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Macrophages and the Viral Dissemination Super Highway

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Abstract

Monocytes and macrophages are key components of the innate immune system yet they are often the victims of attack by infectious agents. This review examines the significance of viral infection of macrophages. The central hypothesis is that macrophage tropism enhances viral dissemination and persistence, but these changes may come at the cost of reduced replication in cells other than macrophages.

Keywords: *Feline corona viruses; Macrophages; Lymphocytic choriomeningitis virus; Human cytomegalovirus; ssRNA*

Introduction

The ability to infect and replicate in macrophages is implicated in the pathogenesis of many viruses, such as influenza virus [1], rabies virus [2], and dengue virus [3]. This review analyzes four viruses in which well-defined mutations have been identified that confer the ability to infect macrophages. The four viruses are lymphocytic choriomeningitis virus (LCMV), feline corona viruses (FCoV), Theiler's murine encephalomyelitis virus (TMEV), and human immunodeficiency virus type-1 (HIV-1). Comparison of the parental and macrophage-tropic forms of these viruses allows the biological effects of macrophage infection to be identified. In addition to these four viruses, human cytomegalovirus (HCMV) pathogenesis will also be examined. HCMV will be used to illustrate how a virus's ability to induce monocyte to macrophage differentiation expands sites of replication and promotes widespread, efficient viral dissemination. The pathways five diverse viruses use to exploit monocytes and macrophages and gain access to internal organs are summarized below (Figure 1). Collectively, these viruses represent extremely disparate branches of the viral family tree, allowing the potential benefits and costs of macrophage tropism to be considered from a broad perspective. The properties of these viruses are summarized in Table 1.

Virus	Class	Parental Strain	Macrophage-Tropic Strain	Gene Changes	Benefit/Cost of Macrophage Tropism
LCMV	(-) sense, ssRNA, enveloped	Armstrong	Clone 13	Env F260L and Polymerase K1079Q or K1079N	Systemic, chronic infection; Increased host susceptibility to other competing pathogens
FCoV	(+) sense, ssRNA, enveloped	FECV	FIPV	Loss of non-structural protein 3c (function unknown)	Systemic infection; loss of replicative ability in intestinal epithelial cells
TMEV	(+) sense, ssRNA, non-enveloped	GDVII subgroup	TO Subgroup	Expression of alternate reading frame protein, L*	Persistence; decreased ability to replicate in neurons
HIV-1	(+) sense, ssRNA - retrovirus, enveloped	Lymph node sequences	Brain Sequences	gp120 envelope T283N	Enhanced macrophage tropism, less dependence on CD4 for entry; increased susceptibility to neutralizing antibodies

Table 1: *Viruses with closely-related non-macrophage tropic and macrophage-tropic strains.*

Lymphocytic Choriomeningitis Virus

LCMV is a prototypic, rodent-borne arena virus (Table 1). It is an enveloped, negative sense, ssRNA virus whose genome is comprised of two segments that employ an ambi-sense coding strategy. The genome is 10,600 nucleotides long, with 4 open reading frames (ORFs): 1 glycoprotein (GP, post-translationally cleaved to GP1 and GP2), and 1 nucleocapsid on the S segment. The polymerase gene and Z gene (a zinc finger protein thought to function in the regulation of viral transcription) are encoded by the L segment [4]. LCMV causes choriomeningitis (i). LCMV infection of laboratory mice has served as one of the classic model systems in which to study host-pathogen interactions [5-10]. Intracerebral inoculation of adult mice with LCMV Armstrong (Arm) strain elicits a robust cytotoxic T lymphocyte (CTL) and humoral immune response; the mice are able to clear the virus 8-10 days post infection [11]. In contrast to this robust CTL response, when newborn mice are intracerebrally inoculated with LCMV Arm they produce no detectable CTL response among effector cells isolated from spleen or lymph nodes (ii). Mice that are infected as newborns develop a persistent LCMV infection with life-long viremia and high titers of virus in all major organs [5].

