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Role of Chemokines in Rabies Pathogenesis and Protection

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Abstract

Chemokines are a family of structurally related proteins that are expressed by almost all types of nucleated cells and mediate leukocyte activation and/or chemotactic activities. The role of chemokines in rabies pathogenesis and protection has only recently been investigated. Expression of chemokines is induced

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by infection with laboratory-adapted, but not street, rabies viruses (RABVs), and it has been hypothesized that expression of chemokines is one of the mechanisms by which RABV is attenuated. To further define the role of chemokines in rabies pathogenesis and protection, chemokine genes such as MIP-1 α , RANTES, IP-10, and macrophage-derived chemokine (MDC) have been cloned into RABV genome. It has been found that recombinant RABVs expressing RANTES or IP-10 induce high and persistent expression of these chemokines, resulting in massive infiltration of inflammatory cells into the central nervous system (CNS) and development of diseases and death in the mouse model. However, recombinant RABVs expressing MIP-1 α , MDC, as well as GM-CSF further attenuate RABV by inducing a transient expression of chemokines, infiltration of inflammatory cells, enhancement of blood–brain barrier (BBB) permeability. Yet, these recombinant RABVs show increased adaptive immune responses by recruiting/activating dendritic cells, T and B cells in the periphery as well as in the CNS. Further, direct administration of these recombinant RABVs into the CNS can prevent mice from developing rabies days after infection with street RABV. All these studies together suggest that chemokines are both protective and pathogenic in RABV infections. Those with protective roles could be exploited for development of future RABV vaccines or therapeutic agents.

I. INTRODUCTION

Rabies continues to present a serious burden for both public health and the global economy. It causes more than 55,000 human deaths, and more than 10 million people undergo postexposure prophylaxis (PEP) every year around the globe (Martinez, 2000; Meslin *et al.*, 1994). Most human cases occur in the developing countries of Asia and Africa, where canine rabies is endemic and resources are limited (Fu, 1997). In more developed countries, human rabies has dramatically declined during the past 60 years as a direct consequence of routine vaccination of pet animals (Lackay *et al.*, 2008). However, wildlife rabies has emerged as a major threat (Morimoto *et al.*, 1996). Despite extensive investigation over more than 100 years, the pathogenetic mechanisms by which infection of street rabies virus (RABV) results in neurological diseases and death in humans are not well understood. Neuronal pathology or damage in the central nervous system (CNS) is limited in rabies patients with only mild inflammation (Miyamoto and Matsumoto, 1967; Murphy, 1977). However, laboratory-attenuated RABV induces extensive inflammation and neuronal degeneration in experimental animals (Miyamoto and Matsumoto, 1967;

Yan *et al.*, 2001). It is only recently that the roles of chemokines in rabies pathogenesis and protection have been investigated. This chapter will summarize recent research activities in this area.

II. CHEMOKINES

Chemokines are a family of structurally related proteins that are expressed by almost all types of nucleated cells and mediate leukocyte activation and/or chemotactic activities (Zlotnik and Yoshie, 2000). The majority of chemokines have molecular masses of 8–14 kDa and share approximately 20–50% sequence homology among each other at the protein level (Gale and McColl, 1999; Zlotnik and Yoshie, 2000). Chemokine proteins also share common gene sequences and tertiary structures, and all chemokines possess a number of conserved cysteine residues involved in intramolecular disulfide bond formation. Chemokines can be divided into four major subfamilies based on cysteine signature motifs: the C, CC, CXC, and CX3C families (Table I) (Gale and McColl, 1999; Zlotnik and Yoshie, 2000). Chemokines in which the C1 and C2 cysteine residues are adjacent are called CC chemokines and include RANTES, MCP-1, TARC, and eotaxin. Many CC chemokines exert their effects on monocytes and macrophages, but CC chemokines have been shown to be important for dendritic cell (DC) chemotaxis and some CC chemokines appear to act preferentially on Th2-type T cells (Gale and McColl, 1999; Zlotnik and Yoshie, 2000). Chemokines in which the C1 and C2 cysteine residues are separated by a single amino acid are called CXC chemokines and include IL-8, IP-10, I-TAC, and SDF-1. CXC chemokines act as chemoattractants for neutrophils and have been shown to be important mediators of T- and B-lymphocyte chemotaxis (Gale and McColl, 1999; Zlotnik and Yoshie, 2000). The C subfamily chemokine, lymphotactin, is a potent T-lymphocyte chemoattractant, and fractalkine is the only member of CX3C chemokine subfamily, which may chemoattract mononuclear leukocytes (Glabinski and Ransohoff, 1999). Chemokines are highly basic proteins and contain at least four cysteine residues that form two disulfide bonds (Ubogu *et al.*, 2006). This property may help mediate stable gradient formation by promoting interactions of chemokines with sulfated proteins and proteoglycans (Cyster, 1999). Chemokines may also be divided into inflammatory chemokines and homeostatic chemokines in terms of biological features and cellular distribution of chemokine receptors (Moser and Loetscher, 2001). The former are secreted by resident and infiltrated cells on inflammatory stimuli or contacting with pathogenic agents. These chemokines are responsible for recruiting cells related to inflammatory reactions

