Chapter 27

Immunosuppressive Mechanisms During Viral Infectious Diseases

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Abstract

For a virus to establish persistence in the host, it has to exploit the host immune system such that the active T-cell responses against the virus are curbed. On the other hand, the goal of the immune system is to clear the virus, following which the immune responses need to be downregulated, by a process known as immunoregulation. There are multiple known immunoregulatory mechanisms that appear to play a role in persistent viral infections. In the recent past, IL-10 and PD-1 have been identified to be playing a significant role in the regulation of antiviral immune responses. The evidence that viruses can escape immunologic attack by taking advantage of the host's immune system is found in LCMV infection of mice and in humans persistently infected with HIV and HCV. The recent observation that the functionally inactive T-cells during chronic viral infections can be made to regain their cytokine secretion and cytolytic abilities is very encouraging. Thus, it would be likely that neutralization negative immune regulation during persistent viral infection would result in the preservation of effector T-cell responses against the virus, thereby resulting in the elimination of the persistent infection.

Key words: Immunoregulation, Persistent infection, IL-10, PD-1, LCMV

1. Virus and Disease

Most viruses that infect humans are usually cleared by the host immune system in a rapid (acute) manner, either (a) by the release of proinflammatory cytokines or (b) by killing the infected cells to limit the replication of the virus [1]. Such acute responses involve a first step innate immune response (macrophages, dendritic cells [DCs], and natural killer [NK] cells) [2], followed by the generation of adaptive immunity (T and B cells). Macrophages and DCs act as phagocytes thus eliminating the infected cells, and NK cells directly kill the infected cells. In adaptive immunity, which kicks

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in 3–5 days after infection, presentation of viral antigen by macrophages and/or DCs or by infected cells that present viral peptides on host MHC molecules results in the activation of T-cells. These T-cells then mount a viral antigen-specific immune response and kill the infected cells either in a cell–cell contact-dependent manner (perforin/granzyme mediated) or through the release of inflammatory cytokines TNF-α or IFN-γ. These cytokines can also act on the virus directly and prevent its replication. Some of the viral antigen-specific T-cells then survive for a very long time creating a memory pool.

Most, if not all, viral infections trigger the production of interferon's (IFNs) by the host immune system. IFNs then primarily limit viral replication to prevent damage to the infected cell. Type-I IFNs are essential part of innate resistance to viral infections as is evident in the case B6 mice infected with MHV-1; absence of type I IFN-mediated signaling in IFN-αβR-KO mice results in progressive loss of body weight, culminating in the death of the mice by day 5 postinfection [3].

Intravenous infection of adult mice with lymphocytic choriomeningitis virus (LCMV)-Armstrong results in an acute infection, which is resolved by virus-specific CD8 and CD4 T cells within 8–10 days [4]. However, unlike in acute viral immune response where antiviral CTLs are present at all times examined; in persistent infections, whether it be the effector phase or memory phase, antiviral CD8 T-cells are rarely found in adult mice or in humans infected early in life [1]. A more detailed discussion of innate and adaptive immune responses in viral infections is beyond the scope of this chapter.

Some virus, for instance HIV and hepatitis B virus (HBV)/hepatitis C virus (HCV) in humans and LCMV in mice, escape killing by the host's immune system, either by avoidance or manipulation and establish persistent infection in the host. To establish a persistent infection, the virus has to overcome several host-dependent factors. One unique strategy that viruses employ is that instead of killing its host cell, the virus causes little to no damage so that it can escape detection by the immune system. The virus then either continues to replicate inside the cell (HIV) or it establishes latency (EBV). Viruses can alter or interfere with the processing of viral peptides by professional antigen presenting cell (APC), a requirement for activation and expansion of the T cells that normally remove infected cells. Additionally, viruses can also inhibit the differentiation of antigen-presenting conventional DCs and can infect effector T and B cells directly [1]. Some viruses also encode miRNAs to regulate their replication or latency or to manipulate or evade host immune responses [5]. During latency, miRNAs keep protein levels of viral genes to a minimum, facilitating evasion of immune surveillance [6]. Finally, viruses also exploit the host's immunoregulatory mechanisms such that

the viral antigen-specific T-cells now become either anergic or are deleted and thus fail to clear the viral infection. This strategy is more detrimental to the host.

