Dendritic Cell Inhibition: Memoirs from Immunosuppressive Viruses

Matthew J. Trifilo,* Bumsuk Hahm,* Elina I. Zuniga, Kurt H. Edelmann, and Michael B. A. Oldstone
Departments of Molecular and Integrative Neurosciences and Infectology, Scripps Research Institute, La Jolla, California

PROLOGUE

In honoring Tom Merigan, I present details of studies from our laboratory on the virus–dendritic cell (DC) interaction that leads to immunosuppression, and I dwell at some length on the initiating mechanism of this interaction, the release of type I interferon (IFN) in DCs. In this prologue, I reminisce about some of my long-time interactions with Tom. Interestingly, it was nearly 30 years ago that Tom Merigan and I (M.B.A.O.) were members of the Virology Study Section of the National Institute of Allergy and Infectious Diseases, and he suggested that we combine our energies and experiences to do an experiment with lymphocytic choriomeningitis virus (LCMV) while studying the generation of type I IFN (Nature 1977;268). The following 5 years led to joint publications on NK cells in humans after IFN therapy (Nature 1979;282) and the actions of IFN on human B cells during measles virus infection and in autoimmune diseases, particularly multiple sclerosis (J Infect 1981;127; 1982;129).

How fitting, then, to honor Tom and his service to the biomedical community and, 30 years after our starting point, to detail current observations from my laboratory on how viruses (LCMV and measles virus) regulate IFN to evade immune recognition. Finally, but not described here, is the fact that the first clinical trials to test type I IFN as a treatment for multiple sclerosis also stemmed from collaboration between my laboratory and those of Tom and of Ken Johnson, who was then at the University of California, San Francisco (Neurology 1984;34). These initial studies led to further work on the administration of type I IFN that evolved into a common treatment for multiple sclerosis.

INTRODUCTION

Altered or reduced immune responses are associated with many life-threatening diseases. Numerous cancers, as well as bacterial and viral infections, can take advantage of weakened adaptive immune responses to promote uncontrolled cellular growth or pathogen replication. Although some forms of immunosuppression are associated with age or genetic defects, evidence is emerging that infections also dramatically reduce functioning of the immune system. For example, HIV-1 infection severely depletes CD4+ T cell numbers and the related activity, a result eventually causing the overall impairment of adaptive immune responses termed AIDS [1]. With >40 million persons infected worldwide, HIV-induced AIDS is responsible for >3 million deaths per year, primarily as a result of opportunistic secondary infections. Another dramatic example of virus-induced immunosuppression comes from human infection with measles virus, which, despite the availability of an effective vaccine, currently infects >30 million persons worldwide, leading to ~1 million deaths annually, usually as a consequence of secondary infections [2, 3]. These examples illustrate the importance of the adaptive immune response and indicate that suppression of this system severely reduces the host’s ability to ward off infection.
The initiation of adaptive immune responses to viruses and bacterial infections relies on the activation of T and B cells by “professional” antigen-presenting cells. To date, DCs have been considered the key element in this process and act as “generals” of the host immune response, eloquently linking innate and adaptive immunity [4]. DCs reside within peripheral tissues and also at tissue interfaces with the environment, where they await encounter with pathogens. On capture of an antigen, DCs migrate to lymphoid organs, such as the spleen and lymph nodes, where presentation of the antigen and activation of naïve T cells occurs [5]. Because DCs are the critical component in the activation of host immune responses and subsequent clearance of pathogens, these cells are ideal targets for inhibition by pathogens that seek to increase their chances of survival within the host [6]. Indeed, numerous viruses and bacteria have devised clever strategies either to evade detection by DCs or to inhibit and/or destroy these cells, resulting in global immunosuppression.