Ahmed., *et al*. [12] set out to determine whether the disseminated, chronic carrier state observed in Arm-infected newborn mice could be explained by the emergence of viral variants arising in the setting of a toleragenic (neonatal) immune system. They isolated and plaque-purified LCMV from both the brain and spleen of 6-8 week old chronically infected mice that had been infected with Arm at birth. Adult mice infected with LCMV isolated from the brains of chronic carrier mice mounted a robust CTL response and cleared the virus within two weeks [12]. In contrast, adult mice infected with virus isolated from the spleens chronic of carrier mice did not generate a detectable CTL response and developed systemic infection, with long-term viral persistence [12]. These results suggested that the virus isolated from the spleens of carrier mice was a variant of the parent LCMV Arm that had acquired the ability to establish persistent infection in fully immuno-competent animals. This variant was named "Clone 13" [12].

Clone 13 differs from Arm at only 5 of 10,600 nucleotides. Two of the mutations result in amino acid changes, nucleotide 856 (S segment, amino acid 260 of the glycoprotein gene) and nucleotide 3267 (L segment, amino acid 1079 of the polymerase gene) [4]. In a study performed by Villarete., et al. [13] newborn mice infected with the parental LCMV Arm were sacrificed at various time points from day 0 to day 250 post-infection and screened for the emergence of Clone 13 variants in various tissues. While the Clone 13 strain was detectable in the brain, the parental Arm remained the predominant strain in this tissue and LCMV titers in brain tissue steadily declined throughout the observation period. By contrast, in spleen, Clone 13 was nearly 90% of the detectable LCMV population by day 32 post-infection, which corresponded with a rise in viral titer that contributed to increased viral load throughout the observation period [13].

Infection of adult mice with LCMV Arm produces a localized, acute choriomeningitis that is cleared within two weeks. Infection of adult mice with LCMV Clone 13 leads to a systemic, multi-organ, persistent infection. To investigate the properties of Clone 13 that altered pathogenesis, Malbouain., *et al*. [14] compared the replication of Arm to Clone 13 strains both *in vitro* and *in vivo*. *In vitro*, Arm and Clone 13 replicated with equal efficiency in fibroblasts, and both strains replicated at comparably low levels in lymphocytes. However, *in vitro* investigation of LCMV replication in tissue macrophages extracted from murine lung (alveolar macrophages) or peritoneum (peritoneal macrophages) demonstrated that the Clone 13 mutations resulting in amino acid changes at codons 260 and 1079 were associated with increased entry and more efficient replication in macrophages, respectively. The study went on to compare *in vivo* dissemination of Arm vs. Clone 13 in adult mice 0-72 hours post infection (prior to the development of B and T cell anti-viral responses), assessing viral titers in liver, spleen, and lung tissues by plaque forming units (PFUs) and northern blot analysis. Titers of Clone 13 were higher in each of the organs. Immunofluorescent (IF) staining of livers from mice infected with Clone 13 demonstrated that F4/80+ macrophages were the first cells to have detectable LCMV protein levels by IF (days 1-3). Hepatocytes did not demonstrate detectable LCMV protein levels by IF until day 5 [14]. The apparent sequential infection of macrophages followed by infection of hepatocytes suggests that the virus may use infected macrophages to gain access to other cell types.

In 1998, α-dystroglycan (a peripheral membrane glycoprotein) was determined to be an entry factor for LCMV [15]. Clone 13 strains bind to the α-dystroglycan receptor with 2-3 logs higher affinity than Arm [16]. Smelt., *et al.* [16] proposes this accounts for the differing behavior of Arm and Clone 13 [16]. The enhanced ability of Clone 13 to enter and replicate in tissue macrophages may be the principal factor leading to enhanced dissemination and viral replication in tissues. Supporting this concept, as early as day 1 post infection,

F4/80+ LCMV infected macrophages were observed in the livers of Clone 13-infected but not Arm-infected mice [14]. As a caveat, while both Arm and Clone 13 replicate in dendritic cells (DCs), Clone 13 infects a higher percentage of DCs and is able to destroy them, leading to a generalized immune suppression that may contribute to viral dissemination [17]. This immune suppression, however, is not observed until 28 days post infection-long after infection of liver macrophages has occurred.

