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The role of dendritic cells in viral persistence

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Abstract

Viruses must modulate or suppress their host's immune system in order to persist. In this review, we discuss the means in which the lymphocytic choriomeningitis virus targets and infects an essential component of the immune system, the dendritic cell, essential to bridging the innate and adaptive immune response. Infection of these cells results in pleiotropic effects that serve to deregulate the host immune response.

Introduction

Nearly 80 years ago, Charles Armstrong isolated the first arenavirus from serial passages of rhesus macaques infected with human brain tissue from fatal cases of St. Louis encephalitis. Clinical observations of lymphocytic infiltrate into the choroid plexus of infected animals led him to name his newly discovered virus lymphocytic choriomeningitis (LCMV) [1]. Fifty years later, our laboratory observed that distinct viral isolates are generated from persistently infected mice in an organ-specific manner. One such viral isolate, Clone 13 (CL-13), was isolated from splenic homogenates of mice infected at birth with Charles Armstrong's original virus strain [2], called Armstrong 53b (ARM 53b), cloned in our laboratory [3]. ARM 53b in adult immunocompetent mice is cleared by a robust cytotoxic T lymphocyte response (CTLs) within two weeks of infection (CTL+P- virus). In contrast, CL-13 induces high titers in the blood, spleen, liver, and brain for over two months and persists indefinitely in the kidneys due to a generalized immunosuppression characterized by T cell dysfunction and loss of virus-specific T cells (CTL-P+ virus) [4–5].

Since the discovery of LCMV, several significant outbreaks of hemorrhagic fevers caused by related New and Old World arenaviruses have occurred. For example, Lassa virus (LASV), an Old World arenavirus of which LCMV is the prototype, causes 300,000 – 500,000 infections and thousands of deaths annually in West Africa [6–7]. The last four decades of research using the ARM 53b/CL-13 system as a model has not only led to a deep understanding of arenavirus infections, but to pivotal discoveries relevant to a variety of persistent infections. LCMV persistence within its natural host, *Mus musculus*, is accomplished through several immunosuppressive mechanisms discussed in this review, including infection and functional dysregulation of dendritic cells (DCs) [8–11] and upregulation of immunosuppressive cytokines and receptors [12–17], ultimately leading to

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functional exhaustion of T cells that are no longer able to respond to and eliminate LCMV-infected cells [18].

Genetic Determinants of Persistent Infection

Though the pathogenesis resulting from ARM 53b and CL-13 infections in adult mice differ substantially, the two isolates only vary by six nucleotides [19–20] causing three amino acid changes, two in the viral glycoprotein [18–22] and one in the RNA-dependant RNA polymerase (RdRp) known as the L protein [21, 23–24]. Importantly, these genetic differences do not affect viral fitness, per se, as both strains grow well in culture, are able to establish a persistent, lifelong infection in immunodeficient mice, and can be transmitted vertically to immunocompetent mice to establish persistently infected mouse carrier colonies [25]. Therefore, the mutations present in CL-13 serve to establish a persistent infection despite the pressure exerted by a fully functional and intact immune response.

While a glutamine encoded by CL-13 at position 1079 (lysine in ARM 53b) is essential for viral persistence [23–24], little is known about its function. The L protein is a high molecular weight protein (~200 kDa) that has been classified into 4 domains. The first domain has recently been found to be responsible for transcription of viral genes and has cap-snatching endonuclease activity [26–27] while the third domain contains the RdRp activity responsible for replication of the viral genome [28–29]. The 1079Q mutation lies amino-terminal to the RdRp domain. In the CL-13 system, the 1079Q mutation has been associated with higher viral titers in macrophages and DCs in vitro ([23] and BM Sullivan, B Nayak and MBA Oldstone, unpublished) and higher genomic copies [20] and infectious virus [30] in DCs in vivo. These phenotypes have not been observed in any other cell types tested thus far suggesting there is no inherent defect in the RdRp activity of the L protein encoded by ARM 53b. Importantly, the 1079Q mutation is not conserved in other CTL-P+ viruses which carry a glutamine, lysine, or asparagine at this position [31]. The 1079Q-mediated increase in viral replication seen in macrophages and DCs has not yet been tested in other CTL-P+ or CTL+P- viruses and thus no conclusion can yet be made as to whether this phenotype is a contributing factor to LCMV persistence. The difficulty in expressing and purifying the L protein has prevented the comparison of L protein function across virus isolates.