Numerous stages during the process leading to proper immune function of DCs have been targeted for inhibition by viruses and bacteria, including antigen detection, DC maturation, DC migration, antigen presentation to lymphocytes by DCs, and effector cytokine release [7]. For example, Toll-like receptors, acting as DC detector systems for viral and bacterial byproducts, have recently been shown to be targets for inhibition by viral infection [8, 9]. Specifically, vaccinia virus was found to block signaling of Toll-like receptors, resulting in the complete inhibition of DC maturation and immunosuppression of the host [10]. Another effective immune evasion strategy is the impairment of antigen presentation by DCs, which aborts T cell stimulation and either prevents activation of or nonproductively activates antigen-specific T cells. The ability to disrupt antigen presentation has evolved in many virus families, including adenoviruses, HIV, and herpesviruses [7, 11]. The fact that broad ranges of viruses with varying genotypes, methods of cell entry, and life cycles have independently adapted mechanisms to disrupt antigen presentation indicates the effectiveness and importance of this strategy for survival of the virus within a host.

Maturation of DCs is a pivotal process in the functioning of antigen-presenting cells and consists of the up-regulation of costimulatory molecules and expression of proinflammatory cytokines. Many viruses, including Ebola virus, Lassa fever virus, herpes simplex virus type 1, and HIV, have adapted strategies to inhibit expression of costimulatory molecules. Further, a number of viruses (hepatitis C virus, HIV, measles virus, and dengue virus) have also adapted mechanisms for inhibiting the secretion of cytokines, such as interleukin (IL)–12 [11–14]. Inhibiting IL-12 production can be advantageous for pathogens, because lowered levels of IL-12 contribute to skewing the T cell response toward Th2 instead of the more effective antiviral Th1 response. The result is blunting of effective CD8+ T cell responses required to combat and clear the pathogen from its host. In addition, the early production of IL-12 by the innate immune system releases a cytokine cascade that includes IFN-γ, and subsequent early inhibition of IFN-γ might also play a role in subsequent viral replication and pathogenesis.

In our laboratory, we have used both measles virus and LCMV to further investigate how viruses manipulate the DC compartment to evade and suppress the host immune response. Measles virus is a highly infectious and pathogenic human virus that does not infect rodents. However, we have adapted transgenic techniques in mice to selectively express the measles virus receptors, either CD46 [15–19] or signaling lymphocyte activation molecule (SLAM) [20], on the surfaces of murine DCs or T cells [21, 22], thereby allowing study of the molecular mechanisms behind measles virus–induced immunosuppression. For LCMV infection, mice readily provide a convenient and powerful model system with which to investigate the mechanisms of virus-induced immunosuppression within the natural host. Here, we describe DC-virus interactions and the consequences in murine models of LCMV and measles virus infection.

**LCMV INFECTION AND DC INHIBITION WITHIN THE NATURAL HOST**

LCMV is a member of the family Arenaviridae, which includes important human pathogens such as Lassa fever, Machupo, Sabia, and Junin viruses. Infection of mice with numerous strains of LCMV, including the prototypic ARM53b (ARM), results in a classical adaptive immune response highlighted by the proliferation and activation of highly effective CD4+ and CD8+ T cells. Of importance, both populations of activated T cells acquire effector functions, including production of antiviral cytokines such as IFN-γ (CD4+ and CD8+ T cells), as well as direct cytolytic activity (CD8+ T cells) that participate in the clearance of virus from the host within 7–10 days after infection [23–25]. Studies of mice persistently infected with ARM since birth identified the emergence of virus variants that present an immunosuppressive phenotype [26]. Of the >50 variants isolated and studied, LCMV clone 13 (CL13) is the model LCMV variant that, in contrast to the parental virus (ARM), fails to clear from immunocompetent mice within 7–10 days but remains a persistent infection that lasts up to 100 days after infection. Surprisingly, virus-specific activated CD4+ and CD8+ T cells are initially generated after CL13 infection to a level comparable to that in wild-type mice infected with ARM. However, a complete collapse of both CD4+ and CD8+ T cell function ensues within 5–7 days after infection [26–29]. Moreover, the immune suppression that follows infection with CL13 is not restricted to LCMV, as is evidenced by the impaired primary cytotoxic T lymphocyte response after exposure to other RNA and DNA viruses, as well as the inability of mice to generate...
antibodies when immunized with soluble and particulate for-

