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Translating insights from persistent LCMV infection into anti-HIV immunity

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Abstract

Human Immunodeficiency Virus (HIV) is a major global health concern with more than 30 million individuals currently infected world-wide. To date attempts to stimulate protective immunity to viral components of HIV have been unsuccessful in preventing or clearing infection. Lymphocytic choriomeningitis virus (LCMV) is an established murine model of persistent viral infection that has been instrumental in illuminating several critical aspects of anti-viral immunity. Although virologically the course of LCMV infection differs significantly from HIV, the immune responses and regulatory mechanisms elicited by these two viruses are markedly similar. In this review we discuss important recent findings in the LCMV model, highlighting the role of host-derived proteins in shaping immune responses to persistent infections, and explore the therapeutic potential of manipulating these pathways to enhance HIV vaccination strategies.

Keywords

LCMV; HIV; T cell exhaustion; persistent viral infection; CD4 T cell; CD8 T cell; immune regulation

INTRODUCTION

The syndrome now known as Acquired Immunodeficiency Syndrome (AIDS) was first identified at UCLA in 1981 (1). In the 30 years since, more than 25 million AIDS related deaths have occurred world-wide. From its characterization as the causative agent of AIDS in 1983 through present day, human immunodeficiency virus (HIV) has proven to be a formidable challenge for clinicians and researchers alike, and currently infects more than 30 million individuals (2, 3). While incredible strides have been made in controlling the virus with combination highly active anti-retroviral therapy (HAART), sterilizing immunity has not been accomplished and the efficacy of therapeutic vaccines remains bleak.

HIV is armed with a multitude of evasion strategies and the virus is exemplary in its ability to escape and mutate in the face of host immunity (4). In fact, the two anti-HIV vaccine attempts to date, one based on stimulating humoral immune responses against HIV-1 glycoprotein components (5) and the other utilizing an adenoviral vector delivery system expressing several HIV proteins to stimulate multiple cellular immune responses (6) have both failed to prevent infection. While the first strategy was effective in generating anti-HIV antibody responses capable of neutralizing laboratory HIV strains, no protective effect was

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found in clinical trials (5, 7). And although anti-HIV cellular immune responses were generated in response to the adenoviral platform, these responses were ultimately ineffective in protection (6). The failure of these two strategies highlights the need for a better understanding of the immune response necessary to prevent and control HIV infection and development of specific tactics to invoke these desired anti-HIV responses.

Lymphocytic choriomeningitis virus (LCMV) infection of mice has proven an invaluable exploratory system for the elucidation of fundamental immunologic mechanisms including, but certainly not limited to MHC restriction of antigen presentation, T cell effector function, contraction, and memory formation (8, 9). The LCMV model is particularly relevant to the study of persistent viral infections and the characterization of T cell dynamics and exhaustion were initially defined in this system and then extended to a variety of human persistent infections including HIV (10). More recently the integral role host proteins play in establishing and maintaining viral persistence has been revealed in the LCMV model. As discussed below, these novel mechanisms are rapidly being tested in HIV infection and may lead to exciting new avenues of immunostimulatory therapies.

One of the key benefits of the LCMV system is the ability of nearly identical virus variants to induce very different clinical outcomes. Infection of mice with the LCMV variant Armstrong results in a rapid expansion of virus-specific effector CD4⁺ and CD8⁺ T cell responses that clear infection within 8–10 days post infection. In contrast, infection with LCMV Clone 13 leads to the emergence of functionally exhausted T cells and decreased antibody responses that are ultimately ineffective at clearing virus, facilitating the establishment of persistent infection (8, 11–15). Exhausted T cells express a unique transcriptional profile leading to reduced proliferative capacity, down-regulation of TCR signaling molecules, the inability to produce multiple immunostimulatory and antiviral cytokines integrally associated with viral clearance during acute infection, and increased expression of inhibitory surface molecules (12, 13, 15–17). Exhausted CD8⁺ T cell responses to persistent LCMV mirror the CD8⁺ T cell responses found in HIV infection, specifically in their inability to proliferate and produce cytokine in responses to viral antigen (10). Both persistent LCMV and HIV infection are dependent on help from CD4⁺ T cells to mount and maintain CD8⁺ T cell and B cell responses (18). Thus conceptually and therapeutically, LCMV and other murine models of persistent infection have proven to be valuable experimental tools to reveal novel aspects of immunity to HIV and other important human viruses (10).