TABLE I Chemokines and chemokine receptors

	Systematic name	Human/mouse ligand	Chemokine receptor
C family		Lymphotactin	XCR1
CC family	CCL1	I-309	CCR8
	CCL2	MCP-1	CCR2, CCR4
	CCL3	MIP-1 α	CCR1, CCR5
	CCL4	MIP-1 β	CCR5
	CCL5	RANTES	CCR1, CCR3, CCR5
	CCL6	C10, MRP-1	CCR1
	CCL7	MCP-3	CCR1, CCR2, CCR3
	CCL8	MCP-2	CCR1, CCR2B, CCR5
	CCL9	MRP-2, MIP-1 γ	CCR1
	CCL11	eotaxin-1	CCR2, CCR3, CCR5
	CCL12	MCP-5	CCR2
	CCL13	MCP-4	CCR2, CCR3, CCR5
	CCL14	HCC-1	CCR1
	CCL15	HCC-2	CCR1, CCR3
	CCL16	HCC-4	CCR1, CCR2, CCR5, CCR8
	CCL17	TARC	CCR4
	CCL18	PARC	Unknown
	CCL19	MIP-3 β	CCR7
	CCL20	LARC, MIP-3 α	CCR6
	CCL21	6Ckine, SLC, exodus-2	CCR7
	CCL22	MDC	CCR4
	CCL23	MPIF-1	CCR1
	CCL24	MPIF-2, eotaxin-2	CCR3
	CCL25	TECK	CCR9
	CCL26	Eotaxin-3	CCR3
	CCL27	ILC, CTACK	CCR10
	CCL28	MEC	CCR3, CCR10
CXC family	CXCL1	GRO α , MSGA- α	CXCR2
	CXCL2	GRO β , MSGA- β	CXCR2
	CXCL3	GRO γ , MSGA- γ	CXCR2
	CXCL4	PF4	CXCR3
	CXCL5	ENA-78	CXCR2
	CXCL6	GCP-2	CXCR1, CXCR2
	CXCL7	NAP-2	CXCR2
	CXCL8	IL-8	CXCR1, CXCR2
	CXCL9	Mig	CXCR3

TABLE I (continued)

	Systematic name	Human/mouse ligand	Chemokine receptor
	CXCL10	IP-10	CXCR3
	CXCL11	I-TAC, IP-9	CXCR3
	CXCL12	SDF-2	CXCR4
	CXCL13	BLC	CXCR5
	CXCL14	BRAK	Unknown
	CXCL15	Lungkine	Unknown
CX3C	CX3CL1	Fractalkine	CX3CR1

(Holman *et al.*, 2010). However, homeostatic chemokines are involved in maintaining trafficking and positioning of immune cells involved in adaptive immunity and antigen presentation in secondary lymphoid organs (Moser and Loetscher, 2001; Sallusto *et al.*, 1999).

Chemokines mediate their effects by binding to the seven transmembrane G-protein-coupled cell-surface receptors (Table I) (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000). Upon binding, the chemokine receptors initiate cellular signaling through changes in intracellular concentrations of calcium and cAMP. Many cellular chemokine receptors can bind more than one chemokine with similar affinities. For example, the chemokine receptors CCR1 and CCR5 may bind RANTES, MIP-1 α , and MIP-1 β , whereas the chemokine receptors CXCR1 and CXCR2 may bind IL-8 (Gale and McColl, 1999; Zlotnik and Yoshie, 2000). Based on the chemokine subfamilies, chemokine receptors have been named CCR1-9, CXCR1-5, XCR1, and CX3R1 (Zlotnik and Yoshie, 2000). Several chemokines can bind to one receptor, and one ligand can bind to more than one receptor (Ubogu *et al.*, 2006). These intricate complex interactions can provide adequate host defenses against infection with pathogens. However, viruses may mimic chemokine receptors to evade host defense mechanisms (Glabinski and Ransohoff, 1999).