Initial approaches to determining the differences between

CL13 infection results in a pronounced reduction in numbers
of DCs within the bone marrow and spleen by 7 days after
infection, which correlates with a reduction in numbers of T
cells. Moreover, DCs remaining within the spleen are unable
to stimulate allogeneic T cell responses, as observed by mixed
lymophocyte reaction assays, whereas DCs from ARM-infected
mice and uninfected mice readily stimulate T cell responses
[28, 29, 37]. These results indicate that CL13 has evolved mul-
tiple strategies for suppressing and altering DC function,
including inhibition of accumulation of DCs within the spleen
and bone marrow, a process now shown to be supported by
both the elimination of mature DCs and the blockade of DC
development, thereby reducing the host’s ability to stimulate
adaptive immune responses [38]. Immunopathological elimi-
ation of DCs and inhibition of DC function after CL13 in-
fection has been reviewed recently [37, 39]. Here, we focus only
on the CL13-mediated blockade of DC development.

DCs are derived from multipotent, undifferentiated pro-
genitors in bone marrow, which proliferate to generate commit-
ted DC precursors. Under appropriate stimulatory conditions,
these intermediate precursor cells differentiate into immature
DCs that then undergo terminal maturation after their en-
counter with pathogens [40]. As mentioned previously, CL13,
but not ARM, reduces numbers of DCs within the host. Because
DC populations are very small fractions within cells of the bone

Figure 1. Inhibition of dendritic cell (DC) development by lymphocytic choriomeningitis virus (LCMV) or clone 13 (CL13) via signal transducer and
activator of transcription (STAT) 2 signaling in vivo. Splenic DCs were isolated from CL13-infected, parent strain (ARM)–infected, or uninfected wild-
type interferon-α/β receptor–deficient (IFN-α/βR−/−), STAT1−/−, STAT2−/−, or STAT6−/− mice after injections with either Flt3 ligand (Flt3-L) or PBS
for 10 days. Representative dot blots indicate the expansion of CD11c+CD8α+ and CD11c+CD8α− DCs within the spleen. Percentages within boxes
indicate the frequency of CD11c+ cells.

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Figure 2. Effect of measles virus on the development of dendritic cells (DCs) expressing human signaling lymphocyte activation molecule (hSLAM) from transgenic mice via type I interferon (IFN)–mediated signal transducer and activator of transcription (STAT) 2 signaling. A, Expression of hSLAM protein on CD4+ T cells, CD8+ T cells, or CD11c+ DCs from spleens of transgenic mice, analyzed by cell cytometric analysis (dark bars). These mice were infected with measles virus at an MOI of 0.5, and measles virus (MV) infectivity was examined by cell cytometric analysis 2 days after infection (light bars). B, Bone marrow cells from transgenic mice and transgenic mice crossed with interferon-α/β receptor–knockout mice that are deficient in a receptor for type I IFNs (Tg/IFN-α/βR), cultured for 2 days in the presence of granulocyte-macrophage colony-stimulating factor and mock-infected (shaded histogram) or measles virus–infected (unshaded histogram) at an MOI of 0.3. Five days after infection, generation of CD11c+ DCs was assessed. C, Transwell experiment, performed with 0.4-μm cell culture inserts containing mock-infected (Tg) or measles virus–infected transgenic bone marrow cells (Tg + MV) in the upper well. Bone marrow cells from STAT1−/−, STAT2−−, or STAT6−− mice were cultured in the bottom well, and their differentiation into CD11c+ major histocompatibility complex class II (MHC-II) DCs was assessed by flow cytometry.

marrow and spleen, we stimulated DC development with Flt3 ligand to study the effects of LCMV infection on DCs. Flt3 ligand is known to induce the expansion of undifferentiated progenitors into DCs within the spleen and bone marrow (~20-fold increase) and to trigger DC maturation in both mice and humans. Similarly, mice infected with ARM after or during treatment with Flt3 ligand also display a dramatic increase in numbers of DCs and DC precursors [38, 41]. In contrast, CL13-infected mice are refractory to the stimulatory effects of Flt3 ligand. Indeed, the observed inhibition of DC development within CL13-infected mice directly correlated with infection of ~20% of plasmacytoid and myeloid DCs within the bone marrow by 15 days after infection (authors’ unpublished data). Moreover, this inhibition was not specific to treatment with Flt3 ligand, because suppression was similar in bone marrow cells cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF), another cytokine important in the development of DCs [38]. Interestingly, addition of CL13 at later time points during culture of bone marrow with GM-CSF failed to disrupt development of DCs. Moreover, within the bone marrow of CL13-infected mice, we observed a marked reduction of Flt3+ lineage-negative DC progenitors, a progenitor cell type that exists early in the life cycle of DC development [41]. Taken together, the results indicate that the ability of CL13 to suppress the number of DCs available for antigen presentation within the spleen is likely dependent on the virus’s ability to directly infiltrate the bone marrow, where it reduces the number of early progenitor cells available for DC differentiation.

