Regulation of Antiviral CD8 T-Cell Responses

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ABSTRACT: A balanced immune response to a viral pathogen leads to clearance of the virus while limiting immune mediated pathology. Control of this process occurs at all stages of the immune response, including during the induction of an antiviral response, clearance of virally infected cells, and the resolution of this response. Regulation of antiviral immune response is further modified when the immune system fails to clear the pathogen and by the nature of chronic infection itself. A number of processes have been implicated in the regulation of antiviral immune responses, such as the limitation of viral antigen load by interferons, apoptosis through cytokine withdrawal or Fas-mediated killing, and control of these responses by regulatory T cells. This review addresses several of these mechanisms.

KEY WORDS: CD8 T cells, antiviral, regulation, T regulatory cell, apoptosis

ABBREVIATIONS: **AICD:** activation-induced cell death; **FASL:** Fas ligand; **HIV:** human immunodeficiency virus; **IFN:** interferon; **IL:** interleukin; **iTr:** induced T regulatory cells; **nTr:** T regulatory cells; **T-bet:** T-box expressed in T cells; **TCR:** T cell receptor; **TGF- β :** transforming growth factor- β ; **TNF:** tumor necrosis factor; **Tr:** T regulatory cells.

I. INTRODUCTION

Inherent to immune responses to infectious agents is the possibility of immune-mediated tissue damage. A balanced CD8⁺ T-cell response leads to successful virus clearance that is accompanied, in most cases, by minimal damage to the infected host. However, in some cases the exaggerated immune response of the host induces severe and sometimes lethal tissue damage that far exceeds the damage that would have been inflicted by virus replication in the absence of the exuberant immune response. For example, severe immunopathology is observed during primary influenza infection, where both innate and CD8⁺ T-cell responses, but not virus replication per se, are thought to mediate most of the damage in the lung.^{1–3} CD8⁺ T lymphocytes have great potential to damage tissues either through cytotoxicity of cells or through cytokines released. This is best

demonstrated by the potentially fatal disease hemophagocytic lymphohistiocytosis, where failure to clear viral infections leads to excessive CD8 T-cell activation, inflammatory cytokine production, and life-threatening, immune-mediated tissue damage.⁴ In addition to the sequelae of acute infection, immunopathology is a significant contributor to the tissue damage observed during persistent viral infections.

Therefore, the immune system has evolved mechanisms to prevent excessive immune pathology while still being able to clear infections. This review will focus on the known mechanisms of regulation of antiviral CD8 T-cell responses. To understand how this occurs, it is important to review how different viral infections are handled by CD8 T cells, since the nature of the virus can greatly affect how the immune response to it is regulated.

II. OVERVIEW OF CD8+ T-CELL RESPONSES IN THE CONTEXT OF ACUTE AND CHRONIC VIRAL INFECTIONS

A. Induction of an Antiviral CD8+ T-Cell Response

The adaptive immune response plays a key role in controlling microbial pathogens. Unlike immune control of bacteria, which in most cases is critically dependent on the development of pathogen-specific humoral responses, CD8+ T cells are paramount for the successful control of a vast majority of viral infections. Following inoculation of a naïve host, virus-specific CD8+ T-cell responses can become evident as early as 4–5 days after infection, with the peak numbers of effector CD8+ T cells usually observed between 7–14 days after infection. The initiation and expansion of antiviral CD8+ T cells is largely dependent on the innate immune response that is rapidly triggered following engagement of several pattern recognition receptors by virion products.^{5–7} Type I interferon (IFN) is a critical component of the innate immune response that, in addition to directly suppressing virus replication, regulates the emerging CD8+ T-cell response.⁸ Type I IFN signaling specifically increases the expression of major histocompatibility complex class I and costimulatory molecules on antigen presenting cells, thus ensuring effective CD8+ T-cell priming.⁹ Furthermore, type I IFN can directly act on CD8+ T cells to augment their proliferation.^{10,11} Other cytokines produced by innate immune cells reinforce the signaling mediated by type I IFN to ensure the expansion and function of cytotoxic virus-specific CD8+ T cells. Interleukin (IL)-12 produced by macrophages and dendritic cells induces expression of the transcription factor T-bet (T-box expressed in T cells).¹² Induction of T-bet expression in CD8+ T cells is essential to the generation of their antiviral cytotoxic effector functions.¹³ Other cytokines expressed by innate immune cells, such as tumor necrosis factor (TNF)- α , IL-15, and IL-18, further stimulate CD8+ T-cell responses. It is important that the features of an antiviral CD8+ T-cell response are largely determined by the nature of the viral infection.

