokines and leukocyte traffic. Nature **392**:565-568.
6. **Balachandran, N. and L. M. Hutt-Fletcher**. 1985. Synthesis and processing of glycoprotein gG of herpes simplex virus type 2. J.Virol. **54**:825-832.
7. **Balan, P., N. Davis-Poynter, S. Bell, H. Atkinson, H. Browne, and T. Minson**. 1994. An analysis of the in vitro and in vivo phenotypes of mutants of herpes simplex virus type 1 lacking glycoproteins gG, gE, gI or the putative gJ. J.Gen.Virol. **75 ( Pt 6)**:1245-1258.

8. **Baranowski, E., G. Keil, J. Lyaku, F. A. Rijsewijk, J. T. Van Oirschot, P. P. Pastoret, and E. Thiry**. 1996. Structural and functional analysis of bovine herpesvirus 1 minor glycoproteins. Vet.Microbiol. **53**:91-101.

9. **Bellner, L., J. Karlsson, H. Fu, F. Boulay, C. Dahlgren, K. Eriksson, and A. Karlsson**. 2007. A monocyte-specific peptide from herpes simplex virus type 2 glycoprotein G activates the NADPH-oxidase but not chemotaxis through a G-protein-coupled receptor distinct from the members of the formyl peptide receptor family. J.Immunol. **179**:6080-6087.

10. **Bellner, L., G. B. Lowhagen, P. Tunback, I. Nordstrom, J. A. Liljeqvist, and K. Eriksson**. 2005. Characterization of T-cell reactive epitopes in glycoprotein G of herpes simplex virus type 2 using synthetic peptides. Arch.Virol. **150**:1393-1406.

11. **Bellner, L., F. Thoren, E. Nygren, J. A. Liljeqvist, A. Karlsson, and K. Eriksson**. 2005. A proinflammatory peptide from herpes simplex virus type 2 glycoprotein G affects neutrophil, monocyte, and NK cell functions. J.Immunol. **174**:2235-2241.

12. **Bennett, L. M., J. G. Timmins, D. R. Thomsen, and L. E. Post**. 1986. The processing of pseudorabies virus glycoprotein gX in infected cells and in an uninfected cell line. Virology **155**:707-715.

13. **Biron, C. A.** 2001. Interferons alpha and beta as immune regulators--a new look. Immunity. **14**:661-664.

14. **Bryant, N. A., N. Davis-Poynter, A. Vanderplasschen, and A. Alcami**. 2003. Glycoprotein G isoforms from some alphaherpesviruses function as broad-spectrum chemokine binding proteins. EMBO J. **22**:833-846.

15. **Cinamon, G., V. Grabovsky, E. Winter, S. Franitza, S. Feigelson, R. Shamri, O. Dwir, and R. Alon**. 2001. Novel chemokine functions in lymphocyte migration through vascular endothelium under shear flow. J.Leukoc.Biol. **69**:860-866.

16. **Cinamon, G., V. Shinder, and R. Alon**. 2001. Shear forces promote lymphocyte migration across vascular endothelium bearing apical chemokines. Nat.Immunol. **2**:515-522.

17. **Colle, C. F., III, C. C. Flowers, and D. J. O'Callaghan**. 1992. Open reading frames encoding a protein kinase, homolog of glycoprotein gX of pseudorabies virus, and a novel glycoprotein map within the unique short segment of equine herpesvirus type 1. Virology **188**:545-557.

18. **Cortes, P. L. and C. J. Cardona**. 2004. Pathogenesis of a Marek's disease virus mutant lacking vIL-8 in resistant and susceptible chickens. Avian Dis. **48**:50-60.

19. **Costes, B., M. B. Ruiz-Arguello, N. A. Bryant, A. Alcami, and A. Vanderplasschen**. 2005. Both soluble and membrane-anchored forms of Felid herpesvirus 1 glycoprotein G function as a broad-spectrum chemokine-binding protein. J.Gen.Virol. **86**:3209-3214.

20. **Costes, B., M. Thirion, B. Dewals, J. Mast, M. Ackermann, N. Markine-Goriaynoff, L. Gillet, and A. Vanderplasschen**. 2006. Felid herpesvirus 1 glycoprotein G is a structural protein that mediates the binding of chemokines on the viral envelope. Microbes.Infect. **8**:2657-2667.

