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The Immune Fulcrum: Regulatory T Cells Tip the Balance Between Pro- and Anti-inflammatory Outcomes upon Infection

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Abstract

Regulatory T cells (Tregs) are indispensable for immune homeostasis and the prevention of autoimmunity. In the context of infectious diseases, Tregs are multidimensional. Here, we describe how they may potentiate effector responses by assisting in recruitment of T cells into the infection site to resolve infection, facilitate accelerated antigen-specific memory responses, limit pathology, and contribute to disease resolution and healing, to the great benefit of the host. We also explore the villainous functions of Tregs during infection by reviewing several diseases in which the depletion or reduction in Treg frequency allows for better generation of effector memory, and results in acute resolution of infection, as opposed to chronicity or severe long-term outcomes. We describe findings generated using mouse models of infection as well as experiments performed using human cells and tissues. We propose that Tregs represent an immunologic fulcrum, promoting both pathogen clearance and damage control by preventing excessive destruction of infected tissues through unchecked immune responses.

1. INTRODUCTION

Thymically selected regulatory T cells (Tregs), defined by the lineage marker forkhead box P3 (Foxp3), quintessentially promote self-tolerance and limit autoimmunity.¹ Thus, aberrant Treg function is extensively implicated in the manifestation of autoimmune syndromes such as inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis (reviewed in Ref. 2). Most severely, in the absence of Treg function, as exemplified by Treg-deficient scurfy mice, or individuals carrying nonfunctional or impaired versions of the *FOXP3* gene, life-threatening multiorgan autoimmunities and lymphoproliferative diseases manifest during early development.^{3,4} In the context of infection, many studies have demonstrated that Tregs act to dampen overexuberant effector immune responses, yet accumulating evidence suggests that Treg function is in fact far more versatile than a simple provision of immune inhibition, as we will highlight here. Tregs thus participate in diverse mechanisms of immunomodulation in a context-dependent fashion. We herein specifically emphasize Treg responses to pathogens, with a focus on their abilities to potentiate appropriate immune

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responses, and propose that Tregs serve as a fulcrum, tipping the immune system between pathogen clearance and protection from collateral damage.

Tregs are broadly categorized into two groups, thymically derived Tregs (tTregs, or natural nTregs) and peripherally induced Tregs (pTregs, or iTregs). Both subsets are defined by their high expression of IL-2R α (CD25) and Foxp3, although peripheral Tregs may be converted to this phenotype from CD25–Foxp3– precursors under the influence of IL-2 and TGF- β .^{5,6} Efforts to distinguish the phenotype of tTregs from pTregs have identified Helios, a member of the Ikaros transcription factor family, as its expression tends to be enriched on tTregs.^{6–9} However, upon certain *in vitro* culture conditions or immunization schemes, low-level induction of Helios expression from peripheral Tregs and Foxp3+ T cells can be detected.^{6,9,10} tTregs may also be differentiated by their increased expression of PD-1, neuropilin-1, and CD73 as compared to pTregs, whereas both subsets may express activation markers including GITR and CTLA-4.^{6,11} Other types of peripheral Foxp3–CD4+ T cells may also exhibit regulatory functions, such as the secretion of IL-10 (Tr1) and TGF- β (Th3)^{12–14}; furthermore, regulatory CD8+ T cells may express Foxp3+ and produce IL-10.^{15,16} Taken together, peripheral Tregs likely represent a highly plastic population, prone to evolution in response to environmental cues or other impetus.¹⁷ Here, we will focus on classic CD4+CD25+Foxp3+ Treg biology.

Human studies largely identify Tregs based on their expression of CD4, CD25, and FOXP3, with low expression of CD127 (IL-7R). Unfortunately, the necessity of intracellular staining for FOXP3 complicates functional assays from human cells, whereas in mice, *Foxp3^{38fp}* reporter mice provide an excellent mechanism of Treg distinction without cellular fixation.¹⁸ Thus, surface markers must suffice for many functional human analyses. Furthermore, FOXP3 is not a definitive and reliable marker for human Tregs, as it can also be expressed on recently activated CD4+ effector T cells.^{19–22} In mice, Tregs are definitively identified based on expression of CD4 and Foxp3, and both mouse and human Tregs may express CD39, CD73,²³ and activation and trafficking markers such as GITR, ICOS, and CTLA-4.²⁴ Activation and proliferation or polarization markers which are not necessarily specific to Tregs such as CD62L, CD69, CD103, CD44, Ki-67, and Tbet (among many others) may also be present, depending on the circumstance.