III. THE ROLE OF CHEMOKINES IN THE CNS WHEN INFECTED BY VIRUSES

A recent review has provided an elegant illustration of the roles of chemokines in the CNS after viral infections (Hosking and Lane, 2010). Viral infections of the CNS can result in a temporal expression of several chemokines and chemokine receptors by CNS resident cells (astrocytes,

microglia, as well as neurons) and by inflammatory cells infiltrated into the CNS (Hosking and Lane, 2010; Nakamichi *et al.*, 2005; Prehaud *et al.*, 2005). Astrocytes and microglia are the dominant source of chemokines following infection with neurotropic viruses (Hosking and Lane, 2010). Robust expression of numerous CC chemokines such as CCL2, CCL3, CCL4, and CCL5 (Zlotnik and Yoshie, 2000) was observed following infection with measles virus (Patterson *et al.*, 2003), mouse hepatitis virus (MHV) (Kim and Perlman, 2005), and human coronavirus (Chen *et al.*, 2010). Infection of rat astrocytes and microglia with paramyxoviruses resulted in rapid expression of mRNA transcripts for CCL5 and CXCL10 (Fisher *et al.*, 1995; Vanguri and Farber, 1994). In some virus infections, chemokine expression was found in a particular cell type. For example, CXCL10 was exclusively secreted by astrocytes in the neural parenchyma, but not by microglia in the brain or recruited bone marrow-derived cell types after infection with lymphocytic choriomeningitis virus (LCMV) (Christensen *et al.*, 2009). Induction of chemokine gene expression is promoted by toll-like receptors (TLRs) when recognizing viral DNA or RNA (Gibson *et al.*, 2002; So and Kim, 2009). For example, TLR2 and TLR3 cooperation leads to the expression of macrophage chemoattractants CCL2 and CCL5 during infection with Theiler's murine encephalitis virus (TMEV) (So and Kim, 2009). However, TLR2 and TLR9 mediate chemokine expression during HSV-1 infection (Aravalli *et al.*, 2008; Lima *et al.*, 2010).

The major activity of chemokines is modulating leukocyte trafficking into the CNS (Hosking and Lane, 2010). Both neuroprotective and neuropathologic effects of chemokine expression in the CNS have been reported, and these are largely due to attracting T lymphocytes and macrophages (Dorries, 2001; Lin *et al.*, 2009). On one hand, infiltration and antiviral activity of T lymphocytes are requisite for viral clearance and survival. For example, CXCL10 expressed in the CNS after infection with neurotropic viruses attracts activated T lymphocytes bearing the receptor CXCR3 (Zhang *et al.*, 2008). It has been reported that in many virus infections such as herpes simplex virus (HSV), MHV, and West Nile virus (WNV), ablation of CXCL10 expression by either depletion with neutralization antibodies or genetic knockout dramatically reduces infiltration of T cell into the CNS, which results in inefficient viral control and severe disease (Stiles *et al.*, 2009; Thapa and Carr, 2008; Zhang *et al.*, 2008). Another chemokine CCL5 and its receptors, CCR5, have also been found to promote leukocyte trafficking into the CNS and control of HSV and WNV infections (Glass *et al.*, 2005; Thapa *et al.*, 2007). CCR5 knockout showed an increased risk for symptomatic WNV infection (Glass *et al.*, 2006). On the other hand, excessive chemokine secretion and accumulation of leukocytes within the CNS lead to the development of neuropathology. It is well known that fatal meningoencephalitis induced by LCMV

infection is mediated by infiltration of virus-specific cytotoxic T lymphocytes (CTLs) (Fung-Leung *et al.*, 1991; Kim *et al.*, 2009). Genetic silencing of CXCL10 or its receptor CXCR3 reduces the infiltration of CD8+ T cells, conferring either partial or near complete protection from immunopathology and death (Christensen *et al.*, 2006; Hofer *et al.*, 2008). Demyelinating disease during MHV infection is largely due to sustained CXCL10 and CCL5 expression, and abrogation of expression of either these chemokine reduces infiltration of immune cells, disease severity, and demyelination (Glass *et al.*, 2004; Liu *et al.*, 2001). There are numerous examples of neuroprotective and neuropathologic activities associated with the expression of chemokines during viral infections (Hosking and Lane, 2010).