Type I IFNs, including IFN-α and IFN-β, are critical for both innate and adaptive immunity and play a major role in protection from viral infection, especially within the first 24 h after infection [42]. In contrast to mice infected with ARM, whose IFN levels begin to decrease within 3–5 days after infection, mice whose DCs support chronic infection by CL13 manifest a significant, sustained production of IFN-α/β from both immature and mature DCs within the spleen and bone marrow for ≥50 days after infection [41]. Although the positive impact of type I IFN in DC maturation and cross-presentation has been well documented [43], experiments using IFN-α/β receptor–deficient (IFN-α/βR−−) mice infected with CL13 surprisingly indicated that the impairment of DC development is dependent on these cytokines [38]. Indeed, IFN-α/βR−− mice
Viruses, including lymphocytic choriomeningitis virus clone 13 and measles virus, have evolved strategies to evade host immune responses by infecting DCs within the bone marrow, resulting in increased local production of type I interferon (IFN). Signaling through signal transducer and activator of transcription (STAT) 2, but not STAT1, activates IFN-stimulated response element (ISRE)–dependent gene transcription. Virus-induced type I IFN then inhibits DC development from precursor cells (PC) within bone marrow. The significant reduction in DC frequency and number affects T cell development and hinders the adaptive immune responses to viral infection. IRF-2, IFN-regulating factor–2; IFN-α/βR, IFN-α/β receptor; JAK, Janus kinase; P, phosphorylation; TYK, tyrosine kinase.

regained sensitivity to Flt3 ligand–mediated DC stimulation regardless of CL13 infection, suggesting that production of IFN-α/β by CL13-infected DCs was critical for suppression of the DC developmental pathway (figure 1). Further evidence that DC suppression was IFN dependent was provided by treatment of mice in vivo with recombinant IFN-β, which resulted in inhibition of DC development after treatment with Flt3 ligand identical to that caused by CL13 infection [41]. Studies using signal transducer and activator of transcription (STAT)–deficient (STAT−/−) mice determined that the signaling cascade responsible for such inhibition was STAT2 dependent and STAT1, STAT4, and STAT6 independent, defining a novel signaling pathway by which IFN can signal directly through STAT2 to mediate the inhibition of DC development (figure 1). Such findings indicate that an immunosuppressive virus can subvert the known antiviral effect of type I IFNs to benefit its own survival. This notion is also supported by recent reports on the role of IFN-regulating factor (IRF)–2 and IFN-α/β that indicate that type I IFN negatively influences the generation of myeloid DCs [44, 45]. Furthermore, a transient reduction in bone marrow cellularity and the concomitant pancytopenia observed after LCMV infection failed to occur in the absence of IFN-α/β receptors [46]. Transient aplasia within the bone marrow and high serum levels of IFN-α/β are relatively common during infection with numerous viruses, including variants of LCMV; however, the impairment of DC development occurs solely during CL13 infection. This selective advantage associated with CL13 infection is likely related to its ability to directly infect DCs and trigger IFN-α/β production to high concentrations within the bone marrow selectively at the site of precursor development [41, 47]. Indeed, the depletion of Flt3−/− undifferentiated DC progenitors as a result of CL13 infection was not observed when the mice lacked IFN-α/β receptors or STAT2 molecules [41]. Consequently, blockade of DC development is an important front of attack that immunosuppressive viruses can use to disable DC defenses and persist in the host.