Feline Corona Viruses (FCoV)

The feline corona Viruses (FCoV) are in the Coronaviridae family. Feline enteric corona virus (FECV) and feline infectious peritonitis virus (FIPV) are often referred to as separate viruses; however, they are actually different strains of FCoV. Mutations in FECV give rise to FIPV [18,19]. The FCoVs are enveloped, positive sense, ssRNA viruses that are spread by oral-fecal transmission. The genome contains 29,000 nucleotides and 11 ORFs. Two ORFs encode replicase proteins, four encode structural proteins (spike, env, membrane, and nucleocapsid), and five encode "accessory" proteins, 3a, 3b, 3c, 7a, and 7b [20]. FECV and FIPV are serologically and morphologically indistinguishable but have markedly different clinical behaviors [19]. FECV replication is restricted to intestinal epithelial cells. Infection is generally benign and may result in asymptomatic enteritis. Among kittens in shelters, catteries, or other multi-cat environments seroprevalence reaches up to 90% [20]. FIPV, the virulent mutant of FECV, has an incidence of 1-5% among shelter or cattery kittens [20]. It can escape the gut and replicate throughout the body, causing systemic inflammatory damage and a "fatal immunopathological disease" [20].

In a study conducted by Harry Vennema, 20 cats with feline immunodeficiency virus (a population in which the incidence of FIPV is about 10-fold higher than in immuno-competent cats) were infected with FECV. Two of them subsequently developed symptoms consistent with FIPV. Sequence analysis of the viruses from these cats revealed two non-identical deletions in the 3c gene. The function of the accessory protein encoded by this gene is unknown [19]. Chang., *et al*. [21] performed a more detailed sequence comparison of FECV and FIPV. The majority of FIPV virulence mutations were located in 3c gene. Many of the mutations led to frame shifts that would presumably knock out 3c function. Later examination of FECV from 28 cats revealed uniformly intact 3c genes and the ability to replicate in intestinal epithelial cells whereas the 3c-inactivated FIPV viruses replicated poorly in these cells, likely reducing their capacity for oral-fecal transmission [21].

If FIPV strains replicate poorly in the gut, can cellular tropism, i.e., macrophage, account for the systemic disease? In a very comprehensive article on FCoV "A review of feline infectious peritonitis virus infection 1963-2008," the author, Niels Pedersen, states that "the acquisition of macrophage tropism appears to be an essential step in the evolution/transformation of an FECV to an FIPV… [and] the relationship between virulence and macrophage/monocyte tropism has been firmly established" [20]. *In vitro*, FIPV was first propagated in Feliscatus whole fetus-4 (Fcwf-4) cells, which were later determined to be a macrophage-like cell line [20]. Virus has been detected in the macrophages associated with the pyogranulomatous lesions of spleen, liver, and kidney, which are characteristically seen in FIPV disease [20]. Severe T cell depletion (postulated to result from apoptosis induced by unidentified "soluble mediators" in the ascitic fluid of FIPV-infected cats [22]), is observed in lymphoid organs where FIPV positive macrophages are detected [23]. Thus, acquiring the ability to replicate in macrophages shifts the clinical manifestations of FCoV infection from benign enteritis to a systemic and fatal inflammatory condition. Of note, soluble products produced by infected macrophages contribute to the immune-mediated pathology observed in FIPV disease. This theme-that virus-infected macrophages release factors that enhance viral infection of surrounding cells and/or promote tissue injury--will be echoed throughout the remainder of the review.