IV. INDUCTION OF CHEMOKINE EXPRESSION IN RABV INFECTIONS

RABV induces a fatal neurological disease in humans and animals, and the roles of chemokines in rabies are just beginning to emerge. Using oligonucleotide microarray, we reported that chemokines were upregulated in the mouse brain after infection with laboratory-attenuated, but not with street virus (Wang *et al.*, 2005). This includes both the CC and CXC subfamilies of proinflammatory chemokines. Among these chemokines, MIP-1 α , RANTES, and IP-10 were increased more than 50- to 100-fold in infected versus sham-infected mice (Kuang *et al.*, 2009; Wang *et al.*, 2005). Further, the protein level of chemokines CXCL10 and CCL5 was dramatically upregulated in neuroblastoma cells after infection with laboratory-attenuated RABV (Masatani *et al.*, 2010). It has also been reported that chemokine CXCL-10 and cytokines (IL-6, IFN- γ) were upregulated at the time of clinical disease in the CNS of mice infected with European bat lyssaviruses (EBLV) types 1 and 2 (Mansfield *et al.*, 2008). Interestingly, a lower but significant increase of CXCL10 was also observed in the salivary glands (Mansfield *et al.*, 2008). CXCL10 has also been reported to be activated by macrophages and microglia infected with RABV (Nakamichi *et al.*, 2004, 2005).

The increased expression of chemokines in RABV infection in the CNS resulted in infiltration of inflammatory cells, induction of apoptosis, and enhancement of blood-brain barrier (BBB) permeability in mice infected with fixed (or laboratory-attenuated) RABV (Fabis *et al.*, 2008; Kuang *et al.*, 2009; Sarmiento *et al.*, 2005; Wang *et al.*, 2005; Zhao *et al.*, 2009). As a consequence, fixed RABV could be cleared from the CNS of mice when infected with low doses (Hooper *et al.*, 2009; Sarmiento *et al.*, 2005). It is, therefore, hypothesized that induction of innate immunity, particularly with chemokines and IFN, is one of the mechanisms for RABV

attenuation. However, mice infected with high dose of fixed RABV die with excessive inflammation in the CNS (Sarmiento *et al.*, 2005; Wang *et al.*, 2005). Therefore, the pathogenetic mechanisms by which street and fixed RABV induce disease are different. In animals infected with a high dose of fixed RABV, it is the expression of chemokines and other innate immune molecules that results in the enhancement of BBB permeability and infiltration of inflammatory cells into the CNS, which is ultimately responsible for the demise of the infected animals (Kuang *et al.*, 2009; Wang *et al.*, 2005). On the contrary, street RABV invades the CNS without stimulating the innate immune responses. Although the exact mechanisms by which street RABV causes rabies are not known, it has been hypothesized that RABV induces CNS dysfunction (Dietzschold *et al.*, 2001). Recently, we have observed that infection of street RABV inhibits the expression of proteins involved in the fusion between neurotransmitter vesicle membrane and the presynaptic membrane, resulting in massive accumulation of neurotransmitter vesicles in presynapses (Dhingra *et al.*, 2007). These observations may also explain why very little neuronal pathology or damage is observed in the CNS of rabies patients (Miyamoto and Matsumoto, 1967; Murphy, 1977), whereas laboratory-attenuated RABV induces extensive inflammation and neuronal degeneration in experimentally infected animals (Miyamoto and Matsumoto, 1967; Yan *et al.*, 2001).