B. CD8+ T-Cell Responses in Acute Infection

In broad terms, acute viral infection is characterized by the virus-host race that leads to the elimination of the losing side. Most acute virus infections are fortunately cleared by virus-specific CD8+ T cells with ensuing antigen depletion, contraction of the CD8+ T-cell response (described in detail below), and establishment of memory CD8+ T cells. The transition from effector to memory CD8+ T-cell responses has been recently reviewed in a number of excellent publications.^{14,15} These memory CD8+ T cells can be extremely long-lived¹⁶ and mediate rapid and effective responses to subsequent viral challenge.

C. CD8+ T-Cell Responses in Chronic Infection

Chronic viral infection presents a distinct challenge to the adaptive immune response that has failed to achieve sterilizing immunity and must now control both excessive virus production and immune-mediated tissue damage to minimize disease in the infected host. The specifics of a CD8+ T-cell response during chronic infection are largely determined by the nature of the chronic infection itself. Certain viruses, with herpesviruses being the prototype, have evolved latent lifestyles that ensure maintenance of the viral genome with little expression of viral genes and minimal, if any, active viral replication. This quiescent colonization of the host is associated with very low levels of viral antigens, appropriate development of memory T cell, and maintenance of highly functional effector CD8+ T cells that are stimulated by the occasional expression of viral antigen due to viral reactivation.

In contrast to quiescent long-term infection discussed above, several viruses, including hepatitis C virus and lymphocytic choriomeningitis virus clone 13, are confined to active replication as the only available viral life cycle. This persistent replication in a chronically infected host evokes sustained and vigorous stimulation of the T-cell receptor (TCR) and upregulation of inhibitory receptors that lead to exhaustion of virus-specific effector CD8+ T cells. Exhaustion of CD8+ T cells is specifically

characterized by decreased cytokine production, attenuated proliferation, and eventual apoptosis.^{17,18} Furthermore, because of continuous antigenic stimulation, the development of memory CD8⁺ T cells is compromised. Because of the high possibility of immunopathology, regulatory T cells also play a role in attenuating CD8⁺ T-cell-mediated tissue damage during persistent viral replication (see below).

Finally, both quiescent and persistent viral life styles can be used by select viruses that establish chronic infection. Human immunodeficiency virus (HIV) is a prototype in this class of agents; it can establish a quiescent infection, where the provirus is maintained as a part of the cellular genome with minimal viral replication. This quiescent infection, prevalent in patients with HIV who are treated with potent antiviral therapy, happens when infected CD4⁺ T cells are able to differentiate into a memory state that does not permit viral replication, thus creating a stable reservoir of latent HIV.¹⁹ In contrast, in the absence of optimal antiviral therapy, HIV undergoes active persistent replication, concomitant with chronic CD8⁺ T-cell stimulation and subsequent exhaustion, as described above.

The immune systems in humans and mice have evolved a highly intricate network of overlapping mechanisms that function to initiate suppression of an immune response to prevent catastrophic immune pathology and/or autoimmunity. Since CD8 cells need viral-derived peptide products to be activated, the nature of the virus and persistence of antigen can have a significant effect on CD8⁺ T-cell responses and how a specific response is regulated. Below we review some of the best studied of these mechanisms in the context of acute and chronic viral infections.