Theiler's Murine Encephalomyelitis Virus (TMEV)

Theiler's murine encephalomyelitis virus (TMEV), a picornavirus, is a non-enveloped, positive sense, ssRNA virus, with internal ribosome entry site (IRES)-driven translation of its genome. The 8.1 kb genome contains a long ORF that is translated into a polyprotein that is cleaved to yield 12 mature proteins. A second, overlapping ORF encodes a 156 amino acid protein known as L* because its coding sequence overlaps that of the L protein, which is at the N-terminus of the polyprotein [24]. While TEMVs share 90% sequence identity at the nucleotide level and 95% identity at the amino acid level, the virus was is divided into two "subgroups" based on disease phenotypes. The GDVII subgroup includes GDVII and FA strains. The TO subgroup includes DA and DeAn Strains [24]. Mice infected with the GDVII subgroup develop acute polioencephalomyelitits. The virus replicates in neurons and is rapidly fatal with a Lethal Dose for 50% of mice

(LD50) of just one PFU [24]. In the few mice that survive, no viral persistence is observed [25]. In contrast, the LD50 for TO subgroup strains is 106 PFU. Despite their diminished "neurovirulence", TO subgroup strains persistently infect brain and spinal cord, causing chronic CNS inflammation and systemic demyelination [24]. TO subgroup strains are often used in mouse models of multiple sclerosis.

Aubert., *et al*. [26] reported that the demylenating lesions in mice infected with the TO subgroup strains contain four major cell types: lymphocytes, microgilial cells (resident brain macrophages), astrocytes, and oliogdendrocytes (the source of myelin in the CNS) [26]. Clatch., *et al*. [27] demonstrated that in a population of mononuclear cells isolated from the CNS of TO subgroup-infected mice, the macrophages (identified using F4/80 and MOMO-II antibodies) were infected with TEMV [27]. In a histological study performed on frozen sections of the brain and spinal cord of TO subgroup-infected mice, Lipton., *et al*. [28] noted that mononuclear inflammatory cell infiltrates first appeared in the spinal cord on day 14 post infection, preceding the earliest signs of demylenating disease, which were observed in 10% of mice by day 22 and in 90% of mice by day 84 [28]. Ninety percent of cells harboring a large TMEV antigen load were macrophages (identified by F4/80, Mac-1, MOMA-II, FA-11, and 2F8 antibody positive staining via IF and negative staining with N418, a mAb that stains murine dendritic cells) [28]. Several confirmatory in vitro studies demonstrated that brain macrophages could be productively infected with TEMV without a significant cytolytic effect [29], and that the DA strain (of the TO subgroup) but not the GDVII strain (of the GDVII subgroup) could productively replicate in the murine macrophage cell line J774-1 [30].

The question still remained as to what genetic elements enabled TMEV TO subgroup strains to infect macrophages and cause a widely dispersed and chronic demylenating disease. Takata., *et al*. [31] addressed this by constructing a series of recombinant chimeric viruses with a DA strain combined with GDVII substitutions for various genes. They compared the ability of the parental DA strain and the chimeras to infect the murine macrophage cell line, J774-1. The ability to infect macrophages was dependent on the DA coding sequence for the L/L* gene region [31]. In the DA strain of TMEV, nucleotide 1080 is a U, resulting in the creation of an AUG codon at the 5' end of the L* gene, whereas the GDVII strain has an ACG codon at this location. Mutation of the AUG to ACG in the DA strain (a change that does not alter the amino acid sequence of the main ORF) rendered the virus unable to replicate in the J774-1 murine macrophages. In contrast, both the parental DA virus (AUG) and the mutant DA virus (ACG) were able to replicate with equal efficiency in BHK-2 cells. Thus, expression of the L* protein was required for infection of macrophages, and macrophage tropism was essential for the disseminated, persistent phenotype of DA strains [31]. Furthermore, macrophages secrete IL-12 in response to TMEV infection, which leads to the development of a Th1 inflammatory response. The accumulation of virus-specific CD^{4+} cells is reported to be a key factor leading to the progressive demylenation of the CNS [32]. This emphasizes that macrophages are both critical for viral dissemination and that their infection contributes to immune-mediated viral pathogenesis.