21. **Crabb, B. S. and M. J. Studdert**. 1993. Epitopes of glycoprotein G of equine herpesviruses 4 and 1 located near the C termini elicit type-specific antibody responses in the natural host. J.Virol. **67**:6332-6338.

22. **Cui, X., L. F. Lee, W. M. Reed, H. J. Kung, and S. M. Reddy**. 2004. Marek's disease virus-encoded vIL-8 gene is involved in early cytolytic infection but dispensable for establishment of latency. J.Virol. **78**:4753-4760.

23. **Demmin, G. L., A. C. Clase, J. A. Randall, L. W. Enquist, and B. W. Banfield**. 2001. Insertions in the gG gene of pseudorabies virus reduce expression of the upstream Us3 protein and inhibit cell-to-cell spread of virus infection. J.Virol. **75**:10856-10869.

24. **Devlin, J. M., G. F. Browning, C. A. Hartley, and J. R. Gilkerson**. 2007. Glycoprotein G deficient infectious laryngotracheitis virus is a candidate attenuated vaccine. Vaccine **25**:3561-3566.

25. **Devlin, J. M., G. F. Browning, C. A. Hartley, N. C. Kirkpatrick, A. Mahmoudian, A. H. Noormohammadi, and J. R. Gilkerson**. 2006. Glycoprotein G is a virulence factor in infectious laryngotracheitis virus. J.Gen.Virol. **87**:2839-2847.

26. **Drummer, H. E., M. J. Studdert, and B. S. Crabb**. 1998. Equine herpesvirus-4 glycoprotein G is secreted as a disulphide-linked homodimer and is present as two homodimeric species in the virion. J.Gen.Virol. **79 ( Pt 5)**:1205-1213.

27. **Engelhardt, T. and G. M. Keil**. 1996. Identification and characterization of the bovine herpesvirus 5 US4 gene and gene products. Virology **225**:126-135.

28. **Favoreel, H. W., G. R. Van de Walle, H. J. Nauwynck, and M. B. Pensaert**. 2003. Virus complement evasion strategies. J.Gen.Virol. **84**:1-15.

29. **Guidotti, L. G. and F. V. Chisari**. 2000. Cytokine-mediated control of viral infections. Virology **273**:221-227.

30. **Jarosinski, K. W. and K. A. Schat**. 2007. Multiple alternative splicing to exons II and III of viral interleukin-8 (vIL-8) in the Marek's disease virus genome: the importance of vIL-8 exon I. Virus Genes **34**:9-22.

31. **Kaashoek, M. J., F. A. Rijsewijk, R. C. Ruuls, G. M. Keil, E. Thiry, P. P. Pastoret, and J. T. Van Oirschot**. 1998. Virulence, immunogenicity and reactivation of bovine herpesvirus 1 mutants with a deletion in the gC, gG, gI, gE, or in both the gI and gE gene. Vaccine **16**:802-809.

32. **Karlin, S., E. S. Mocarski, and G. A. Schachtel**. 1994. Molecular evolution of herpesviruses: genomic and protein sequence comparisons. J.Virol. **68**:1886-1902.

33. **Keil, G. M., T. Engelhardt, A. Karger, and M. Enz**. 1996. Bovine herpesvirus 1 U(s) open reading frame 4 encodes a glycoproteoglycan. J.Virol. **70**:3032-3038.

34. **Kimman, T. G., N. de Wind, N. Oei-Lie, J. M. Pol, A. J. Berns, and A. L. Gielkens**. 1992. Contribution of single genes within the unique short region of Aujeszky's disease virus (suid herpesvirus type 1) to virulence, pathogenesis and immunogenicity. J.Gen.Virol. **73 ( Pt 2)**:243-251.

35. **Kongsuwan, K., M. A. Johnson, C. T. Prideaux, and M. Sheppard**. 1993. Identification of an infectious laryngotracheitis virus gene encoding an immunogenic protein with a predicted M(r) of 32 kilodaltons. Virus Res. **29**:125-140.

36. **Lalani, A. S., J. W. Barrett, and G. McFadden**. 2000. Modulating chemokines: more lessons from viruses. Immunol.Today **21**:100-106.

37. **Liu, J. L., S. F. Lin, L. Xia, P. Brunovskis, D. Li, I. Davidson, L. F. Lee, and H. J. Kung**. 1999. MEQ and V-IL8: cellular genes in disguise? Acta Virol. **43**:94-101.