III. INTRINSIC CONTROL OF CD8⁺ T-CELL RESPONSES IN ACUTE AND CHRONIC VIRAL INFECTIONS THROUGH MECHANISMS OF APOPTOSIS

It has long been appreciated that apoptosis is essential to the development of immune cells and in the maintenance of tissue homeostasis.²⁰ Given the remarkable rate and magnitude of CD8⁺ T-cell expansion in

response to both acute and chronic viral infections, virus-specific CD8⁺ T cells would eventually overwhelm the host if left unchecked. Regulation of apoptosis in specific immune cells is a major mechanism for facilitating the suppression of immune responses following clearance of acute viral infection as well as maintaining tissue homeostasis and limiting immune pathology during chronic viral infection.

Apoptosis results from the cleavage and subsequent activation of a family of cysteine proteases called caspases that go on to cleave specific cellular proteins to facilitate cell death.²¹ In humans and mice, there are 2 different yet eventually convergent pathways of apoptosis that are known to play a role in regulating the CD8⁺ T-cell response to viral infection.²² These 2 signaling cascades are known as the intrinsic, or mitochondrial, pathway, which is mediated by BCL-2 proteins, and the extrinsic pathway, which is induced through ligation of the FAS “death receptor” with FAS ligand (FASL), its cognate ligand. The relative contributions of the intrinsic and extrinsic apoptosis pathways to the regulation of CD8⁺ T-cell responses are defined by the nature of the viral infection (acute vs. persistent chronic vs. quiescent chronic) and the extent of antigenic stimulation that can vary from virus to virus and tissue to tissue.

A. BCL-2 Proteins and the Intrinsic or Mitochondrial Pathway

The outcome of whether a cell lives or dies by the intrinsic or mitochondrial pathway of apoptosis is the result of a balance between antiapoptotic and proapoptotic members of the BCL-2 family.^{23–25} These proteins function to regulate the permeability of the outer mitochondrial membrane that is required for the activation of an “apoptosome” protein complex. The apoptosome consists of apoptotic protease-activation factor 1, cytochrome c, and caspase 9 and is required for activation of cell death-inducing “executioner” caspases.^{26–28}

B. FAS Receptor Signaling and the Extrinsic Pathway

The FAS receptor, also known as Apo-1 or CD95, is a type 1 transmembrane protein belonging to a

subset of the TNF receptor family known as the “death receptors.”²⁹ All death receptors, including FAS, TNF-related apoptosis-inducing ligand receptor, and TNF-receptor 1, are characterized by an 80 amino acid intracellular death domain that is essential for induction of apoptosis by the extrinsic pathway.^{30,31} Activation of FAS by its physiological ligand FASL triggers apoptosis in the FAS-expressing cell by recruiting adaptor/docking proteins via the cytoplasmic FAS death domain and the ensuing recruitment/activation of caspases that leads to cell death.^{32,33}

C. Regulation of Apoptosis During Acute and Chronic Viral Infections

During the normal primary lymphocyte response to an acute viral infection, signals from the TCR, costimulatory molecules, and growth factors stimulate the expression of antiapoptotic BCL-2 proteins (i.e., BCL-2, Bcl-X) that promote cell survival and proliferation necessary for the development of a robust and specific antiviral T-cell response and the efficient clearance of the viral antigen from the host. By definition, an acute viral infection culminates in sterilizing immunity and clearance of the viral antigen from the host. The resulting decrease in antigenic stimulation is accompanied by a decrease in production of prosurvival cytokines such as IL-2.^{34,35} Antiviral CD8+ T cells experiencing this “cytokine withdrawal” exhibit a decrease in intracellular antiapoptotic BCL-2 proteins as well as an increase in proapoptotic BH3-only proteins BCL-2 interacting member of cell death (Bim) and p53 upregulated modulator of apoptosis (Puma).^{2,36,37} In this way, clearance of the virus triggers suppression of the immune response by tipping the balance of BCL-2 proteins within antiviral CD8+ T cells to favor the induction of apoptosis via the intrinsic pathway. While other proapoptotic BCL-2 proteins antagonize prosurvival signaling, Bim and Puma exhibit the highest affinity for antiapoptotic BCL-2 proteins and therefore are thought to be especially instrumental in mediating the contraction of a virus-specific CD8+ T-cell response.^{38,39}