Human Immunodeficiency Virus (HIV-1)

The human immunodeficiency virus type-1 (HIV-1) is an enveloped, positive sense, ssRNA retrovirus in the lentiviral genus. Acute HIV-1 can present with a mononucleosis-like syndrome; however symptoms often go unnoticed. Chronic, untreated HIV-1 infection leads to a progressive decline in CD^{4+} T cell counts, progressive failure of the immune system, and infection with opportunistic agents. The neuropathology of HIV-1-associated dementia (HAD) is especially relevant to this review. HAD is a constellation of cognitive and motor deficits, including short-term memory impairment, reduced concentration ability, leg weakness, and behavioral symptoms [33]. The use of combination antiretroviral (cART) therapy has significantly reduced the incidence of HAD; however, the increased longevity of cART-treated patients and has led to an increase in the prevalence of HAD. Currently, the prevalence of HAD is estimated to be about 10% of HIV-1-infected individuals. Another ~30% of HIV-1-infected patients exhibit symptoms of the more subtle form of HAD, minor cognitive motor disorder (MCMD) [33]. The poor penetration of antiretroviral drugs into the central nervous system (CNS) may contribute to neuro-AIDS and allow the brain to act as a significant HIV-1 reservoir.

HIV-1 variants in brain are genetically distinct from the HIV-1 outside the CNS. Within an individual, phylogenetic analysis shows that HIV-1 sequences in the brain compartment are more closely related to each other than to sequences from peripheral sites [34-37]. The clustering of brain HIV-1 sequences is especially evident in the V3 loop of the HIV-1 envelope protein gp120 [34-37]. This region

contains the primary determinants of HIV-1 cellular tropism [38], suggesting that a switch in cellular tropism may be responsible for the ability of HIV-1 to propagate in brain and cause HAD. In the CNS, expression of the cell surface molecule and HIV-1 receptor, CD4, has only been documented on peri vascular macrophages and microglial cells. These are the only cells in the CNS shown to support productive HIV-1 infection [33]. After the [iii] autopsy, Peters., *et al*. [39] extracted HIV-1 from both the brain and lymph nodes of patients with neurological complications and compared the envelope protein genes. The brain-derived envelope proteins exhibited macrophage tropism and increased ability to infect cells that express low levels of CD4 [39].

To develop a molecular explanation for how HAD could be associated with HIV-1 sequences that confer macrophage tropism and the ability to infect cells with low CD4 levels, Dunfee., *et al*. [40] Compared HIV-1 envelope sequences from brain and lymphoid organs. They focused on 26 residues that were demonstrated to directly contact the CD4 receptor in the HXB2 gp120 crystal structure (1GM9). This analysis revealed that a T283N mutation in the C2 region of gp120 was present at a higher frequency in HIV-1 from brain (24/28) than in HIV-1 from lymphoid organs (4/8). Introducing the N mutation at position 283 enhanced macrophage and microglial tropism and decreased dependence on surface CD4 expression for viral entry. Through examination of gp120-CD4 crystal structures and a confirmatory kinetic experiment, they determined that amino acid 283 interacts directly with Q40 on CD4 and showed that 283N increased affinity for the CD4 receptor 2.5 fold. Introducing a Q40A mutation abolished this increase in affinity. The study concluded with an analysis of 481 brain envelope sequences from 66 AIDS patients. This investigation showed that 283N was present in 41% of HAD patients, but in only 8% of non-HAD patients ($p < 0.001$) [40].