38. **Lubinski, J., T. Nagashunmugam, and H. M. Friedman**. 1998. Viral interference with antibody and complement. Semin.Cell Dev.Biol. **9**:329-337.

39. **Lusso, P.** 2000. Chemokines and viruses: the dearest enemies. Virology **273**:228-240.

40. **Martins-Green, M.** 2001. The chicken Chemotactic and Angiogenic Factor (cCAF), a CXC chemokine. Int.J.Biochem.Cell Biol. **33**:427-432.

41. **McFadden, G., A. Lalani, H. Everett, P. Nash, and X. Xu**. 1998. Virus-encoded receptors for cytokines and chemokines. Semin.Cell Dev.Biol. **9**:359-368.

42. **McGeoch, D. J., F. J. Rixon, and A. J. Davison**. 2006. Topics in herpesvirus genomics and evolution. Virus Res. **117**:90-104.

43. **Mettenleiter, T. C., B. G. Klupp, F. Weiland, and N. Visser**. 1994. Characterization of a quadruple glycoprotein-deleted pseudorabies virus mutant for use as a biologically safe live virus vaccine. J.Gen.Virol. **75 ( Pt 7)**:1723-1733.

44. **Middleton, J., S. Neil, J. Wintle, I. Clark-Lewis, H. Moore, C. Lam, M. Auer, E. Hub, and A. Rot**. 1997. Transcytosis and surface presentation of IL-8 by venular endothelial cells. Cell **91**:385-395.

45. **Murphy, P. M.** 1993. Molecular mimicry and the generation of host defense protein diversity. Cell **72**:823-826.

46. **Nakamichi, K., Y. Matsumoto, and H. Otsuka**. 2002. Bovine herpesvirus 1 glycoprotein G is necessary for maintaining cell-to-cell junctional adherence among infected cells. Virology **294**:22-30.

47. **Nakamichi, K., K. Ohara, D. Kuroki, and H. Otsuka**. 2000. Bovine herpesvirus 1 glycoprotein G is required for viral growth by cell-to-cell infection. Virus Res. **68**:175-181.

48. **Parcells, M. S., S. F. Lin, R. L. Dienglewicz, V. Majerciak, D. R. Robinson, H. C. Chen, Z. Wu, G. R. Dubyak, P. Brunovskis, H. D. Hunt, L. F. Lee, and H. J. Kung**. 2001. Marek's disease virus (MDV) encodes an interleukin-8 homolog (vIL-8): characterization of the vIL-8 protein and a vIL-8 deletion mutant MDV. J.Virol. **75**:5159-5173.

49. **Patel, J. R. and J. Heldens**. 2005. Equine herpesviruses 1 (EHV-1) and 4 (EHV-4)--epidemiology, disease and immunoprophylaxis: a brief review. Vet.J. **170**:14-23.

50. **Pellett, PE and Roizmann B.** Herpesviridae. 5th edition, 2479-2499. 2007. Philadelphia, Lippincott, Wilkinson and Wilkins. Fields Virology.

51. **Richman, D. D., A. Buckmaster, S. Bell, C. Hodgman, and A. C. Minson**. 1986. Identification of a new glycoprotein of herpes simplex virus type 1 and genetic mapping of the gene that codes for it. J.Virol. **57**:647-655.

52. **Schall, T. J. and K. B. Bacon**. 1994. Chemokines, leukocyte trafficking, and inflammation. Curr.Opin.Immunol. **6**:865-873.

53. **Seet, B. T. and G. McFadden**. 2002. Viral chemokine-binding proteins. J.Leukoc.Biol. **72**:24-34.

54. **Su, H. K., R. Eberle, and R. J. Courtney**. 1987. Processing of the herpes simplex virus type 2 glycoprotein gG-2 results in secretion of a 34,000-Mr cleavage product. J.Virol. **61**:1735-1737.

55. **Thomsen, D. R., C. C. Marchioli, R. J. Yancey, Jr., and L. E. Post**. 1987. Replication and virulence of pseudorabies virus mutants lacking glycoprotein gX. J.Virol. **61**:229-232.

56. **Tortorella, D., B. E. Gewurz, M. H. Furman, D. J. Schust, and H. L. Ploegh**. 2000. Viral subversion of the immune system. Annu.Rev.Immunol. **18**:861-926.