While HIV and LCMV are inherently different viruses with respect to genetic composition, replicative strategies and mechanisms of infection, immunologically speaking they elicit in many ways similar anti-viral responses. Both viruses initially trigger productive T cell responses but are unable to clear infection likely due to multiple factors including the selective loss of high affinity responders and continued propagation of functionally exhausted T cells (19–24). Similar congruencies can be found in the host-derived immunoregulatory strategies invoked by the two viruses in order to subvert immunity. In this review we discuss the important recent contributions of the LCMV model toward understanding the immune response against HIV infection and the potential promise of manipulating several host immunomodulatory proteins to enhance anti-HIV vaccine strategies.

HOST IMMUNOREGULATORY FACTORS AND THE RESTORATION OF ANTIVIRAL IMMUNITY

The immune system has exquisitely evolved not only to aggressively attack invading pathogens but also to control and dampen these responses limiting immunopathology and

bystander autoimmunity. From the moment of infection it is a race between viral replication and anti-viral immune responses (25). In the case of acutely resolved infections, innate immune responses are successful in recruiting and instructing adaptive immune effectors that can clear virus efficiently and contract appropriately leaving no residual pathogen or cytotoxic molecules. However, for reasons that are not well understood, when initial viral insult is too robust or the virus is too efficient at evasion, host immune components are unsuccessful in eliminating antigen. In this case, prolonged immune responses must be attenuated to avoid chronic inflammation and destructive immunopathology. Unfortunately, these efforts to repress undesired immune outcomes also lead to the demise of virus-specific responses. In the past few years, multiple fundamental discoveries in the LCMV system have illuminated the mechanisms by which virus-specific cells initially lose function permitting viral persistence, as well the master role of host-derived immunoregulatory proteins in directly inhibiting anti-viral immunity and sustaining the immunosuppressive state (18, 26–30). Excitingly these same mechanisms also appear to limit anti-HIV immune responses, opening up novel therapeutic avenues to boost HIV-specific immunity and control virus infections.

THE PROGRAMMED DEATH RECEPTOR 1 PATHWAY OF IMMUNOSUPPRESSION

The degree to which host proteins control the establishment of immunosuppression in the face of persistent viral infection wasn't truly appreciated until 2005 with the seminal discovery of the role of the host protein programmed death (PD) ligand 1 (PD-L1) in the maintenance of T cell exhaustion in LCMV (26). PD-1 is an inhibitory member of the CD28 co-stimulatory family of proteins (31). PD-1 expression is increased on virus-specific CD8+ T cells in LCMV and HIV infections correlating with the degree of T cell exhaustion (26, 32–35). Treatment of LCMV persistently infected mice with blocking antibodies to PD-L1 resulted in expansion of potent CD8+ T cells that were capable of lowering virus replication in several organs (26). This strategy was also effective in restoring CD8+ T cell responses in persistently infected CD4+ T cell-depleted mice which exhibit an enhanced level of T cell exhaustion, suggesting that a similar approach may also be efficacious in the presence of HIV induced CD4+ T cell depletion. Similarly, in vitro PD-L1 blockade effectively stimulated proliferation and production of multiple cytokines by exhausted HIV specific CD8+ T cells (33–35). However, whether PDL-1 blockade will efficiently restore HIV-specific T cell function in vivo is not clear.

It was recently demonstrated that in vivo PD-1 blockade during Simian Immunodeficiency Virus (SIV) infection resulted in a similar immune restorative effect as observed during persistent LCMV infection (36). Interestingly, PD-1 blockade also enhanced B cell and antibody responses to SIV indicating that immune restoration and enhanced viral control is likely a multi-factorial process. This concept is further substantiated by the fact that multiple cell types express PD-1 during HIV infection (37). Thus, PD-1/PD-L1 blockade could potentially affect the functional responses of numerous cell types cumulating in enhanced immune dynamics and controlled viral replication

In addition to its important potential therapeutic role, the correlation of PD-1 expression to functional exhaustion has facilitated further identification, quantification and description of T cell exhaustion during HIV infection, when functional assays can be difficult and samples limiting. The ability to amplify anti-viral immunity by blocking host regulatory factors, independent of targeting viral proteins, may be especially advantageous in HIV infection given the high mutability of the virus. Thus, by facilitating host immune control of HIV infection it may be possible to circumvent the problems of drug resistance due to viral mutation.