Murine models of Treg cell ablation have provided excellent tools for the examination of Treg function, and loss of function, which logistically cannot be achieved in human studies *in vivo*. Germline *Foxp3* dysfunction results in catastrophic autoimmunity, thus necessitating conditional knockout strategies for Foxp3+ Tregs. Foxp3-diphtheria toxin receptor (Foxp3-DTR) mice were created by inserting the human DT receptor under the control of the *Foxp3* locus, thus allowing for the selective ablation of Foxp3-expressing cells upon diphtheria toxin (DT) administration.²⁵ Similarly, bacterial artificial chromosome (BAC)-transgenic “depletion of regulatory T cells” (DEREG) mice were created, again reliant on DT-mediated Foxp3 ablation.²⁶ Both strategies allow for the specific and inducible depletion of Foxp3+ Tregs at desired times, although neonatal but not adult DEREG mice exhibit spontaneous autoimmunity upon DT-ablation of Tregs.^{25–27} Thus, it appears that Treg elimination is incomplete in adult DEREG mice, or cannot be sustained due to the repopulation of Tregs lacking the DT-sensitive transgene.^{27,28} Prior to the availability of Foxp3-DTR and DEREG

mouse models, it was also common to deplete Tregs using monoclonal antibodies against CD25. Whereas many of these studies laid important groundwork for the field, CD25 can be expressed on activated T cells, as well as Tregs, thereby complicating the clarity of these results.

2. ACUTE INFECTIONS IN DELICATE TISSUES

2.1 The Lung

The lung is a site of significant antigen exposure, where inhaled allergens and pathogens are constantly introduced, and must be quickly controlled to maintain homeostasis and avoid airway consolidation. In this regard, sentinel alveolar macrophages maintain the airways in a tolerogenic state, partially via active TGF- β , tethered to the surface of neighboring alveolar epithelial cells.^{29,30} However, upon the introduction of a pathogen, a rapid and tightly regulated immune response must be generated where, ideally, the infection can be cleared with minimal collateral damage, since overly exuberant responses can compromise air exchange and are therefore dangerous to the host.

2.1.1 Influenza Virus—Influenza virus infection elicits a strong proinflammatory cytokine response with abundant lymphocyte and innate cell infiltration, and whereas T cell and antibody responses are generally effective at controlling the virus, serious sequelae may result from acute infection.³¹ Such morbidities become especially pronounced in more susceptible hosts, or during seasons when emerging or highly pathogenic strains are introduced. Tregs thus provide an irreplaceable mechanism for dampening influenza-associated pathology, controlling the magnitude of the immune response, and promoting healing. These dampening Treg functions have been well elucidated in animal models.^{32–35} During primary influenza infection of mice, Tregs accumulate in the lung prior to the influx of antigen-specific CD4+ and CD8+ T cells and suppress effector T cell responses.^{32–34} These responses are retained *ex vivo*, and the adoptive transfer of influenza-exposed Tregs provides protection to infected recipients. More dramatically, the adoptive transfer of Tregs ameliorates influenza virus-associated morbidity and prolongs survival in immunodeficient *Rag1*^{−/−} mice, whereas effector CD4+ T cells do not facilitate the same protection.³² As *Rag1*^{−/−} mice are significantly immunocompromised, the repletion of Tregs is insufficient to control viral load, yet Treg introduction delays the accumulation and organizes infiltrating monocytes, providing protective effects for tissue preservation, potentially through CCL8 suppression.³²

Treg-mediated suppression appears to be antigen specific, as several groups have identified influenza-specific Tregs that acquire functional specialization by secreting IL-10, and upregulating activation markers CTLA-4, GITR, CD44, CD69 and ICOS, and the T_H1-associated transcription factor, Tbet.^{33,34} In line with this evidence, Brincks *et al.* have shown that influenza-specific Tregs additionally acquire a memory phenotype, and are uniquely adept at trafficking to the lung and draining lymph nodes (dLNs) upon rechallenge with a heterologous virus strain.³⁶ Memory Tregs then control the intensity of memory T cell responses and protect the host from collateral damage in an MHC II-dependent, antigen-specific manner. These results cannot be recapitulated by replacing memory Tregs with

naïve Tregs, further implicating long-term immune-shaping effects of Tregs on both primary and memory responses to influenza virus.³⁶