Persistent viral infections, as would be expected, are a severe problem to humans, as is evident in the case infection with HIV; persistent infection with HIV leads to the exhaustion of CD4 T-cells thereby rendering the host susceptible to a variety of secondary infections, which ultimately lead to the demise of the host. However, such persistent infections, if are actively sought by the host immune system, due to the continued activation of T-cells can lead to severe immune pathology, wherein the host's immune system destroys the host's own tissue.

There is a copious amount of data that are published, which provides evidence for the deleterious effects of an over-active immune system causing sever immune pathology in persistent viral infections. For instance, overexuberant immune response is implicated in fatal H5N1 influenza [7] and a dysregulated immune response contributes to clinical disease in patients with SARS [8, 9]. Similarly, in mice, excessive T-cell responses contribute to the tissue damage that occurs during the process of virus clearance in infections with LCMV, HSV, RSV [10–12], and MHV-1 [13].

1.1. LCMV as a Model to Study Immune Regulation

In our laboratory, we have extensively used LCMV as a model for establishing persistent infection in mice [14–20]. It is easier to dissect the various immunoregulatory mechanisms involved in viral infections using LCMV due to the availability of two different strains; one that establishes acute infection (LCMV-Armstrong) and is readily cleared from the host and the other that establishes persistent infection (LCMV-clone 13) for a very long time. Armstrong and clone 13 viruses share identical CD4 and CD8 T-cell epitopes but are phenotypically very distinct, replicate in different cell types, and have different clearance rates [21]. Further, since LCMV is not a cytolytic virus, it allows to distinctly examine the effects caused by the immune response-mediated damage to the host tissue [22].

Persistent viral infections ultimately result in immunosuppression in the host where the CTLs lose functionality by either functional inactivation and/or physical deletion [1]. Several different mechanisms, which act either individually or in combination, including clonal exhaustion, secretion of immunosuppressive cytokines such as IL-10 and TGF-β, and overexpression of programmed death 1 (PD-1) on activated T-cells, contribute to the failure of T-cell responses during persistent viral infections. In LCMV-clone 13 model of persistent infection, multiple immunoregulatory mechanisms are activated, including infection/impairment of DCs, and a global inactivation of the virus-specific T-cell response [23–27]. The antiviral CD4 and CD8 T-cells are either physically deleted or persist in an inactivated state and are

nonresponsive toward viral antigens [23–27]. With respect to the significance of such inactivation in human patients, in HIV infection, CD4 T cells that can produce TNF-α, interleukin 2 (IL-2), and IFN-γ are conspicuously absent. Consequently, in HIV+ patients, the presence of functionally active CD4 T-cells that can produce the cytokines TNF-α, IFN-γ, and IL-2 directly correlates with low viremia [28]. Similarly, in mouse models of persistent infection, a significant three- and fourfold decrease in TNF-α and IL-2 producing cells, respectively, was observed in the spleens of clone 13 infected mice by day 100 postinfection [23].

However, unlike it was believed before, it is increasingly apparent that in persistent infections, the effector T-cells are not deleted but exist in a functionally inactive state. Thus, it was interesting to note that despite prolonged periods of inactivation, the remaining CD4 and CD8 T-cells still retain the ability to recover their functional activity [29]. This suggests that, in persistent viral infections, the host's immune system can still be manipulated such that the host can reestablish immunity against the virus. Thus, functional preservation of CD4 and CD8 T-cells during viral persistence is the ultimate mode of achieving strong antiviral immunity and viral clearance.

2. Immune Regulation

2.1. General

As discussed previously, for a virus to establish persistence in the host, it has to exploit the host immune system such that the active T-cell responses against the virus are curbed. On the other hand, the goal of the immune system is to clear the virus, failing which the immune responses need to be downregulated so as to avoid unnecessary damage to the host tissue, by a process known as immunoregulation. The various known immunoregulatory mechanisms comprise (a) activation or induction of Tregs, (b) expression of inhibitory molecules such as programmed cell death-1 (PD-1) on activated T-cells, and (c) production of suppressor cytokines such as TGF-β or IL-10. In the recent past, among the major immunoregulatory molecules identified so far [15, 30–32], IL-10 [15, 32] and PD-1 [30] appear to be playing a significant role in the regulation of antiviral immune responses and are thus being actively investigated. The confounding aspect of all these different factors is that they not only regulate antiviral immune responses but they are also involved in the regulation of autoimmune responses.