V. OVEREXPRESSION OF CHEMOKINES CAN BENEFIT THE HOST IF THE EXPRESSION IS TRANSIENT WHILE IT HARMS THE HOST IF THE EXPRESSION IS PERSISTENT DURING RABV INFECTIONS

To further explore the role of chemokines in RABV infections, chemokines MIP-1 α , RANTES, or IP-10 were individually expressed in the genome of RABV HEP-Flurry strain (Zhao *et al.*, 2009, 2010). It was found that although the expression of MIP-1 α further reduced RABV pathogenicity, expression of RANTES or IP-10 enhanced RABV pathogenicity in the mouse model. The differences in pathogenicity induced by these recombinant RABVs are not due to the rate of virus replication, but rather due to the level and the duration of the expression of chemokines (Zhao *et al.*, 2009). HEP-MIP1 α induced the expression of MIP-1 α in the mouse model but subsided quickly. In addition, only low to moderate levels of other chemokines were induced. Likewise, only low and transient infiltration of inflammatory cells (macrophages, neutrophils, and T cells) was observed in the infected mice. In contrast, HEP-RANTES and particularly HEP-IP10 induced not only high and persistent expression of the intended chemokines but also high expression of other chemokines. High and persistent infiltration of inflammatory cells was also observed in the CNS, which

could produce neurotoxins, free radicals, and proinflammatory cytokines, causing CNS destruction (Hooper *et al.*, 2009; Zhao *et al.*, 2009). HEP-MIP1 α enhanced the BBB permeability temporarily, while HEP-RANTES and HEP-IP10 induced more extensive and prolonged enhancement of BBB permeability. Further, HEP-IP10 induced BBB permeability to the extent that allowed large molecules (10 kDa) to enter the CNS. Although the consequence is not entirely clear, this may have allowed more inflammatory cells or other toxic substances enter into the CNS. Thus, these studies indicate that transient expression of chemokines may help attenuate RABV, whereas high and persistent expression of these chemokines, particularly IP-10, may be harmful to the host during RABV infections.

VI. CHEMOKINES EXPRESSION CORRELATES WITH THE ACTIVATION OF DENDRITIC CELLS AND ENHANCEMENT OF ADAPTIVE IMMUNITY

As chemokines play roles as attractants of naïve and effector T cells (Moser and Loetscher, 2001), these recombinant RABV expressing chemokines were tested for their ability to enhance adaptive immunity (Zhao *et al.*, 2009, 2010). Although overexpression of MIP-1 α further attenuated RABV (Zhao *et al.*, 2010), it enhanced the adaptive immune responses by stimulating the production of high levels of virus-neutralizing antibodies (VNA). As MIP-1 α is one of the major chemoattractants for monocytes, especially immature DCs and macrophages (Barouch *et al.*, 2003; Maurer and von Stebut, 2004; McKay *et al.*, 2004), it is possible that expression of MIP-1 α recruits/activates DCs. Indeed, it was found that overexpression of MIP-1 α resulted in the induction of a strong innate immune response at the local site and recruitment/activation of DCs as well as B cells in the draining lymph nodes and the peripheral blood, leading to the production of high levels of VNA (Zhao *et al.*, 2010). DCs are the most potent antigen presenting cells (APCs) (Clark, 1997), which process antigen, migrate to the T cell zone and stimulate the activation of antigen-specific naïve T cells. Activated T cells stimulate the proliferation and differentiation of antigen-specific naïve B cells into antibody-producing plasma cells (Dubois *et al.*, 1999).

To confirm that recruitment/activation of DCs is the major step in the induction of VNA in RABV immunization, DC-recruitment/activation molecules such as macrophage-derived chemokine (MDC) and granulocyte-macrophage colony-stimulating factor (GM-CSF), in addition to MIP-1 α , were individually expressed in the RABV LBNSE strain (Wen *et al.*, 2011). MDC is known to preferentially attract Th2 cells and regulatory T cells via CCR4 (Iellem *et al.*, 2001; Imai *et al.*, 1999; Yoshie *et al.*, 2001). It is also a potent chemoattractant for additional cell types