FAS or FASL are minimally involved in apoptosis induced by cytokine withdrawal. In contrast,

FAS-FASL signaling significantly contributes to the suppression of CD8+ T-cell responses through a phenomena known as activation-induced cell death (AICD).²² AICD occurs when repeated antigenic stimulation of previously activated effector CD8+ T cells through the TCR leads to upregulation of FAS signaling and eventually apoptosis.^{32,40–42} While cytokine withdrawal is thought to be the most significant factor in apoptosis-mediated suppression of the CD8+ T-cell response following the clearance of an acute viral infection, where a decline in survival factors often precludes persistent restimulation of activated CD8+ T cells, regulation of immune responses during chronic viral infection clearly involves AICD-mediated apoptosis through the FAS-FASL pathway.

It is becoming increasingly evident that both Bim and FAS-mediated regulation of apoptosis synergize to control immune responses in the context of both acute and chronic viral infections. Studies examining viral infection in mice deficient in Bim, FAS-FASL signaling, or both highlight the differential requirements for these pathways in suppressing CD8+ T cell responses to different types of viral infection.^{2,43–48} During quiescent chronic infection with the mouse gammaherpesvirus 68, Bim-mediated induction of apoptosis through the intrinsic pathway is further enhanced by FAS-mediated activation of the protein BH3-interacting domain death agonist (Bid) to achieve optimal suppression of virus-specific CD8+ T cells.^{43,48} In contrast, Bid is dispensable for suppression of CD8+ T-cell responses mounted against acute influenza virus infection or following the acute phase of infection with the alphaherpesvirus HSV-1.^{2,43} It is likely that the relative contributions from Bim and FAS-mediated pathways to the overall regulation of antiviral CD8+ T-cell responses vary from infection to infection, where differences in the biology of the virus, the types of cell(s)/tissue infected, and the strength/duration of antigenic stimulation contribute significantly to the signals received by antiviral CD8+ T cells (as well as surrounding cells). In fact, the programmed deletion of CD4+ T cells, B cells, and other antigen-presenting cells that support an antiviral CD8+ T-cell response has been shown to support the suppression of immune responses to

certain types of chronic infection and is thought to work in concert with induction of apoptosis within the CD8⁺ T cells themselves.^{47–50} Furthermore, regulation of apoptosis can be further modified by some viral gene products expressed during infection that have been shown to target key components of the intrinsic or extrinsic apoptosis pathways and can certainly affect the nature of the immune response to that pathogen.^{51–53}

IV. EXTRINSIC CONTROL OF ANTIVIRAL CD8⁺ T-CELL RESPONSES BY REGULATORY T CELLS

Although contraction of CD8 cells following acute viral clearance through cytokine withdrawal or through FAS/FASL-mediated apoptosis are critical to control CD8 responses, T regulatory (Tr) cells also are involved in this process. Not only are Tr cells a population of cells that are essential to the prevention of autoimmunity, they also are important for the control of immune responses to foreign antigens.^{54–56} Tr cells have been divided into natural (nTr) or induced (iTTr) based on their developmental location and antigen specificity. nTrs are generated in the thymus as a committed regulatory cell population that expresses the lineage-specific transcription factor Foxp3, and these cells are self-antigen specific.^{57–59} iTTr cells can be generated in vitro from Foxp3-negative CD4⁺ T lymphocytes by TCR activation in the presence of transforming growth factor- β (TGF- β) and IL-2.^{60,61} iTTr cells also are generated in vivo from peripheral Foxp3⁻ CD4⁺ cells, resulting in a population of cells that have regulatory properties but with a distinct TCR repertoire compared to nTrs. Furthermore, nTrs and iTTrs act in synergy to induce peripheral tolerance.^{62,63}