Ashley Haase is credited with being the first to hypothesize that lentiviruses use a "Trojan horse" strategy to enter the CNS, infecting cells circulating in the peripheral blood and using them for portage cross the BBB [41]. Macrophages and microglial cells are situated at the perivascular region of the brain, poised to come into direct contact with virus-infected cells crossing the BBB [33]. While the exact mechanism of neuronal damage and dementia remains unknown, the "bystander effect" hypothesis suggests that the production of pro-inflammatory cytokines and chemokines, secondary to the infection of CNS macrophages, may lead to immune-mediated damage to the CNS [33]. As further evidence that direct infection of macrophages leads to an expansion of the viral niche, Swingler., *et al*. [42] Demonstrated that HIV-1-infected macrophages that express Nef intersect with the macrophage CD40 ligand pathway, causing the release of a paracrine factor that renders resting T cells permissive to HIV-1 infection [42]. Thus, macrophages are not only critical for the dissemination of HIV-1 to the brain, but also are likely to promote the infection of T cells.

Human Cytomegalovirus (HCMV)

Human cytomegalovirus (HCMV) is an enveloped, dsDNA, β herpes virus. Expression of the many HCMV genes occur in three phases. Immediate early gene expression, whose products regulate viral DNA replication, is followed by early and then late gene expression, which results in the expression of viral structural proteins [43]. HCMV is ubiquitous. It establishes life-long persistence after primary infection and is associated with a wide range of clinical presentations, with more severe disease occurring in patients with immunological impairments. HCMV causes retinitis in patients with HIV-1/AIDS and promotes graft rejection in both solid and hematopoietic organ transplant recipients. It is the leading cause of CNS damage and deafness in neonates [44]. In immunocompetent hosts, HCMV is associated with mononucleosis and is a proposed risk factor for the development of atherosclerosis, secondary to virus-mediated endothelial injury [44]. These examples establish that HCMV can access and infect several different organ systems. To do this, it needs an effective mechanism for dissemination.

In the previous four examples, distinct molecular signatures were associated with a shift in tropism, leading to the infection of macrophages and the propagation of virus throughout the body and/or to an increase in the ability to establish persistent infection. In contrast, strong evidence indicates that all strains of HCMV infect monocytes, enabling hematogenous spread and seeding of multiple organs [44]. In the blood of patients with acute HCMV infection, monocytes contain viral DNA and are the primary cell type that is infected [45]. HCMV DNA and/or RNA are present in peripheral blood cells but not the plasma of healthy HCMV-positive donors [43]; HCMV transmission can be reduced by depleting blood of leukocytes [43], demonstrating that white blood cells remain infected even in people lacking disease symptoms. Detection of HCMV-positive blood leukocytes is the most accurate predictor for the development

of CMV disease in CMV-negative transplant recipients receiving allografts from HCMV-positive donors [46]. Inoculation of rat CMV-infected monocytes into naive animals results in systemic infection and detectable CMV in salivary glands within four weeks [47]. Clearly, there is an association between HCMV DNA (as well as immediate early gene products) in monocytes and the spread of HCMV to multiple organ systems. Interestingly, however, monocytes are not productively infected with HCMV. Rather, monocytes express only immediate early gene products and they have not been observed to produce any infectious virus particles [48]. Monocytes survive only briefly in the blood, whereas the replication cycle of HCMV takes days to weeks to complete *in vivo* [49]. This disparity may contribute to the failure of circulating monocytes to support productive HCMV infection.

If monocytes are incapable of producing HCMV, can macrophages facilitate the establishment of productive infection and spread to target organs? Both alveolar macrophages and monocyte-derived macrophages express immediate early and late gene products and they produce infectious viral particles that can be quantified in plaque assays [13,50]. While the monocyte cell line THP1 is blocked at the phase of immediate early gene expression, this block can be overcome by driving these cells to differentiate into macrophages using phorbol ester [51]. In their differentiated state, the cells produce immediate early and late gene products as well as infectious viral particles, which can be detected by electron microscopy and infectious center assays. As it turns out, HCMV is fully armed to induce the differentiation of monocytes into macrophages. Using a transwell assay to model trans endothelial migration, Smith., et al. [49] demonstrated that monocytes infected with HCMV or monocytes exposed to UV-inactivated HCMV displayed morphological characteristics of differentiated macrophages. These cells exhibited increased migration, increased the expression of adhesion and motility molecules such as β1 integrin, occluding, and ZO-1, and positive F-actin staining. The newly differentiated macrophages supported productive HCMV infection [49]. This demonstrates that HCMV infection can induce monocytes to differentiate into macrophages, enhancing spread and penetration into extra vascular tissues.