including DCs (Chantry *et al.*, 1999; Godiska *et al.*, 1997). MDC produced by DCs attracts CCR4-bearing activated (or memory) T cells to enhance immune responses and increase effector functions (Wu *et al.*, 2001), and it may allow for T–B cell interaction with subsequent formation of germinal centers (Schaniel *et al.*, 1998). GM-CSF regulates the production and functional activation of hemopoietic cells such as monocyte/macrophages and all granulocytes (Metcalf, 2008) and is a cytokine responsible for the recruitment, activation, and maturation of APC (Hamilton and Anderson, 2004). Each of these recombinant viruses stimulated more maturation/activation of murine bone marrow-derived DCs *in vitro* and more recruitment and/or activation of DCs, mature B cells as well as T cells in the periphery than the parent virus, which leads to higher levels of VNA and better protection (Wen *et al.*, 2011). Thus, our data suggest that the expression of chemokines can result in recruitment/activation of DCs, thus enhancing RABV immunogenicity and protection. Chemokines have been used as an adjuvant by incorporating into vaccine preparations to stimulate innate and adaptive immune responses (Han *et al.*, 2009; Kutzler *et al.*, 2010). Coadministration of chemokine and DNA encoding viral protective antigens increases trafficking of mature DCs into the secondary lymphoid tissues, presenting processed viral antigen to naïve T cells and provides protective immunity against virus challenge. RANTES, MCP-1, MIP-1 β , and TRANCE have been used together with a truncated secreted version of the RABV glycoprotein in plasmid expression DNA vaccine to enhance immune responses (Pinto *et al.*, 2003). Together, these observations suggest that recombinant RABV expressing chemokines could be developed as potential vaccine candidates.

VII. RECOMBINANT RABV EXPRESSING CHEMOKINES/ CYTOKINES CAN BE USED EFFECTIVELY TO PREVENT THE DEVELOPMENT OF RABIES

These recombinant RABV expressing chemokines/cytokines were also tested to determine whether they have the ability to prevent animals from developing rabies. Adult mice were infected with a lethal dose of street RABV and then treated with recombinant RABV at different time points after infection (Wang and Fu, unpublished data). As shown in Fig. 1, 60–70% of the mice intracerebrally treated with recombinant RABV expressing MDC, IP-10, MIP-1 α , or GM-CSF at day 4 after infection with street RABV (a Mexican dog virus, DRV) were protected from developing rabies. The protection rate in mice treated with live recombinant RABV was significantly higher than that in sham-treated mice (20%). In contrast, treatment with UV-inactivated RABV did not provide significantly better protection than sham-treated mice despite the fact that VNAs

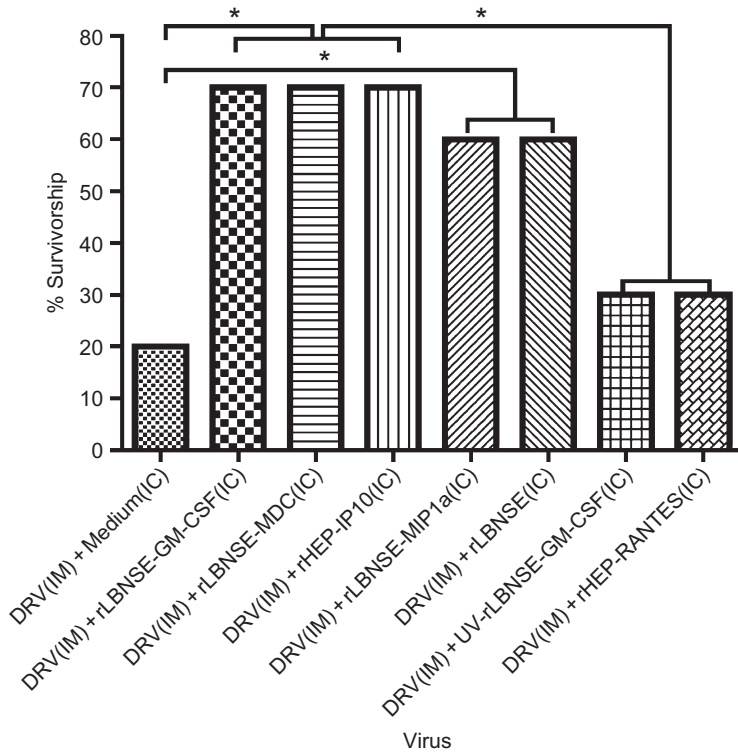


FIGURE 1 Recombinant RABVs expressing chemokines/cytokines prevent the development of rabies in the mouse model. ICR mice (4–6 weeks of age) were infected intramuscularly with street DRV and treated intracranially 4 days later with various recombinant RABVs or medium. Mice were observed daily for 2 weeks, and the survivorship was calculated and analyzed statistically.