The evidence that viruses can escape immunologic attack by taking advantage of the host's immune system is found in LCMV infection of mice [15, 30, 32] and in persistently infected with HIV and HCV [33–37]. Thus, it is important to understand the effectors involved in active immunoregulation, such that we can

define how a virus exploits the host's immunoregulatory mechanism and thereby design drugs that can circumvent this virally induced immunoregulation.

2.2. Immune Regulation by Innate Immunity

This aspect of the immune system has been recently reviewed, for details refer McGill et al. [2]. The innate immune system mainly comprises macrophages, DCs, and NK cells. Macrophages and DCs are primarily involved in antigen presentation to the T-cells, with DCs usually being the more efficient APC. NK cells are primarily involved in the direct killing of virally infected cells as part of the innate immune protection. However, as with adaptive immune system, hyperactive innate immune system could very well lead to tissue/organ damage. A direct evidence for innate immune-mediated pathology is evident in the case of fatal H5N1 influenza [7]. Here, we will focus only on those cell populations that exhibit immune regulatory function.

2.2.1. Macrophages

Although macrophages are not the most efficient of APCs, they are very efficient phagocytes. And thus, it is believed that macrophages may be a predominant cell population contributing to IAV-associated immunopathology [39]. The first evidence for the role of macrophages in immune regulation was evident in alveolar macrophages that exist in a relatively quiescent state during homeostasis but have a regulatory phenotype in the lungs [40]. In addition, Dillon et al. showed that stimulation of macrophages through the TLR2 receptor induces macrophages that promote immunological tolerance through the production of TGF-β1 [41]. And finally, macrophages have been shown to suppress the induction of innate and adaptive immunity [42–44].

2.2.2. Dendritic Cells

DCs are probably the most efficient APCs of the immune system, both in animal models and humans. Therefore, the initiation of effective Ag-specific immunity to pathogens is a hallmark of DC function. However, it was also demonstrated that DCs can induce and maintain self-antigen-specific tolerance in the periphery [45]. Further, DCs can actively induce T-cell anergy [46, 47], T-cell suppression [47, 48], and generation of Tregs [49]. As they did for macrophages, Dillon et al. also showed that stimulation through the TLR2 receptor on DCs induces regulatory DC [41]. Further, TLR2 activation in mice results in IL-10 upregulation and Treg survival [50, 51]. Interestingly, incubation of DCs in the presence of IL-10 generates DC with tolerogenic properties [52]. Thus, DCs, when activated through the TLR2 receptor, appear to acquire regulatory phenotype and indeed are able to suppress the activation antigen-specific T-cells.

In support for the role of DC in immune regulation in viral infections, in in vitro experiments, it was found that the infection

of DCs with high MOI results in truncated CD8 T-cell responses and increased IL-12p40 production [53]. Further, such high MOI infection also results in an increased production of the antiinflammatory cytokines IL-10 and TGF-β [54]. Finally, in IAV infections, rather than inducing T-cell activation, when influenza antigen-bearing immature DCs encounter naïve T cells, they induce tolerance to viral antigens [55, 56]. The immunoregulatory role played by DCs in other viral infections needs to be explored.

Thus, under certain conditions, for instance activation through TLR2, both DCs and macrophages appear to acquire an immunoregulatory function. However, whether macrophages and DCs can exhibit similar activity in a majority of persistent viral infections needs to be determined. Be as it may, it would be interesting to speculate that since viral infections result in TLR2 activation, at least in infections with some viruses (chronic infections), such TLR2 activation induces tolerogenic DCs and/or macrophages, which prevent further activation of antiviral T-cells.

2.3. Immune Regulation by Adaptive Immunity

2.3.1. CD4+ Tregs

One of the most extensively studied cell population in immunology is CD4+CD25+ regulatory T-cells (Tregs), because of their demonstrated importance in the events leading to autoimmunity. Thus, doing a formal detailed review of Tregs takes a very extensive review of literature and is therefore beyond the scope of this review article. But, for those who are interested, for a detailed review of Tregs, refer to Sakaguchi et al. [57].