57. **Tran, L. C., J. M. Kissner, L. E. Westerman, and A. E. Sears**. 2000. A herpes simplex virus 1 recombinant lacking the glycoprotein G coding sequences is defective in entry through apical surfaces of polarized epithelial cells in culture and in vivo. Proc.Natl.Acad.Sci.U.S.A **97**:1818-1822.

58. **Trapp, S., N. Osterrieder, G. M. Keil, and M. Beer**. 2003. Mutagenesis of a bovine herpesvirus type 1 genome cloned as an infectious bacterial artificial chromosome: analysis of glycoprotein E and G double deletion mutants. J.Gen.Virol. **84**:301-306.

59. **Van de Walle, G. R., M. L. May, W. Sukhumavasi, J. von Einem, and N. Osterrieder**. 2007. Herpesvirus chemokine-binding glycoprotein G (gG) efficiently inhibits neutrophil chemotaxis in vitro and in vivo. J.Immunol. **179**:4161-4169.

60. **Van de Walle, G. R., K. Sakamoto, and N. Osterrieder**. 2008. CCL3 and viral chemokine-binding protein gG modulate pulmonary inflammation and virus replication during equine herpesvirus type 1 (EHV-1) infection. J.Virol. **82**:1714-1722

61. **van Maanen, C.** 2002. Equine herpesvirus 1 and 4 infections: an update. Vet.Q. **24**:58-78.

62. **von Einem, J., P. M. Smith, G. R. Van de Walle, D. J. O'Callaghan, and N. Osterrieder**. 2007. In vitro and in vivo characterization of equine herpesvirus type 1 (EHV-1) mutants devoid of the viral chemokine-binding glycoprotein G (gG). Virology **362**:151-162.

63. **Webb, L. M. and A. Alcami**. 2005. Virally encoded chemokine binding proteins. Mini.Rev.Med.Chem. **5**:833-848.

Table I. Genera and members of the subfamily *Alphaherpesvirinae*

| Genus | Virus | Abbreviation | Host | Gene/Protein with Virokine/Viroceptor/ vCKBP function | Ref. |
|---|---|---|---|---|---|
| **Simplexvirus** | Human herpesvirus type 1 | HSV-1 | human | | |
| | Human herpesvirus type 2 | HSV-2 | human | gG peptides (immunomodulator?) | **8,9,10** |
| | Bovine herpesvirus type 2 (bovine mammilitis) | BHV-2 | cattle | | |
| | Ateline herpesvirus type 1 | AtHV-1 | spider monkey | | |
| | Cercopithecine herpesvirus type 1 | CeHV-1 | macaque | | |
| | Cercopithecine herpesvirus type 2 | CeHV-2 | macaque | | |
| | Cercopithecine herpesvirus type 16 | CeHV-16 | baboon | | |
| | Saimiriine herpesvirus type 1 | SaHV-1 | marmoset | | |
| | Macropodid herpesvirus type 1 | MaHV-1 | wallaby | | |
| | Macropodid herpesvirus type 2 | MaHV-2 | wallaby | | |
| **Varicellovirus** | human herpesvirus type 3 (Varicella zoster virus) | VZV | human | | |
| | Cercopithecine herpesvirus type 9 | CeHV-9 | macaque | | |
| | Equid herpesvirus type 1 (equine abortion herpesvirus) | EHV-1 | horse | gG (viroreceptor/vCKBP) | **13,56,57,58** |
| | Equid herpesvirus type 3 (equine coital exanthema virus) | EHV-3 | horse | gG (vCKBP) | **13** |
| | Equid herpesvirus type 4 (equine rhinopneumonitis virus) | EHV-4 | horse | | |
| | Equid herpesvirus type 6 | EHV-6 | donkey | | |