were induced in these mice. Surprisingly, recombinant RABV expressing RANTES did not protect mice from developing rabies in these mice. Treatment with recombinant RABV by other routes (intramuscular, intradermal, or intranasal) was less effective (Wang and Fu, unpublished data). It was found that intracerebral treatment of mice with these recombinant RABVs induced significantly higher levels of chemokine/cytokine expression in the CNS and in the periphery, infiltration of inflammatory and immune cells into the CNS, and enhancement of BBB permeability than sham-treated mice or mice treated with UV-inactivated RABV. These studies indicate that there are two important factors for protection: VNA in the periphery and enhanced BBB permeability. To demonstrate this is the case, mice were treated with a chemokine (chemoattractant protein-1, MCP-1) with a dose known to enhance BBB permeability. Indeed, this treatment increased the protective efficacy of UV-inactivated RABV, but not in

sham-treated mice. These data confirm that chemokines can induce infiltration of inflammatory cells in the CNS and thus enhance the BBB permeability, which allows immune effectors (VNA) enter into the CNS to clear the virus and prevent the development of rabies.

BBB is a separation of circulating blood and CSF in the CNS and protects the CNS tissues from circulating cells and factors (Pachter *et al.*, 2003). The enhancement of BBB permeability and inflammatory cells infiltration is often associated with pathological changes in the CNS. However, transiently increased BBB permeability has been found to be helpful in clearance of the attenuated RABV from the CNS (Phares *et al.*, 2006). Highly pathogenic RABV is correlated with the inability of infected animals to enhance BBB permeability and deliver immune effectors into the CNS (Roy *et al.*, 2007). Further studies have shown that lethal infection with pathogenic RABV could be prevented by increasing BBB permeability in infected animals through the induction of an autoimmune CNS inflammatory response that facilitates immune effectors entry into the CNS tissue and promotes virus clearance (Roy and Hooper, 2007). Chemokines can help enhance the BBB permeability by inducing inflammatory responses in the CNS, thus aiding immune effectors enter into and clear the virus from the CNS. One of the questions that remains unanswered in these studies is whether the immune effectors (in this case, VNA) need to be produced in the CNS as has been proposed (Hooper *et al.*, 1998, 2009) or whether VNA produced in the periphery and transported to the CNS is just as effective. Future studies should be directed to address this issue.

VIII. SUMMARY

This chapter summarizes recent studies on the role of chemokines in rabies pathogenesis and protection. It has been found that laboratory-adapted RABV is capable of inducing chemokine expression as part of innate immune responses, which is beneficial to the host by initiating infiltration of inflammatory cells into the CNS, enhancing the BBB permeability, and clearing the virus from the CNS (Kuang *et al.*, 2009; Sarmiento *et al.*, 2005; Wang *et al.*, 2005). This is especially important when animals are infected with low doses of laboratory-adapted RABV. However, street RABV fails to induce the expression of chemokines and other innate immune molecules, leading to unblocked invasion of the virus into the CNS (Kuang *et al.*, 2009; Sarmiento *et al.*, 2005; Wang *et al.*, 2005). However, excessive expression of chemokines and other innate immune molecules could induce neurological diseases by inducing extensive inflammation in the CNS when animals are infected with high doses of fixed RABV (Kuang *et al.*, 2009; Sarmiento *et al.*, 2005; Wang *et al.*, 2005). Thus,

expression of chemokines has both protective and pathogenetic roles in RABV infections. This contention has been further confirmed by overexpression of some of the chemokines (Zhao *et al.*, 2009). Overexpression of MIP-1 α further attenuates RABV, while overexpression of RANTES and IP-10 increases RABV pathogenicity (Zhao *et al.*, 2009). However, overexpression of MIP-1 α enhances the immunogenicity of RABV, and the recruitment/activation of DCs is the possible mechanism for the enhanced immunogenicity (Zhao *et al.*, 2010). Indeed, overexpression of chemokines or cytokines with the ability to activate DCs increased RABV immunogenicity and provided better protection (Wen *et al.*, 2011; Zhao *et al.*, 2010). Further, recombinant RABV expressing chemokines/cytokines can be used to prevent the development of rabies in the mouse model (Wang and Fu, unpublished data). Therefore, recombinant RBVs expressing chemokines/cytokines could have the potential to be used not only for pre- and postexposure immunization but also for therapy in clinical rabies.

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