While the importance of Treg-mediated immune suppression should not be overlooked, particularly in delicate lung tissue, unexpected non-suppressive roles for Tregs during influenza infection are beginning to be elucidated as well. For example, León *et al.* demonstrated that Treg depletion impairs the germinal center (GC) response to influenza virus challenge by impairing T follicular helper (T_{FH}) cell function.³⁷ In this context, Tregs compete for IL-2, which is inhibitory to T_{FH} function, and in their absence, an overabundance of IL-2 impedes the correct organization of the effector response in the lung and dLN. This work highlights the multidimensional role for Treg function in the lung, placing emphasis on the ability of Tregs to orchestrate prudent effector responses by encouraging GC formation, and regulating primary and memory responses to influenza virus.

2.1.2 Respiratory Syncytial Virus—Respiratory syncytial virus (RSV) infection is another prevalent respiratory illness and constitutes a major concern in infants due to its potential to result in severe outcomes, such as bronchiolitis.³⁸ Additionally, early childhood RSV infections are known to drive strong T_H2-dominated responses, placing affected children at a higher risk for asthma later in childhood.^{39,40} Attempts at developing a safe vaccine against RSV have failed to date, most famously in the case of a formalin-inactivated RSV vaccine candidate that unexpectedly enhanced the acquisition and severity of natural infection in vaccinees.⁴¹ It is thought that the generation of inappropriate antibody epitopes and a lack of local CD8+ T cell responses played a role in this failure, though studies in mice using a similar vaccine demonstrated that a “crash” in Treg presence may also be contributory.⁴² In this regard, immune responses to RSV are enigmatic in that natural infection drives incomplete immunity, allowing for viral reinfection, and while passive antibody transfer can be protective, natural antibody titers do not always correspond with protection.³⁸ Effector CD8+ T cell-mediated immunity appears to better correlate with protection and viral control (although some caveats exist for these rules), but paradoxically, T cell responses can contribute to enhanced immunopathology and disease severity.³⁸ These scenarios strongly imply a necessity for regulatory mechanisms.

Many groups have shown that RSV infection elicits the expansion and activation of Tregs in the lung parenchyma, airway spaces (as assessed by bronchoalveolar lavage), and dLN.^{43–46} Upon Treg ablation, animals lose significantly more body weight and experience a protracted recovery period.^{43,44,46} Perhaps not surprisingly, the peak of weight loss correlates with disease severity and has been reported to be accompanied by innate immune cell infiltrate, and activated NK cells, and T cells which express high levels of CD69 and secrete IFN- γ .⁴³ In other reports, Treg depletion results in strong T_H2-driven immune responses, with IFN- γ and IL-13-producing CD4+ T cell recruitment, and abundant eosinophilia.⁴⁶ There exists some disagreement regarding the effect of Treg ablation on viral load, although the timing of analysis post-RSV challenge seems to impact these conclusions.^{43–47} To distill several reports, it appears likely that whereas the peak titer of RSV may remain unaffected, viral clearance may be delayed (although not inhibited) by Treg ablation. Emphasis on the timing of response has been further highlighted by several

groups who report that the depletion of Treg cells prior to RSV challenge impairs the early egress of RSV-specific CD8⁺ T cells from the dLN into the lung.^{44,45} Such a delay in the antiviral response permitted a protracted infection course, despite a later influx of polyfunctional CD8⁺ T cells producing IFN- γ and TNF- α .^{44,45} Thus, Tregs serve an important role during acute RSV infection by facilitating the early trafficking of antigen-specific CD8⁺ T cells into the effector site. Moreover, Ruckwardt *et al.* report that not only are CD8⁺ antigen-specific T cell responses delayed in accumulating in the lung in Treg-depleted mice, but the viral epitopes to which the responses are directed are also altered, tipping the balance toward an increased frequency of immunodominant epitope-specific CD8⁺ T cells.⁴⁵

Interestingly, Treg ablation apparently exerts an opposite effect on memory responses to RSV, as memory CD8⁺ RSV-specific T cell responses are increased in frequency in mice depleted of Tregs during acute RSV infection.⁴⁵ The explanation of acute versus memory discrepancies has yet to be formally revealed. However, it seems reasonable to assume that since the antigen-specific CD8⁺ T cell responses normalize at later timepoints during acute infection, and that an increased production of cytokines and associated damage ensues, stronger, and possibly dysregulated, CD8⁺ T cell responses in Treg-ablated mice could lead to an increased frequency of memory T cells. Taken together, we conclude that Tregs provide essential control of early effector cell recruitment, and later limitation of excessive cellular infiltrate and cytokine production in the lung, thereby promoting efficient and appropriate effector immunity, and aiding the resolution of inflammation.