In addition to unraveling the suppressive pathways induced by IFN, LCMV infection has also provided a useful model for study of DC differentiation [48]. Specifically, we found that both ARM and CL13 infection can program bone marrow plasmacytoid DCs to differentiate into myeloid DCs. Although further work is required to fully understand the biological significance of this event in the context of a viral infection, our data point out a previously unrecognized developmental plasticity of bone marrow plasmacytoid DCs and highlight the power of the LCMV model for studying the effects of viruses on DC development and differentiation.
MEASLES VIRUS–INDUCED IMMUNOSUPPRESSION

Because it is so important to understand the mechanisms viruses use to cause human disease, our laboratory also focuses on the human pathogen measles virus. As mentioned above, measles virus infection incites pronounced immune suppression that allows secondary infections to prevail, sometimes fatally [3, 49]. Previous reports have determined that measles virus can preferentially infect human DCs generated in vitro from monocytes and CD34+ progenitor cells isolated from human peripheral blood, a profile reminiscent of that observed in LCMV CL13 infection of mice [50–56]. Infection by measles virus can alter the phenotype of DCs, reduce their functions, and promote their death, all of which have been observed as up-regulation of tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) expression, inhibition of CD40L–dependent differentiation, global suppression of T cell stimulatory capabilities, and increased apoptosis.

The study of measles virus pathology has been complicated by the fact that the virus does not readily infect rodent cells. Furthermore, measles virus can enter cells via at least 2 known cellular receptors—CD46 [16–18] and human (h) SLAM, expressed on DCs [20]—making it difficult to separate the individual roles played by either receptor in pathogenesis and immunosuppression via measles virus. To dissect the behavior of each measles virus receptor and determine how each participates in measles virus–mediated immunosuppression, we created transgenic mice expressing either the CD46 receptor on all nucleated cells [15] or the hSLAM restricted to DCs (hSLAM-Tg) [21]. Here, we focus on the hSLAM-Tg model and the impact of measles virus infection on DCs. Specifically, hSLAM protein expression was placed under the transcriptional control of the murine CD11c promoter, restricting its expression to CD11c+ DCs and no other cells, including CD4+ and CD8+ T cells, within transgenic mice (figure 2A). Construction of this system provided murine bone marrow–derived (in vitro) and splenic (in vivo) DCs with novel susceptibility to measles virus. Interestingly, measles virus infection significantly inhibited the development of DCs from hematopoietic bone marrow stem cells, including a significant reduction in proliferation and expression of costimulatory molecules (B7-1, B7-2, and CD40) when cultured in the presence of either GM-CSF (figure 2B) [41] or Flt3 ligand [41]. Similar to results observed with the CL13 system, measles virus–induced DC suppression was linked to the IFN pathway, because transgenic bone marrow cells deficient in the IFN-α/β receptor were able to divide and mature to levels comparable to those observed in wild-type cells cultured in either GM-CSF or Flt3 ligand. Furthermore, treatment of the culture with recombinant IFN-β mimicked the suppressive effect observed following measles virus infection in a dose-dependent manner, yet again demonstrating the potent impact of type I IFN on the DC developmental pathway. Because these results sharply contrasted with previous reports underscoring the importance of type I IFN in enhancing, not suppressing, DC function and the adaptive immune response [42, 57, 58], we next compared the effects of type I IFN on precursor versus immature DCs. Not only did type I IFN dramatically enhance DC activity, but this effect clearly was dependent on the developmental state of the DC population. Unlike the powerful suppressive activity observed when IFN was added to precursor cells, the addition of recombinant IFN to immature DCs resulted in functional maturation and boosted their ability to stimulate T cell responses. This 2-pronged activity of type I IFN depended on the developmental state at which the cytokine encountered DCs and/or their precursors. These results strongly support the notion that the location and concentration of IFN are critical in the type of response evoked and indicate that viruses with the ability to selectively target precursor DCs, or the cells immediately surrounding them, have a selective advantage over the host by turning a commanding, immune-stimulatory pathway against itself (figure 3).