Numerous suppressive mechanisms for Tr cells have been demonstrated; these have been historically divided based on whether contact between the Tr and target cells is necessary. Several molecules have been implicated in the contact-dependent suppression by Tr cells, including cytotoxic T lymphocyte antigen-4 engagement of B7 molecules on target cells,^{64,65} surface expressed TGF- β ,^{60,66} and cytotox-

icity of target cells through the perforin/granzyme pathway.⁶⁷ Contact-independent suppression is largely mediated by cytokines, such as IL-10,^{68,69} secreted TGF- β ,⁷⁰ and IL-35.⁷¹

A. Tr Cells in the Control of CD8⁺ T-Cell Responses

Although the majority of studies have examined the role of Tr cells on the function of CD4⁺ T cells and antigen-presenting cells, studies also have examined the function of Tr cells on CD8⁺ T-cell responses. It is not surprising that similar mechanisms of action have been demonstrated in the control of CD8⁺ T-cell responses. Tr cells have the potential to regulate the induction of an antiviral CD8⁺ T-cell response as well as the contraction phase of a CD8⁺ T-cell response. In chronic viral infections, Tr cells have the potential to be detrimental by promoting viral persistence or, alternatively, be protective by preventing immune pathology. Finally, Tr cells have the possibility to beneficially modulate the nature of the antiviral CD8 response and in some cases promote the generation of memory CD8⁺ T cells.

B. Inhibition of CD8⁺ T Cells by Tr Cells

Initial in vitro experiments evaluating a potential role for Tr cells in human CD8⁺ T-cell responses demonstrated that co-culture experiments with CD4⁺CD25⁺ cells led to contact-dependent inhibition of proliferation, IL-2 production, IFN- γ production, and perforin/granzyme expression.^{72,73} Tr cells thus are able to modulate the cytotoxic potential of CD8⁺ T cells and natural killer cells.⁷⁴ Other studies demonstrated that human Tr cells can kill activated but not resting CD8⁺ T cells in a contact-dependent manner that may utilize granzymes and perforin.⁷⁵ Granzyme B--deficient Tr cells also were less effective at suppressing CD8⁺ T cells in co-culture studies.⁷⁶

Initial in vivo studies in mice demonstrated that there is an age-dependent increase in memory CD8⁺ T cells that correlates with a decrease in CD4⁺CD25⁺ Tr cells.⁷⁷ Several studies have investigated the role of Tr cells in models of viral infection. Depletion of

TR cells with an anti-CD25 monoclonal antibody (PC-61) augmented the expansion of virus-specific CD8⁺ T cells in mice infected with the Sendai virus without affecting viral titers, and this effect was partially dependent on granzyme B expression in TR cells.⁷⁶ In feline immunodeficiency virus, vaccination with inactivated virus led to the development of virus-specific CD4⁺ TR a manner that was dependent on surface-expressed TGF- β .⁷⁸

Although these studies look predominantly at CD8⁺ T cells, in these models TR cells could indirectly affect antiviral CD8⁺ T cells as well. Proper activation of CD8 T cells requires dendritic cell activation and antigen presentation, and TR cells have profound effects on dendritic cells.^{79–81} TR cells prevent dendritic cell maturation, and immature dendritic cells promote CD8 T-cell tolerance.^{82,83} TR cells promote tolerogenic dendritic cells through a variety of mechanisms, including TGF- β , IL-10, and cytotoxic T lymphocyte antigen-4.⁸⁴