There exist mainly two types of Tregs, the natural Tregs (nTregs) that originate from the thymus and the induced Tregs (iTregs) that arise in the periphery. Tregs, either natural or induced, are characterized by the expression of the transcription factor Foxp3. Thus, it was found that the ectopic Foxp3 expression in normal T-cells enables these cells to exhibit suppressor function in vitro and in vivo [58]. Although the significance still needs to be determined, it was recently demonstrated that the expression of Foxp3 in Treg cells does not destine the T-cell to be a Treg for life, since a subset of Treg cells appear to downregulate Foxp3 expression (exFoxp3 cells) [59].

nTregs constitute 4–5% of peripheral CD4 T cells and are important in the maintenance of immunological self-tolerance in the periphery [60]. CD25+ T cells appear in the periphery after day 3 and rapidly increase to adult levels within 2 weeks (reviewed in [61, 62]). CD4+CD25+ Tregs are actively involved in the regulation of antiviral immunity in order to limit immune pathology in the host [63–67] but thereby they also inhibit viral clearance [68–70].

Tregs have been implicated to play a role in a number of different viral infections, in both mice and humans, including but not limited to Friends' virus or HSV (in mice) and HCV and HIV (in humans) [12, 68–72]. To further demonstrate the importance of Tregs in viral immunity (or immune regulation), Tregs are

relatively deficient in patients with severe dengue infection when compared to those with mild disease [73].

Tregs exert their suppressive function through a combination of various mechanisms including cell-contact (killing and/or functional modulation of APC and/or responder T cells (reviewed in [74–76])) and soluble factors (secretion of immunosuppressive cytokines, IL-10 and TGF-β [77, 78]). However, it appears that direct cell-contact-mediated suppression is critical for the action of Tregs. Tregs inhibit, via CTLA-4, the upregulation of CD80 and CD86 by immature DC upon antigenic stimulation and also downregulate the expression of CD80/CD86 by mature DC [79, 80], thus indirectly affecting antiviral CD4 and CD8 T-cell responses. Another plausible mechanism by which Tregs mediate their regulatory activity has been proposed; since Tregs cannot produce IL-2 and thus cannot expand in an autocrine fashion [81], but constitutively express high levels of IL-2R (CD25), they likely deprive effector T-cells of IL-2 in the vicinity and thus provide hindrance to the activation/expansion of responder T cells [82].

As such, the take home point is that natural CD4+CD25+ Tregs have only a minimal impact on viral elimination per se but they play an important part in limiting collateral tissue damage usually caused by strong antiviral T-cell responses [12, 66]. Thus, they are one of the most important players in mediating immune regulation, either in antiviral immunity or in autoimmunity.

2.3.2. PD-1/PD-L1

Based on few recent findings [30, 31, 35, 36, 38, 83–86], it is increasingly apparent that the PD-1/PDL-1 axis plays a major role in regulating immune responses during antiviral T-cell responses. PD-1 is an inhibitory receptor of the CD28 family and has two ligands PD-L1 and PD-L2, but most of the immunoregulatory activity of PD-1 is brought upon by its interaction with PD-L1 (reviewed in [87]). Accordingly, PD-L1$^{-/-}$ mice infected with LCMV clone 13 died owing to immunopathologic damage [30].

During chronic infection with LCMV-clone 13, PD-1 is upregulated at both RNA and protein level, in exhausted LCMV-specific CD8 T cells. Further, PD-1 expression continued to increase in exhausted CD8 T cells and the high level of expression was sustained resulting in the failure of these to clear infection [30, 31]. Blocking the activity of PD-1, using an antibody against PD-L1, enhanced virus-specific CD8 T cell proliferation, production of IFN-γ, and their lytic ability [30]. And further, blocking PD-L1 results in enhanced epitope-specific responses to therapeutic vaccination [84].

In our laboratory, using LCMV-induced T1D model, the RIP-GP-LCMV mice, we found that the virus-induced upregulation of PD-L1 in prediabetic mice prevents the expansion of diabetogenic CD8+ T-cells, thus confirming the importance of PD-1 in regulating antiviral CD8 T-cell responses [85].

Interestingly, such PD-L1 upregulation also resulted in synergistic enhancement in the capacity of virally enhanced CD4+CD25+ Tregs to mediate immunoregulation [83].