| | | | | | |
|---|---|---|---|---|---|
| | Equid herpesvirus type 8 | EHV-8 | donkey | | |
| | suid herpesvirus type 1 (pseudorabies virus) | PRV | pig | | |
| | Bovine herpesvirus type 1 (infectious bovine rhinotracheitis) | BHV-1 | cattle | gG (vCKBP) | **13** |
| | Bovine herpesvirus type 5 (bovine encephalitis herpesvirus) | BHV-5 | cattle | gG (vCKBP) | **13** |
| | Bubaline herpesvirus type 1 | BuHV-1 | water buffalo | | |
| | Ovine herpesvirus type 1 (sheep pulmonary adenomatosis-associated herpesvirus) | OvHV-1 | sheep | | |
| | Caprine herpesvirus type 1 | CapHV-1 | goat | gG (vCKBP) | **13** |
| | Cervid herpesvirus type 1 | CerHV-1 | reindeer | gG (vCKBP) | **13** |
| | Rangiferine herpesvirus type 1 | RanHV-1 | reindeer | gG (vCKBP) | **13** |
| | Phocid herpesvirus type 1 | PhoHV-1 | seal | gG (unknown) | |
| | Felid herpesvirus type 1 | FeHV-1 | cat | gG (viroreceptor/vCKBP) | **18,19** |
| | Canid herpesvirus type 1 | CaHV-1 | dog | gG (unknown) | |
| **Mardivirus** | gallid herpesvirus type 2 (Marek's disease herpesvirus) | MDV | chicken | vIL-8 (virokine) | **17,21,35,46** |
| | gallid herpesvirus type 3 (Marek's disease herpesvirus 2) | GaHV-3 | chicken | | |
| | Meleagrid herpesvirus type 1 | HVT | turkey | | |
| **Iltovirus** | gallid herpesvirus type 1 (Infectious laryngotracheitis virus) | ILTV | turkey<br>chicken | gG (vCKBP?) | **23,24** |
| | Psittacid herpesvirus type 1 (Pacheco disease virus) | PsHV-1 | parrot | | |