IL-10 MEDIATED IMMUNOSUPPRESSION

In addition to cell surface proteins, soluble factors dramatically impact the outcome of T cell activation. In persistent LCMV infection, the cytokine IL-10 was shown to play a major role in initially restricting T cell responses (27, 28). IL-10 was initially characterized as a CD4⁺ T cell-produced Th2 cytokine with the ability to indirectly repress Th1 responses (38, 39). It is now evident that IL-10 has multiple important immunoregulatory roles and a plethora of cell types including DC, B cells, macrophages, CD4⁺ T cells, CD8⁺ T cells, NK cells as well as innate and adaptive regulatory T cells have been shown to produce it (39, 40). Infection of mice with a persistent, but not an acutely cleared variant of LCMV leads to sustained expression of IL-10 by multiple immune cell subsets (27, 28). When IL-10 activity is neutralized early following infection with a persistent strain of LCMV (i.e. Clone 13) CD4⁺ and CD8⁺ T cell effector functions are not exhausted, but instead are maintained, and an otherwise persistent virus is rapidly cleared. This was the first identification that a single factor was responsible for derailing the immune response to permit viral persistence and importantly, that sustaining T cell immunity could facilitate the clearance of a persistent infection. Additionally, IL-10 blockade later during an established persistent LCMV infection causes enhanced virus-specific T cell responses indicating that IL-10 continues to suppress anti-viral immunity throughout persistent infection (41, 42).

There is considerable evidence that serum levels of IL-10 are elevated in HIV infection (43–45). Increased expression of IL-10 in HIV is also correlated with diminished T cell activity and increased virus replication (46). In vitro blockade of IL-10 restores the function of exhausted CD4⁺ and CD8⁺ T cells isolated from infected individuals (45–48). Interestingly, IL-10 expression is tightly correlated to both LCMV and HIV replication indicating a conserved mechanism whereby the immune system utilizes IL-10 to modulate its function in response to levels of virus replication. It will ultimately be interesting to determine how the immune system reads and translates virus replication into immunosuppression and how best to utilize this information to boost anti-HIV immune responses while limiting immunopathology.

CD27 AND RESTRICTION OF ANTIVIRAL ANTIBODY RESPONSES

CD27 is a member of the tumor necrosis factor receptor (TNFR) family of co-stimulatory molecules and is expressed primarily on naïve and activated CD4⁺ and CD8⁺ T cells as well as B cells and NK cells. The ligand for CD27 is CD70, which is expressed transiently by activated T and B cells, and in a highly regulated manner on APC populations (49). Traditionally engagement of this receptor/ligand pair is considered to augment T and B cell responses (50). However, in the case of LCMV Docile infection, and perhaps HIV infection as well, CD27-CD70 interactions have been found to be detrimental to the formation of neutralizing antibody responses (51). LCMV infection induces elevated expression of CD70 on splenocytes resulting in chronic CD27 signaling to CD4⁺ T cells. These cytokines then mediate the destruction of marginal zone architecture and subsequently the ability to mount productive antibody responses. Disrupting CD27/CD70 interactions with blocking antibodies to CD70, or analyzing antibody responses in CD27 deficient mice reveal the emergence of potent neutralizing antibody responses and resolution of an otherwise persistent infection.

The debilitated neutralizing antibody responses seen in persistently infected LCMV mice are similar to the antibody responses observed in HIV infected individuals (52, 53). Although the role of CD27/CD70 interactions in the aberrant neutralizing antibody responses found in HIV infection is not clear. CD70 expression is found to be elevated in HIV infection suggesting that a similar suppressive mechanism of antibody responses may be elicited (54,

55). Further, the role this receptor/ligand pair played in the failure of the subunit vaccine initiative is unknown and disruption of CD27/CD70 interactions may enhance future vaccine attempts aimed at eliciting anti-HIV antibody responses.

CD4 T CELL HELP AND THE POTENTIAL TO AMPLIFY ANTIVIRAL RESPONSES

In opposition to the suppressive factors that limit T cell responses during viral persistence it is becoming evident that distinct factors are induced during persistent infection that actively sustain residual levels of anti-viral immunity (18). Although residual levels of anti-viral activity are insufficient to clear infection, they continue to exert control over virus replication. As with the identification of host-regulatory factors in suppressing immunity, the formal role of individual stimulatory factors are being defined in the LCMV system and their immunologic relevance to humans tested in HIV infection.