C. Control of CD8⁺ T-Cell Responses by TR Cells: The Role of IL-2

IL-2 is a pleiotropic cytokine with both immune promoting and immune inhibitory functions. Studies have demonstrated that IL-2 is required for the development of memory CD8⁺ T cells, and treatment of mice with IL-2 results in the expansion and development of effector function of CD8⁺ T cells.^{85–87} CD8 T cells that cannot respond to IL-2 *in vivo* can expand on a primary challenge but fail to generate memory.⁸⁸ However, IL-2 is a key cytokine for the survival and function of TR cells.^{89–91} This dichotomous function of IL-2 is demonstrated in humans and mice lacking IL-2 receptors, resulting in a mixture of both cell-mediated and humoral autoimmunity as well as susceptibility to viral infections.^{92–95} TR cells are efficient at competing for IL-2 *in vivo* since they constitutively express the high affinity IL-2 receptor, and this competition is thought to limit the expansion of CD8⁺ T cells.^{90,96} This competition also has been shown to promote the apoptosis of lymphocytes through a Bim-dependent mechanism.⁹⁷

D. Beneficial and Detrimental Effects of TR Control of Antiviral CD8⁺ T-Cell Responses

The suppression of CD8 antiviral responses by TR cells can have beneficial as well as detrimental effects. In chronic viral infections with persistently replicating pathogens, such as hepatitis B virus, HIV, or hepatitis C virus, virus-specific TR cells are generated that inhibit CD8⁺ T cell responses and may contribute to antigen persistence.^{98–106} This apparent detrimental response of TR cells is likely designed to prevent tissue damage by CD8⁺ T cells. Since immune-mediated damage is a major mechanism of tissue damage during a viral infection, control of this damage is necessary to prevent organ dysfunction. TR cells have been shown to prevent immune-mediated damage in several experimental models.^{107–110} These studies demonstrate that a critical balance is necessary to prevent CD8-mediated tissue damage while allowing for viral clearance, which has been demonstrated in respiratory syncytial virus infections.¹¹¹ In situations where this balance is offset, such as in cases of chronic viral infections, the function of TR cells may be detrimental. To add more complexity to this issue, recent data have argued that TR cells promote effective CD8⁺ T-cell responses as well. Although TR cells clearly inhibit CD8⁺ T-cell responses, this effect can also lead to more effective immunity by selecting for specific CD8⁺ T-cell clones. During priming of CD8 responses, TR cells have been shown to promote formation of CD8⁺ T-cell memory by competing for IL-2.¹¹²

V. CONCLUSION

CD8 T-cell responses are critical for controlling viral infection but can cause severe immunopathology if left unchecked. Aspects of CD8 T-cell responses, such as the specificity, magnitude, effector function, or duration, can differ markedly depending upon the nature of the pathogen that elicited the response. Regulation of specific antiviral CD8 T-cell responses is achieved through the coordination of overlapping mechanisms that function to balance

FIGURE 1: Proposed mechanisms of the regulation of antiviral CD8 T-cell responses. Early events in viral infection trigger innate immune mechanisms that support the expansion of virus-specific CD8 T cells to effectively combat a particular infection. Upon clearance of the infectious virus (and viral antigen), these signals change to attain immunological memory and facilitate the contraction of a CD8 T-cell response to limit damage to host tissue. In some instances, sterilizing immunity cannot be achieved and a particular viral infection is considered to be chronic. Regulatory T cells and intrinsic and extrinsic mechanisms of apoptosis work in concert to limit viral specific CD8 T cells. APC, antigen presenting cells; IFN, interferon; IL, interleukin; TR, T regulatory cell

virus-host interactions to achieve minimal detriment to the host (Figure 1). It is clear that in addition to the CD8 T-cell regulation mechanisms reviewed above, other important but yet unidentified factors are likely to be involved; the identification of such factors is an important direction of future research. A better understanding of CD8 T-cell regulation in the context of viral disease can lead to better immune control of viral infection and allow mitigation of immunopathology that may, in some instances, be of greater detriment than the virus itself.

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