The downstream molecular mechanisms for measles virus–induced inhibition of DC development were further investigated by the use of knockout mice specifically defective in genes involved in the type I IFN signaling pathway. Type I IFN is known to require activation of both STAT1 and STAT2 to induce transcriptional activation of numerous type I IFN–induced genes [59–61]. In contrast, and similar to the aftermath of CL13 infection, measles virus–induced type I IFNs required the expression of STAT2, but not other STAT molecules (figure 2C), for DC inhibition. This STAT2-specific inhibition of DC generation occurred after direct treatment of precursor DCs with recombinant IFN-β as well as after measles virus infection, suggesting the existence of a precursor DC–specific IFN signaling pathway. Surprisingly, IFN-induced STAT2 activation led to the activation of IFN-stimulated response element–mediated gene transcription even in the absence of STAT1 expression. Considering that STAT1-independent, STAT2-dependent signaling is a unique form of IFN signaling for inhibition of DC development, we envision therapeutic potential in targeting STAT2-selective signaling to increase the efficacy of IFN. This technology holds promise in the development of drugs to conquer diseases caused by immunosuppression or persistence of virus. The molecular mechanism controlling STAT2-specific DC inhibition is currently under investigation.

Opposing the concept of measles virus as an inhibitor of precursor DCs are reports that measles virus infection induced maturation of DCs generated from human blood cells, as determined by expression levels of the costimulatory molecules B7-1, B7-2, and CD40. Similarly, the phenotypic maturation of bone marrow–derived immature DCs obtained from transgenic mice was also induced by measles virus infection in our model (authors’ unpublished data). However, amounts of B7-
1, B7-2, and CD40, as well as major histocompatibility complex class I and II proteins, were significantly reduced on the surface of CD11c+ DCs isolated from spleens of transgenic mice [21], indicating that measles virus suppressed maturation of splenic DCs suggesting that measles virus maintains numerous strategies for altering DCs, depending on their localization and status of differentiation and/or maturation.

Measles virus–dependent DC inhibition results in a global reduction in T cell activity in humans, as demonstrated by diminished antigenic, mitogenic, and allogeneic responses. Similarly, expression of the hSLAM receptor in measles virus–infected DCs from transgenic mice resulted in aborted allogeneic responses, as determined by reduced mixed lymphocyte reactions and decreased expression of T cell activation markers, including CD44, CD25, and CD69. Measles virus–infected DCs also actively decreased expression of T cell activation markers, including CD44, CD25, and CD69. Measles virus–infected DCs also actively inhibited mitogen-stimulated T cell proliferation. Although the precise mechanism is not known at this time, type I IFN, tumor necrosis factor-α, lymphotoxin-α, and lymphotoxin-β secretion from T cells do not appear to directly participate in the observed measles virus–mediated suppression of T cell activation, because T cells selectively lacking any of these molecules were still suppressed by measles virus–infected DCs.

Taken together, the results indicate that measles virus has evolved to redundantly inhibit multiple DC populations by either reducing the total number of DCs available to stimulate T cells or directly inhibiting the functional maturation of immature DCs, whereby the virus can effectively shut down the adaptive immune response, enabling survival within the host. Using a novel murine model to study the interface between DCs and measles virus, we have uncovered a unique strategy that several viruses (including LCMV and measles virus) appear to apply to their advantage to turn a highly effective proinflammatory molecule into a potent DC inhibitor. This strategy, selective targeting of a unique type I IFN/STAT2–specific signaling pathway, appears to attack the precursor DC population of cells within the bone marrow. This pathway is effective only at reducing the numbers of newly produced DCs; therefore, measles virus has also devised several as-yet-undefined methods of inhibiting immature DCs that may already exist within lymphoid tissue and, on infection with virus, likely stimulate sufficient T cell responses. Collectively, suppression of immature and precursor DCs dramatically halts the adaptive immune response, making this novel “one-two punch” on DCs a lethal combination.

CONCLUSIONS

For a virus or any infectious agent to persist, it must use unique strategies to either evade or suppress the host’s immune response. LCMV and measles virus, although structurally and genetically different, share a weapon to promote their survival within a host. Both measles virus and LCMV CL13 specifically inhibit DCs and their precursors for infection and subsequently inhibit the maturation and/or differentiation of these cells, which represents a novel maneuver that allows a virus to avoid host surveillance and, thereby, survive. These models of virus infection provide powerful tools to dissect signaling pathways involved in DC maturation and differentiation as well as suggest a potential use for DC-based immunotherapies. The benefits will be innovations in methods to inhibit harmful immune responses, such as those that cause autoimmune diseases, and to overcome the immunosuppressive effects that accompany many chronic viral infections.

References

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