CD4+ T cells are considered to be the central mediators of adaptive immunity. Their necessity for optimal class switching and affinity maturation of B cell responses has been well described (56). CD4+ T cells play important roles in several phases of CD8+ T cell responses as well (57–61). One particularly debilitating feature of HIV is its capacity to infect and deplete the host pool of CD4+ T cells. Progression to AIDS and death are strictly correlated with diminished CD4+ T cells counts (62). In LCMV as well, there is a stringent dependence on CD4+ T cell help for the optimal generation of immune responses as well as the control of persistent LCMV infection (63, 64). In the absence of CD4+ T cell help, virus-specific CD8+ T cells suffer a drastically enhanced deletion and functional exhaustion and anti-LCMV specific antibody responses are not mounted. The culmination of these functional deficits is increased virus titers and the inability to control infection. Unlike CD4+ T cell help for B cell responses, the precise mechanisms of help for the generation of optimal CD8+ T cell responses are not well defined (65, 66). Identifying specific CD4+ T cell-derived proteins involved in supporting memory CD8+ T cell and B cell responses may provide a productive strategy by which to simultaneously enhance multiple arms of the immune response to control infection and to optimize vaccine design towards preventing infection.

THE CYTOKINE IL-2 AND ATTEMPTS TO FUEL CD8+ T CELL IMMUNITY

IL-2 is the signature cytokine of CD4+ T cells and its requirement for the *in vitro* generation and proliferation of CD8+ T cells has been immunology dogma for more than 30 years (67, 68). It has recently been suggested that IL-2 has important roles not only in amplifying CD8+ T cell responses but also in shaping the quality of them as well (69). Important roles for this cytokine in the primary expansion and generation of lasting memory CD8+ T cell responses have been described in LCMV and other models (70, 71). The inability of CD8+ T cells to respond to IL-2 correlates with increased functional exhaustion during persistent LCMV infection (72). However, IL-2 expression is rapidly suppressed by both CD4+ and CD8+ T cells in persistent LCMV and HIV infection (73). Thus, it is unclear whether the source of IL-2 signals that sustain residual CD8 T cell activity originate early in persistent infection when T cells are still producing the cytokine (12) or whether the low amount of sustained IL-2 producing T cells present during the persistent stage of infection provide sufficient amounts to sustain CD8+ T cells. Alternatively, an as yet unidentified non- T cell source of IL-2 may exist.

Therapeutic administration of IL-2 during LCMV persistence increases anti-viral immunity and decreases virus titers (74). In HIV infection, IL-2 administration increased CD4+ T cell counts, however the impact of this effect is more difficult to interpret, and varied with

respect to levels of CD4⁺ T cells at the onset of treatment (75). Nonetheless, the benefit of sustained IL-2 producing CD4⁺ T cells is well established (76–78). Thus, although the mechanisms remain elusive IL-2 is at the very least, an important correlate to sustaining immune competence during persistent LCMV and HIV infections. Determination of how IL-2 achieves this goal will lead to both important biologic insights as well as potential immune enhancing therapies to control HIV infection.

IL-21 ACTIVITY IN VIRAL PERSISTENCE

IL-21 is a member of the common gamma chain family of cytokines and has pleiotrophic effects on a variety of cell types including those of both myeloid and lymphoid lineage (79). We along with two other groups recently identified a necessity for IL-21 in the maintenance of CD8⁺ T cell responses during persistent LCMV infection (80–82). The inability to receive IL-21 signals resulted in enhanced depletion and functional exhaustion of CD8⁺ T cells. Virus-specific CD4⁺ T cells are the dominant producers of IL-21 in vivo (81). The diminished CD8⁺ T cell responses found in the absence of IL-21 mirror those described in the total absence of CD4⁺ T cells, indicating that IL-21 is an important aspect of help during viral persistence. IL-21 producing HIV specific CD4⁺ T cells are also detected in HIV infected individuals and similar to LCMV correlate with CD8⁺ T cell competence and the ability to control viral replication (83). Decreased levels IL-21 are also linked with increased disease progression (84). The therapeutic utility of IL-21 is being investigated, but based on the stimulatory effect of IL-21 in both CD8⁺ T cells and B cells it is possible that IL-21 therapy may simultaneously enhance multiple antiviral effector functions.