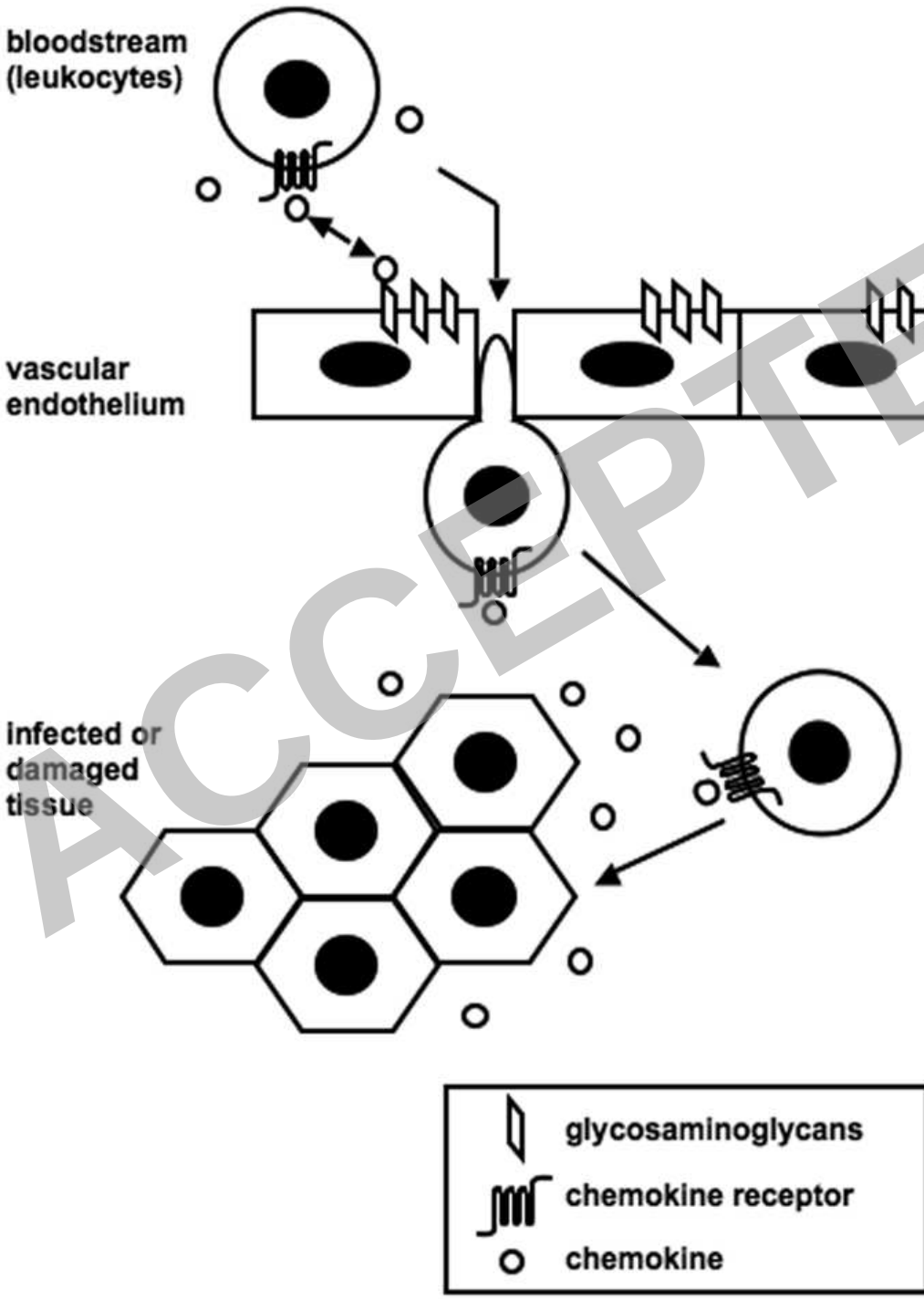




Figure 1: Van de Walle *et al.*

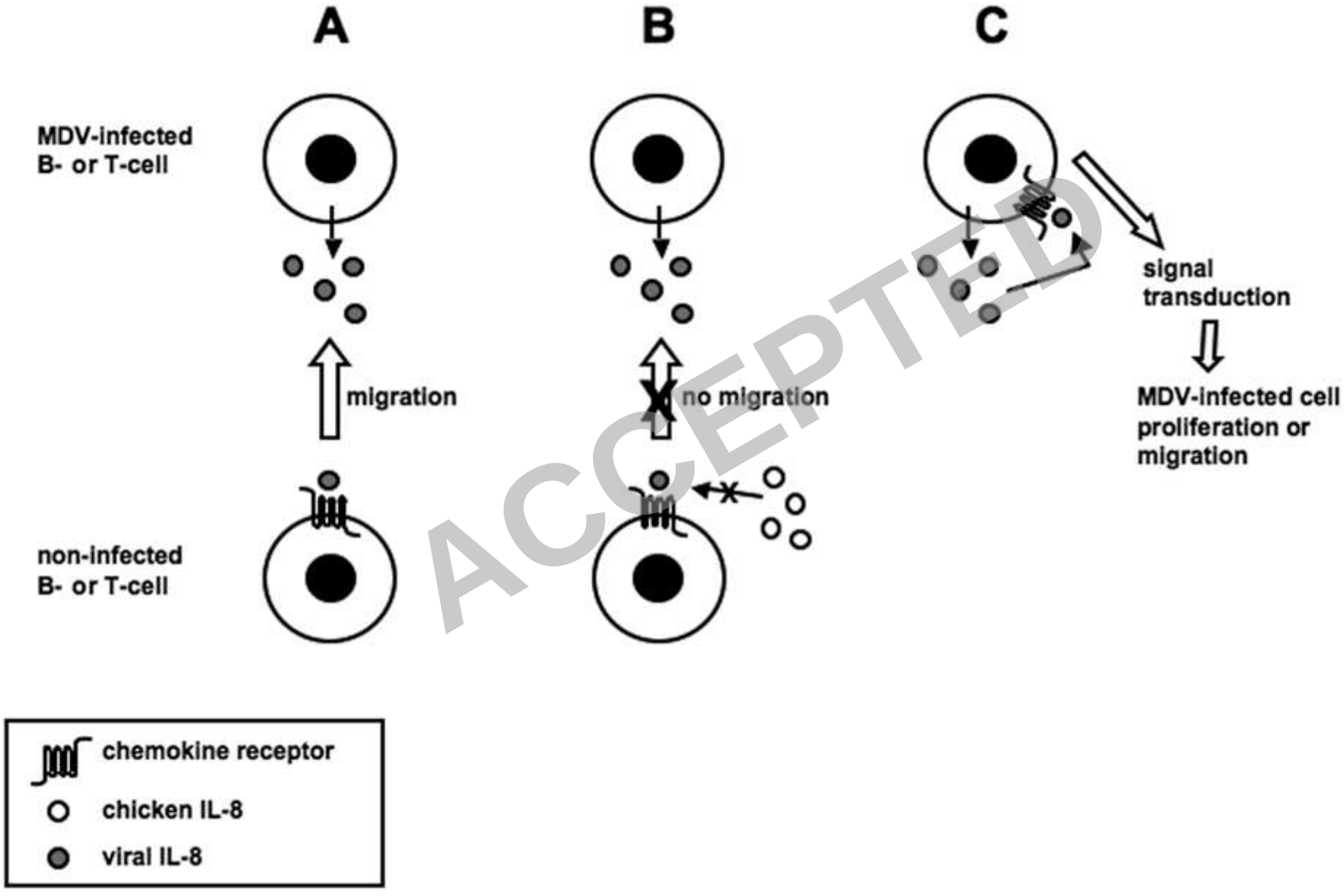