Discussion

The ability to infect and replicate in macrophages is a critical mechanism that viruses exploit in order to disseminate. Given the demonstrated advantages, what accounts for the failure of more viruses to evolve macrophage-tropic strains? Like everything else, the infection of macrophages may come at a price. Macrophage tropism might carry the risk that the infected cells would become activated and turn into effective antigen presenting cells, enhancing adaptive immune responses and ultimately promoting viral clearance. This process might be relatively less likely in the CNS (with its dearth of lymphocytes), perhaps accounting for the tendency for macrophage tropic strains to colonize the CNS compartment. In the periphery, macrophage tropic strains might be forced to down-modulate their level of replication and infectious viral particle production in order to diminish the threat of a robust anti-viral immune response. This accommodation might reduce their ability to achieve the high titers needed for efficient transmission to a new host. The enhanced ability of certain macrophage tropic strains to establish persistent infection may help to mitigate the loss of high-level replication. Persistence may be an essential component of fitness among viruses that are transmitted relatively inefficiently, e.g., though intimate contact. When opportunities for transmission are few and far between, staying power may be required for a virus to persist long enough to move into a new host. Ironically, macrophages, the stalwart garbage collectors who comprise the body's cleanup crew, are all too often co-opted by viruses. Understanding the details of how viruses exploit macrophages may point the way to strategies to contain these infectious agents, opening the door to the control of deadly, persistent viruses that evade the immune system and that have thus far thwarted many vaccine development efforts.

Summary and Conclusion

In summary, five viruses were discussed: lymphocytic choriomeningitis virus (LCMV), feline corona viruses (FCoV), Theiler's murine encephalomyelitis virus (TMEV), human immunodeficiency virus type-1 (HIV-1), and human cytomegalovirus (HCMV). While these viruses are heterogeneous, they share a particular feature: small changes that allow for infection of macrophages promote viral dissemination. LCMV Clone 13 infects macrophages and leads to a systemic, multi-organ infection. FIPV infection of macrophages leads to systemic inflammation, which is often fatal. In FCoV, macrophage tropism was essential for the disseminated persistence of DA strains. In HIV-1, a switch in cellular tropism may be responsible for the ability of HIV-1 to propagate in brain and cause HAD. In HCMV,

infection of monocytes drives differentiation into macrophages, enhancing spread and penetration into extra vascular tissues. This may come at a cost to viral fitness, highlighting the role of cellular tropism in the paradoxical balance between viral fitness and virulence.

Footnotes and Supplemental Information

[(i)]This is defined in the Gale Encyclopedia of Medicine as "cerebral meningitis in which there is marked cellular infiltration of the meninges, often with a lymphocytic infiltration of the choroid plexuses" {Gale Encyclopedia of Medicine}.

[(ii)]LCMV-specific CTL responses in adult mice infected with the Armstrong strain are so characteristically vigorous that the LCMV model was fundamental in determining how cytotoxic T lymphocytes (CTLs) interact with MHC class I molecules in order to lyse virally-infected cells and furthermore, to characterize CTL dynamics in the clearance of viral infection [10-15]. Newborn mice infected with LCMV Armstrong strain produce comparable humoral immune responses when compared to adult mice infected with LCMV Armstrong strain [5]. Additionally, spleen cells from carrier mice actively suppress LCMV-specific CLT responses of spleen cells from normal adult mice, but have no effect on the LCMV-specific CLT responses [5].

[(iii)]Astrocytes have also been show to be susceptible to HIV-1-infection, and in vitro infection of oliodentrocytes and brain microvascular endothelial cells has also been demonstrated [33]. Of note, neurons are not susceptible to HIV-1 infection and do not express CD4 [33].

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