The viral glycoprotein is synthesized as a single amino acid chain that is subsequently cleaved by the host protease, site-1 protease (S1P), into GP1, GP2 and a signal peptide which form a complex at the surface of the virion [32–33]. GP1 has been shown to bind the cognate host receptor, α -dystroglycan (α DG), a specificity shared by all Old World arenaviruses and Clade C New World arenaviruses [34–36]. Recently, two independent reports confirmed that a leucine at position 260 in the viral spike glycoprotein, GP1, encoded by CL-13 is essential for long-term persistence while either an asparagine (encoded by ARM 53b) or an aspartic acid (encoded by CL-13) at position 176 of GP1 does not contribute to the persistent phenotype [19–20].

High-Affinity Interaction with Host Receptor Determines Cellular Tropism and Pathogenesis

Essential to LCMV pathogenesis is the ability of GP1 to bind with high affinity to host cells expressing a form of α DG that is post-translationally modified by the host glycosyltransferase, LARGE. The nascent polypeptide, dystroglycan, is cleaved into two non-covalently associated proteins: α DG, which serves as a docking site for extracellular matrix (ECM) proteins, and β DG, which contains a single transmembrane domain and anchors α DG to an intracellular complex linked to the actin cytoskeleton via the protein

dystrophin [37]. Binding of GP1 and the ECM protein laminin to α DG requires extensive glycosylation of the α DG mucin-like domain in the form O-mannose, a rare form of glycosylation in mammalian cells. The enzyme POMGnT1, which adds N-acetylglucosamine to the O-mannosylated protein and the glycosyltransferase [38], LARGE, have been shown to be essential to this process [39]. Nevertheless, overexpression of LARGE can bypass defects in the biosynthesis of O-linked mannose precursors, leading to expression of an α DG that binds LCMV as well as laminin, suggesting that the modifications made by LARGE are critical to the synthesis of functionally glycosylated α DG [36]. While laminin and GP1 bind overlapping sites on the α DG mucin-like domain, the molecular interactions are likely distinct as the former relies on the presence of divalent cations while the latter does not [9]. The nature of this binding is essential to the ability of CL-13 to persist as while both ARM 53b and CL-13 can bind α DG, CL-13 binding occurs at 2.5 logs higher affinity than ARM 53b [10, 31] and can therefore compete with and displace laminin, leading to receptor binding and subsequent fusion of the virus with the host membrane [9, 40] (Fig 1).

High affinity to α DG is strictly conserved among CTL-P+ LCMV isolates and is also a property of LASV. Over 50 viral isolates encompassing both CTL-P+ and CTL+P- phenotypes have been sequenced and many have been tested for the ability to bind α DG. In all cases, CTL-P+ viruses like CL-13 have been shown to have a high affinity to α DG as well as encode a small aliphatic amino acid (leucine>>isoleucine>valine) at position 260 of GP1. Conversely, CTL+P- viruses like ARM 53b have a weak affinity to α DG and encode a phenylalanine at that position [19, 31].

Highlighting the importance of α DG, a recent genome-wide association study examining specific single-nucleotide polymorphisms in populations living in areas of West Africa endemic to LASV has found high linkage scores to two essential components involved in α DG biosynthesis and expression: Dystrophin, the loss of which abrogates α DG surface expression, and LARGE, which had the highest linkage score found in this study [41–42]. We are currently studying how these genetic changes alter dystroglycan expression and cellular infection.

The high affinity for α DG conferred by the 260L mutation allows the virus to infect and deregulate an essential component of the immune response, the DC [8, 11, 31, 43]. These cells are responsible for bridging the innate and adaptive immune response and are critical for inducing the T cell response that ultimately clears LCMV infection. Biochemical characterization of α DG in the spleen revealed that the overwhelming majority of functionally glycosylated α DG among splenocytes is expressed by DCs [31]. Consistent with this observation, CTL-P+ viral infections lead to infection of the majority of splenic DCs and replicate primarily in the white pulp of the spleen where DCs traffic to initiate adaptive immune responses. Conversely, CTL+P- viruses do not infect DCs to high levels and replicate primarily in the red pulp [8, 31]. Importantly, recent data from our laboratory using mice with cell specific knock-out of S1P show that mice with DCs deficient in S1P do not establish a persistent infection while mice with a T cell deficiency in S1P can be persistently infected by CL-13 (DL Popkin and MBA Oldstone, unpublished).

Disruption of DC function as a strategy for persistence

There are a diverse number of strategies by which a virus can escape, hinder, or hide from the immune response. However, from virus' perspective, disrupting or subverting DC functions is one of the most beneficial strategies because DCs are central to both innate and adaptive immunity. Viral alterations of DC function ultimately can result in decreased T cell activation or functionality, thus, giving the virus time and space to replicate. Viruses can

subvert DC function in a variety of mechanisms that, when used in conjunction, can cripple the immune response. Some of these mechanisms include: 1) downregulation of major histocompatibility (MHC) and costimulatory molecules, 2) downregulation of inflammatory cytokines, and 3) production of immunosuppressive cytokines (Fig 2). Many of these mechanisms were first observed using the ARM 53b/CL-13 model of infection which has been essential to studying non-cytolytic mechanisms of persistence.