Figure 2: Van de Walle *et al.*

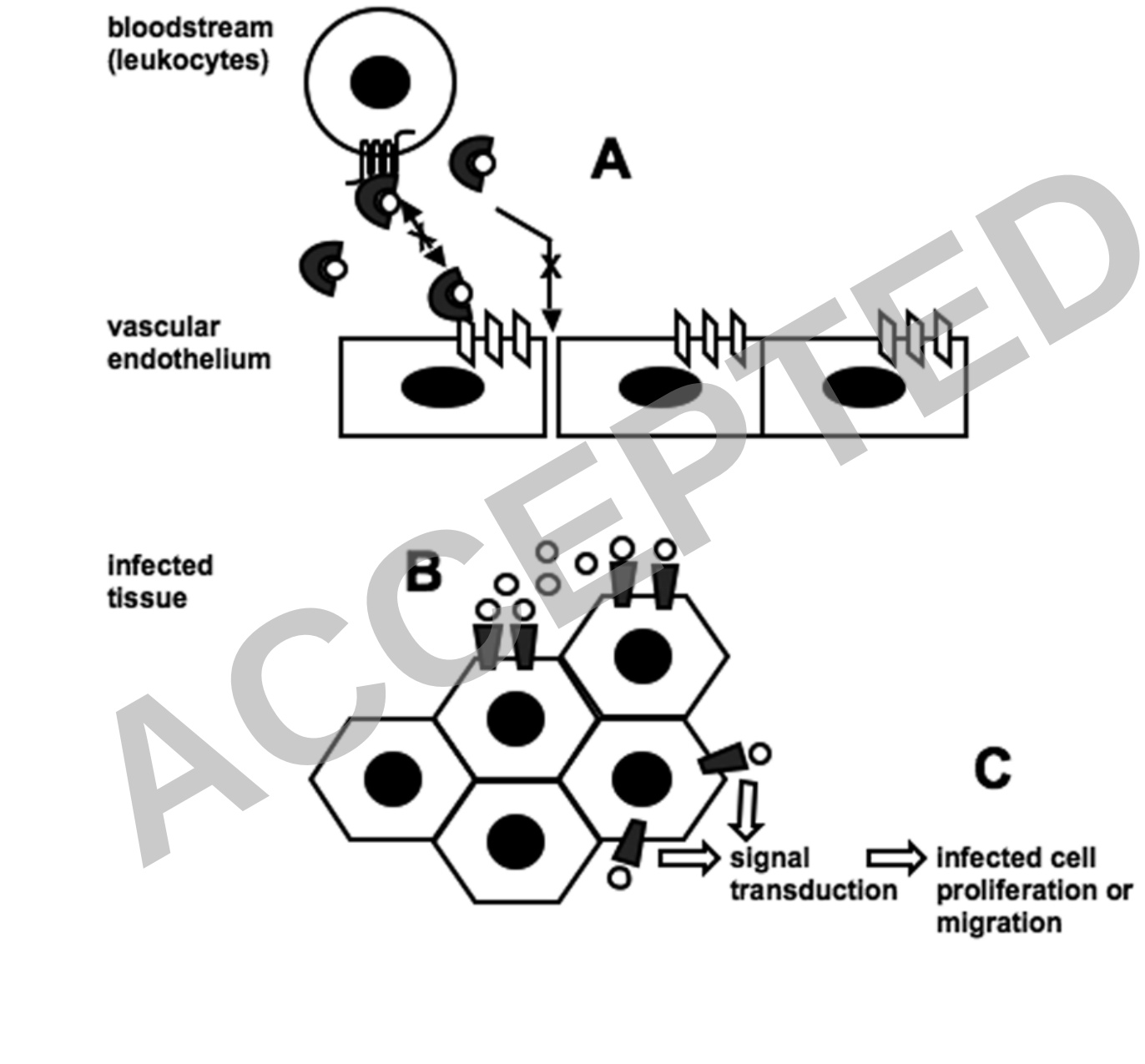




Figure 3: Van de Walle *et al.*