THE IMPORTANCE OF TIMING AND THE INFLUENCE OF THE INDIVIDUAL

Since the primary function of host immunoregulatory molecules is to prevent immunopathology and damage to the self, the timing and amount of blocking interference and cytokine enhancements are critical considerations and must be tailored to stimulate effective immune responses without allowing the outgrowth of harmful uncontrolled activation. Blocking immunoregulatory molecules or attempting to heighten stimulation of responses in the acute phase of infection for example, may result in undue immunopathology or death. This is evident when IL-21 treatment is administered during the early phase of persistent LCMV infection (82). Although dramatically elevated virus-specific CD8 T cell responses were generated and significant reduction in viral titers achieved following IL-21 therapy, mice suffered from severe immunopathology and were ultimately moribund. Similarly, infection of PDL1 knock-out mice with persistent LCMV was universally fatal (26). In contrast, attempts to restore exhaustive function after viral persistence has been established appear to be better tolerated in vivo in mice. Results from PDL1 blocking experiments in rhesus macaques confirm that parameters can be achieved to enhance immunity in the absence of uncontrolled immunopathology (36). Short-term PD-1 blockade (4 treatments over 10 days) provided long-term restoration of T cell responses and enhanced SIV control. Animals treated in this study showed no detectable increase in serum levels of anti-nuclear antibodies, an assay of autoimmunity. However, this was only a short-term treatment regimen in a limited number of animals, and the outcome of longer-term therapy and administration to a diverse human cohort is unknown, but promising.

As humans we are an extremely genetically diverse species and individual variation in sensitivity to immunoregulatory molecules must also be considered. Excitingly, emerging data suggests that genetic polymorphisms in the IL-10 promoter that result in lower IL-10 production are associated with clearance of HCV infection and enhanced virus control during chronic HCV, HBV, HIV and EBV infections (85–89). The disparity between individuals to PDL1 or IL-10 blockade in terms of the level of functional restoration

achieved by blocking these factors in vitro highlights the complexity of signaling events in different individuals infected with the same pathogen (46, 48). This concept is also apparent in by cancer therapy trials in which different individuals have vastly different responses to CTLA-4 blockade, despite having the same type of tumor (90). While there is a strong indication that blockade or enhancement of regulatory factor function may be effective therapeutically at several points during the course of persistent infection it is clear that the effects of manipulating any given pathway may be variable and unique to the individual.

FUTURE PROSPECTS: SYNTHESIZING THE OPTIMAL COCKTAIL

This review discusses a handful of host-derived proteins that may be beneficial candidates for therapeutic manipulation and optimization of vaccine protocols. This list however, is far from exhaustive. It is clear that multiple pathways function to suppress and sustain anti-viral immune responses in the face of persistent infection (18). Furthermore, the signature of immunoregulatory pathways at play is likely unique to any given infection. The capacity of PD-1/PD-L1 interactions to suppress CD8 T cell responses in a variety of diverse persistent infections including HIV and SIV, as well as both RNA (HCV), and DNA (HBV) viruses highlights this molecule in particular as a promising therapeutic tool (33–36, 91, 92). However, the concerted operation of multiple immunoregulatory pathways does suggest the potential for increased efficacy of multi-armed strategies at bolstering immune responses. This has been demonstrated in the LCMV system with the combinatorial effects of dual PD-1 and IL-10 blocking treatments leading to markedly enhanced T cell function and viral control (41). Similarly, simultaneous blockade of PD-L1 and another inhibitory receptor Lag-3 during persistent LCMV infection, further enhanced T cell responses despite Lag-3 blockade alone having only minimal effect (29, 93). Combinatorial strategies that also include a stimulatory component, such as cytokines or vaccines, may further enhance immunity by simultaneously alleviating suppression and rendering cells susceptible to positive signals that are then provided. Ideally, it may be possible to manipulate the cocktail such that the precise components of suppression that hinder anti-viral responses are blocked, while the stimulatory messages are promoted. Ultimately, by identifying the correct factors such a combinatorial strategy may be devised to sufficiently enhance the quality and quantity of vaccine-induced immunity to prevent HIV infection, and to therapeutically control or overcome established HIV infections.

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