Finally, in humans infected with HIV who have increased PD-1, an association exists between in vivo T-cell unresponsiveness and restoration of T-cell function in vitro with antibody to PD-L1 [36, 85, 86]. Thus, the PD-1–PD-L1 axis is crucial for sustaining suppression of CD8 T cells during persistent infection.

2.3.3. CTLA-4

Costimulation through the CD28 molecule is essential for the generation of efficient antiviral T-cell responses. Consequently, the prevention of costimulation during the chronic phase of clone 13 infections diminishes antiviral CD8 T cell responses and prevents control of viral replication [88].

CTLA-4, an inhibitory receptor belonging to the B7-CD28 family of costimulatory molecules (reviewed in [89]), is constitutively expressed on murine Foxp3+ Treg, whether thymus derived or induced in the periphery [90–92] and can mediate the suppression of effector T-cell responses. CTLA-4 is also upregulated on activated T cells, and competes with CD28 for binding to the same ligands, B7-1 (CD80) and B7-2 (CD86). However, instead of inducing activation of T-cells, such as CD28, CTLA-4 antagonizes the production of IL-2 and inhibits T-cell activation.

While the importance of CTLA-4-mediated regulation T-cell responses in tumor immunology is well known, its role in persistent viral infections is still debatable. In mice infected with LCMV, CTLA-4 expression or lack thereof by T cells has little or no effect on antiviral immunity, either in the effector or in memory generation during acute viral infection [30]. And, similar to acute infection, CTLA-4 was also not involved in T-cell exhaustion in mice chronically infected with LCMV clone 13 [30].

The strongest argument for the involvement of CTLA-4 in the regulation of antiviral immunity comes from the studies of HIV-infected humans (reviewed in [93]) or SIV-infected monkeys [94]. Arguing for a role of CTLA-4 in antiviral immunity, it was found that CTLA-4 expression was high in HIV-specific CD4 but not CD8 T cells in humans with acute HIV infection but low in people who were able to spontaneously control infection [95]. More specifically, consistent with the finding that CTLA-4 engagement downregulates IL-2 secretion [96], HIV-Gag-specific CD4 T cells that produced IL-2 in addition to IFN-γ had less CTLA-4, while those CD4 T cells that produced only IFN-γ had higher CTLA-4 expression [95]. And finally, it was shown that blockade of CTLA-4 with antibodies resulted in an augmentation of both SIV-specific CD4 and CD8 efffector T-cell responses [94].

Thus, CTLA-4 plays a major role in immunoregulation by preventing costimulation through CD28 receptor leading to inefficient activation of CD8 T-cells, in some, if not all, persistent viral infections. Hence, therapeutic approaches aimed at preventing the action of CTLA-4 during some persistent infections, where CTLA-4 is known to play a role, will be beneficial to a subgroup of patients.

2.3.4. TGF-β

With the discovery that Tregs make an immunoregulatory cytokine TGF-β, quite a few laboratories performed extensive analysis of the role played by this cytokine in immune regulation (reviewed in [97]). It is now well known that TGF-β controls various aspects of the inflammatory response through the regulation of chemotaxis, activation, and survival of T cells, NK cells, and APCs and is crucial for the control of antiviral immunity (reviewed in [97]). TGF-β also exhibits highly potent inhibitory effects on cytolytic functions, cytokine production, and proliferation of T-cells (reviewed in [98]). Conversely, TGF-β also exhibits antiapoptotic effects [99–101] on T-cells best exemplified in the case of the effector T-cells. When cultured in the presence of TGF-β and IL-2 effector, CD4 T-cells exhibit enhanced accumulation (owing to increased proliferation and survival) and even better prolonged expansion [99]. And finally, TGF-β, produced by Tregs, can act in an autocrine fashion to induce further expansion of Tregs from which it is produced. Also, TGF-β can induce the conversion of naïve CD4+CD25– T cells into CD4+CD25+ T cells by the induction of Foxp3 [102]. Thus TGF-β is extensively used to induce the expansion of Treg in in vitro cultures. Data from our laboratory as well as others have now shown that such in vitro expanded Tregs are able to adoptively confer protection in virus-induced T1D model (reviewed in [103]).