There are three main types of DCs found within the spleens and lymph nodes of mice: 1) CD11b⁺ CD8α⁻ DCs (myeloid), 2) CD8α⁺ DCs (lymphoid), and 3) CD11b⁻ B220⁺ DCs (plasmacytoid [pDCs]). Viruses can target each of these populations to accomplish immune disruption. In both CD8α⁻ and CD8α⁺ DCs, CL-13 causes decreased cell-surface expression of MHC and T cell costimulatory molecules. Presentation of antigen on MHC in combination with costimulatory molecules is absolutely necessary for T cell activation. Downregulation of MHC causes a reduction of the DC's capacity to stimulate T cells [31, 43]. In CL-13-infected DCs, MHC class II is not sequestered intracellularly but rather expression is abrogated to nearly undetectable levels. Interestingly, this effect on MHC expression can last beyond clearance of the virus from the serum and most organs (>120 days) [43], indicating that there may be a negative effect on DCs that continues beyond the span of infection.

CL-13 also targets the pDC population. Type I interferon is a potent anti-viral cytokine and pDCs are the main producers of type I interferon (IFN) secreting high levels soon after LCMV infection [44–45]. In the CL-13 model, they are one of the first targets of infection and virus replicates to higher levels within this cell population [8, 18–20, 31]. Furthermore, CL-13 can interfere with the ability to secrete type I IFN [46]. Although both ARM 53b and CL-13 are able to abrogate IFN secretion, only CL-13 does so over a much longer length of time. Lack of IFN hampers not only antiviral immunity but results in a defect in responding to secondary challenges by other viruses.

In addition to targeting inflammatory cytokines for downregulation, viruses also upregulate anti-inflammatory cytokines. Normally, the function of negative immune regulators is to prevent immune pathology caused by an excessive immune response. However, during infection, tempering the immune response often prevents complete clearance of the pathogen leading to persistence. Negative immune regulators associated with viral persistence include IL-10, programmed death-1 (PD-1), T cell immunoglobulin mucin-3 (TIM-3), and Lymphocyte-activation protein 3 (LAG-3).

Many viral pathogens exploit the IL-10 pathway, including several relevant human pathogens. IL-10 has been found to be upregulated in human immunodeficiency virus while HCMV goes one step further and encodes a viral form of IL-10 [47]. In addition, high serum IL-10 levels correlate with higher viral loads in chronic Hepatitis B virus (HBV) infected individuals [48]. Th17 cells from these individuals also secrete IL-10 upon stimulation with HBV antigen [49] and blockade of IL-10/IL-10R ex vivo can restore IFN-γ secretion by NK cells that is normally suppressed during active infection [48] though the exact role and contribution of IL-10 to HBV infection remains controversial.

During CL-13 infection, IL-10 is primarily expressed by DCs with a negligible or small percentage expressed by CD4⁺ T cells and B cells. Only minimal expression of IL-10 by DCs has been observed in ARM 53b-infected mice. IL-10 upregulation results in a decrease in virus-specific T cells and a loss of T cell functionality as measured by their ability to produce IFN-γ, IL-2, and TNF-α. In comparison, both antibody blockade of IL-10 and gene-specific deletion of IL-10 results in the maintenance of a robust T cell response leading to clearance of CL-13 infection [12]. Furthermore, during HIV infection, IL-10 production by

DCs targets them for lysis by NK cells [50] showing that IL-10 can also directly impact the DC population. Thus, by subverting the use of one cytokine, it is possible for viruses to impact several cell populations and cause immunosuppression. Studies are ongoing to discover the specific molecular mechanisms by which IL-10 is induced and the immune cells on which IL-10 acts.

PD-1, a member of the CD28 receptor family, acts to negatively regulate T cell signaling by binding its cognate ligands PD-L1 and PD-L2 found on the surface of several cell types [51]. High levels of PD-1 are found on the surface of exhausted CD4⁺ and CD8⁺ cells during persistent LCMV infection and blockade of PD-1 signaling leads to early clearance [13]. The effect of PD-1 is not limited to arenavirus infections as PD-L1 blockade in HBV transgenic mice leads to increased numbers of IFN- γ producing CD8⁺ T cells in the liver [52].