While the role of TGF-β in regulating immune responses is extensively known in a variety of autoimmune, infectious (bacterial) and other diseases evidence of TGF-β role in regulating antiviral immunity is scarce. Still, in chronic infections, such as influenza, HIV, HBV, HCV, and others, there is evidence of increased TGF-β production. In a virus-induced T1D mouse model, data from our laboratory showed that on one hand TGF-β suppresses naïve CD8+ T cell activation, while on the other hand it enhances the survival and function of antigen-experienced/memory CD8 T cells [104]. Most importantly, systemic administration of TGF-β was shown to protect from T1D [105].

Interestingly, several recent studies have convincingly demonstrated that TGF-β plays an essential role in the development of a newly discovered highly proinflammatory T-cells, the IL-17-secreting T cells (Th17) [106]. Although, TGF-β cannot induce

the conversion of naïve cells to Th17 by itself, together with IL-6, TGF-β facilitates the differentiation of T-cells into Th17 cells [107]. The most important aspect of TGF-β-induced effect is that TGF-β in conjunction with IL-2 induces the differentiation of naïve T-cells into Foxp3+ Tregs and this IL-2 inhibits the differentiation into Th17 cells [108].

Thus TGF-β is a very interesting cytokine, in that, in the presence of IL-2, it induces the generation of regulatory T-cells, CD4+ Foxp3+ Tregs, but the same TGF-β, in the presence of IL-6, induces the generation of proinflammatory Th17 T-cells. Hence, TGF-β apparently is very important in the regulation of antiviral immunity because while Tregs (as discussed above) are clearly involved in preventing tissue damage due to immune pathology, Th17 cells are highly proinflammatory and are known to be playing a significant role in autoimmune diseases. Thus, this dual functionality of TGF-β needs further elucidation.

2.3.5. IL-10

One of the most studied regulatory cytokine other than TGF-β is IL-10. IL-10 exerts its suppressive effect on macrophages [109] and T cells by blocking CD28 and ICOS signals in a rapid signal transduction cascade [110].

The best example for the role of IL-10, in regulating immune responses in viral infections, was provided in the last few years by a couple of laboratories, including data from our own laboratory. It is known that by 9 days after infection with LCMV clone 13, which establishes persistent infection in adult mice, both virus-specific CD4+ and CD8+ T-cells become unresponsive. Data from our laboratory showed that the use of an antibody to the IL10 receptor results in very efficient control of viral persistence, even leading to the elimination of virus. By removing IL-10 inhibitory effect, in this case on T-cell anergy, we were able to induce host resistance such that the host could now resolve persistent LCMV infection [15]. This was followed by further proof that such IL-10 acts on effector T-cells [32]. When IL-10 was blocked with antibody to IL-10 receptors, T-cell numbers increase sufficiently to clear virus from blood and tissues; these T-cells that were anergized because of the persistent virus infection now regained their ability to produced proinflammatory cytokines like TNF-α, IFN-γ and thus were able to reacquire their normal effector T-cell functions [32].

There was a significant increase in the frequency of IL-10 producing cells in both spleen and liver during persistent clone 13 infection [23]. Accordingly, significantly more IL-10 RNA was found in the spleens of clone 13-infected mice than that of Armstrong-infected mice [32]. CD4+CD25+ Foxp3+ regulatory T-cells are thought to be the major producers of IL-10. However, in clone 13 persistent infection, although CD4 T-cells

produced some IL-10 initially, they are not responsible for the majority of IL-10, since IL-10 was produced in significant quantities even after the functional inactivation of CD4 T-cells; further these CD4 T-cells stopped making any IL-10 after functional inactivation [32]. Interestingly, instead of the CD4 T-cells, it appears that DCs are the major producers of IL-10 in clone 13 infection [32]. Further, using IL-$10^{-/-}$ mice, the authors found that the lack of IL-10 resulted in the preservation of viral antigen (NP396-404)-specific CD8 T-cell responses that are normally lost during persistent infection [32]. And, as we had reported originally [15], antibody blockade of IL-10 prevented viral persistence [32], thus definitely showing that IL-10 plays a major role in immunoregulation during persistent viral infection with LCMV.

Although it appears that IL-10 may be acting directly on T-cells, the involvement of Tregs in immunoregulation but the their failure to make IL-10 during persistent LCMV infection suggests that other than acting on T-cells, IL-10 may also be playing an immunoregulatory role by directly altering the antigen-presenting capabilities of the APCs. However, this hypothesis needs further testing since it was found that APC functionality is normal in terms of antigen presentation in clone 13 infection [23].

3. Concluding Remarks

Persistent viral infections are a real threat to human health during a variety of viral infections with HIV, HBV, and HCV. The major role players responsible for the immunosuppression that happens during persistent infections have been identified as PD-1/PD-L1 and IL-10, with TGF-β and CTLA-4 also playing a role to some extent (Fig. 1). The recent observation that the functionally inactive T-cells during chronic viral infections can be made to regain their cytokine secretion and cytolytic abilities by neutralizing IL-10 is very encouraging [32]. More importantly, when IL-10 [111] or PD-L1 [84] was first neutralized, therapeutic vaccination against ongoing persistent LCMV infection was highly effective. Further, IL-10R blockade resulted in the ability of an otherwise ineffective DNA vaccination to be successful such that the DNA vaccination elicited a fourfold increase in the number of functional CD8 T cells [111]. Thus, it would be likely that neutralization of either IL-10 or PD-1/PD-L1 (depending on the virus) during the persistent viral infection would result in the preservation of effector T-cell responses against the virus thereby resulting in the elimination of the persistent infection.

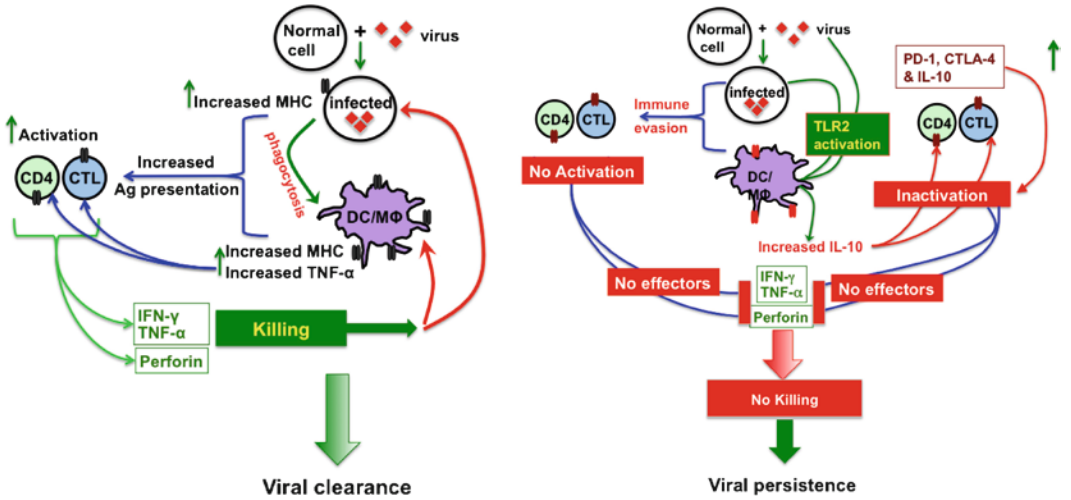

Fig. 1. Immune regulation during persistent viral infection. In an acute infection (*left*), when a virus infects a normal cell, it is phagocytosed by antigen presenting cell (APC) (dendritic cell [DC] or MΦ). The APCs efficiently present the viral antigens to CD4 and CD8 cells, which either release proinflammatory cytokines (TNF-α, IFN-γ) or upregulate perforin, which kills the infected cells, ultimately leading to the elimination of the virus. However, in persistent infection (*right*), due to a number of reasons, including miRNAs and molecular mimicry, viruses evade immune recognition. Thus, the CD4 and CD8 cells are not sufficiently activated and the virus avoids immune-mediated elimination. On the other hand, APCs (MΦ and DCs), upon TLR2 activation, upregulate IL-10; IL-10 exhibits significant immunosuppressive effects leading to the inactivation of CD4 and CD8 T-cells. Further, activated T-cells also upregulate CTLA-4 that prevents costimulation, thereby leading to inactivation. And, finally, virus antigen-specific T-cells also upregulate PD-1 which, upon engagement with its ligand PD-L1, signals the death of that T-cell. These events, help to avoid immune response mediated host tissue damage but at the cost of establishing viral persistence.

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