IL-10 and PD-1 appear to function by separate pathways indicating that there is redundancy in regulating the immune system. Viruses have taken advantage of these multiple negative regulatory pathways to induce persistence. Blockade of IL-10 and PD-L1 in mice persistently infected with LCMV results in a substantial increase in functional virus-specific CD4⁺ and CD8⁺ T cells compared to blockade of either negative immune regulator alone. In addition, mice deficient in IL-10 do not show altered PDL1 levels early during CL-13 infection and vice versa [53]. However, crosstalk between IL-10 and PD-1 may occur during HIV infection, as high levels of PD-1 expression on monocytes correlate with high plasma levels of IL-10. Furthermore, inducing PD-1 signaling on monocytes from infected patients using an anti-PD-1 antibody leads to a ten-fold increase in IL-10 secretion while the addition of exogenous IL-10 had no effect on PD-1 expression by these cells. Because PD-1 is not expressed by myeloid DCs or plasmacytoid DCs in these HIV⁺ individuals, negative immune regulatory mechanisms likely differ between cell types [54].

Two other negative immune regulators, TIM-3 and LAG-3, are coexpressed with PD-1 on exhausted T cells. TIM-3 has been shown to be upregulated on T cells during chronic HCV [55–56] and LCMV infection [16]. During LCMV infection, a majority of virus-specific CD8⁺ T cells coexpress Tim-3 and PD-1 and coexpression is associated with increased CD8 T-cell exhaustion compared to expression of either one alone. Although blockade of TIM-3 does not improve viral control, simultaneous blockade of both TIM-3 and PD-1 synergistically improves both CD8⁺ T cell responses and viral control in CL-13-infected mice [16]. Similar to TIM-3, LAG-3 blockade or deletion of LAG-3 alone does not improve T cell responses or viral control [57] while simultaneous blockade of PD-1 and LAG-3 results in synergistic alleviation of T cell exhaustion and viral control [17], suggesting that LAG-3 is dependent upon the PD-1 pathway.

Persistent virus blocks DC homeostasis

During a viral infection that targets DCs, DCs must be replenished. To maintain DC homeostasis, a series of DC progenitors in the bone marrow proliferate and differentiate in a manner dependent upon FMS-like tyrosine kinase ligand (FLt3-L) (reviewed in [58]). Administration of FLt3-L in a naïve mouse results in peripheral expansion of the DC population (~20-fold) [59–61], however, in a CL-13 infected mouse this treatment results in little to negligible expansion (<1.5 fold) [43]. Similarly, in vitro, CL-13 and measles virus infection impedes differentiation of bone marrow cells into DCs [43, 62]. Recent work in our laboratory suggests that the block in differentiation occurs very early in the DC progenitor pathway. The inhibition of DC differentiation is dependent on type I IFN as deletion of IFN α and IFN β or their receptor alleviates the defect in differentiation [43, 62]. This IFN-dependent mechanism in both CL-13 and measles virus functions in a STAT-1

independent, STAT-2 dependent manner whereas normal type I IFN signaling is STAT-1 dependent [62]. Thus, viruses can subvert a pathway that is central to anti-viral signaling to impede the ability of the host to replenish DCs, further hampering activation of T cells.

Though virus infection can prevent DC repopulation by the normal pathway, the immune system responds to the depletion of DCs by maintaining the ability to differentiate between DC subsets. During virus infection, pDCs (CD11c+CD11b-B220+) can transform into myeloid DCs (CD11c+CD11b+B220-) [63]. The ability to convert between these two DC types is important because CD11b+ DCs are better antigen-presenting cells than pDCs. Though viruses have a multitude of mechanisms to target DCs, the immune response has mechanisms to cope.

Conclusions

Although nearly 80 years of research on LCMV has lead to many insights on viral persistence, significant questions remain to be addressed regarding the role of DCs in this process. Recent breakthroughs in understanding the DC progenitor pathway should allow a definitive dissection of the mechanism by which LCMV impairs DC differentiation and new discoveries may arise from closer examination of early and late viral events. The intersection between the acute phase and persistent phase of infection has been heavily examined, however, both very early events in infection and late events during clearance of persistent infection will likely offer new discoveries in mechanisms of persistence and the means by which the immune system is able to fight back.

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Highlights

- Viral persistence is dependent on high affinity interactions between α DG and GP1
- LCMV specifically targets DCs through interactions with α DG
- LCMV disrupts DC function and homeostasis leading to viral persistence

Figure 1. Displacement of the extracellular matrix protein laminin from α -dystroglycan leads to subsequent viral infection

A) The weak affinity of the Armstrong 53b isolate for α -dystroglycan is unable to compete with laminin for binding and thus cannot efficiently infect dendritic cells. B) The strong affinity between LCMV Clone 13 and α -dystroglycan mediated by a leucine at position 260 can compete off laminin from the heavily glycosylated region of the mucin domain causing efficient infection of the target cell.

Figure 2. Mechanisms to disrupt DC function during persistent LCMV infection

Dendritic cells are targeted for infection by CL-13. Consequently, multiple events occur that all ultimately lead to a general immunosuppression that not only allows persistence of the virus but leads to poor responses to secondary infections with other pathogens.