2.1.3 Mycobacterium tuberculosis—*Mycobacterium tuberculosis* (Mtb) infects tolerogenic alveolar macrophages, where it slowly replicates until the bacteria kill the overwhelmed cell, or T cell responses are finally generated to command the phagocyte to mount an antibacterial response.⁴⁸ Mtb is a master at manipulating immune responses, and acute exposure may result in chronic infection. In this regard, the exact requirements for successful bacterial clearance and disease resolution remain enigmatic, although many lines of evidence indicate that robust, early effector T cell responses are critical for disease resolution.^{48–50}

Upon infection of mice, Tregs (including recently identified Mtb-specific Tregs⁵¹) have been shown to expand in the dLN and lung early during infection and upregulate activation markers such as CTLA-4, GITR, and ICOS.^{51,52} Human peripheral and pleural Tregs similarly respond and expand upon exposure to *M. tuberculosis*,^{53–55} and several groups have reported a correlation between chronically elevated frequencies of Tregs in tuberculosis patients with active disease, compared to far lower frequencies in individuals who resolve infection, or who were never infected.^{53,56,57} The theme from these publications would implicate Treg responses as inhibitory to pathogen clearance and disease resolution. In contrast, Wergeland *et al.* reveal that increased frequencies of Tregs are accompanied by increased frequencies of activated (HLA-DR+CD38+) T cells in the blood of Mtb-infected individuals during the acute versus latent stage of infection, which were both elevated over frequencies in naïve individuals.⁵⁸ These data may simultaneously argue for Treg-mediated protection against the detrimental effects of chronic immune activation, which notoriously predisposes individuals to unrelated health complications.

Investigations using mouse models of Treg ablation have revealed additional complexities. Treg ablation has been reported to either reduce Mtb bacterial load in the lungs⁵² or have little effect on bacterial clearance.⁵⁹ Ozeki *et al.* have reported that the timing of Treg depletion appears to be most important, as a transient, early Treg ablation results in superior bacterial clearance and decreased granuloma formation at 2 weeks postinfection, but elicits no effect on later bacterial load or lung pathology, as assessed at 3 and 5 weeks postinfection.⁶⁰ Moreover, Leepiyasakulchai *et al.* show that in the lungs (but not LNs) of highly susceptible DBA/2 mice, despite sustained high Mtb load, the frequency of Tregs decreases at 3 weeks postinfection, and this declination continues through chronicity.⁶¹ Thus, expanded Treg frequency may not always be directly linked to the inhibition of pathogen clearance, and here the authors suggest a role for both CD103+ dendritic cells (DCs) and Tregs in promoting immunity toward Mtb challenge.⁶¹

2.1.4 Fungal Infections—Immunocompetent individuals rarely acquire pathogenic fungal infections of the lung; however, for susceptible individuals such as those who are immunosuppressed due to HIV infection, bone marrow transplantation, or chemotherapy, infections with organisms such as *Candida*, *Pneumocystis*, *Aspergillus*, *Cryptococcus*, and *Coccidioides* are more common. Our discussion of the fungal-Treg axis will be very brief, as current literature regarding a role for Tregs is fairly limited. There are, however, some lines of evidence for both Treg-mediated mitigation of pulmonary inflammation^{62,63} and Treg-mediated inhibition of antifungal responses.⁶⁴ Using a mouse model of *Pneumocystis* pneumonia and immune reconstitution inflammatory syndrome (IRIS), McKinley *et al.* report that both anti-CD25-mediated depletion of regulatory T cells in wild-type mice, and the reconstitution of *Pneumocystis*-infected SCID mice with CD4+CD25[−] T cells elicit proinflammatory cytokine production, whereas the reconstitution of SCID mice with Tregs has no effect on disease exacerbation, but augment *Pneumocystis* burden.⁶² As *Pneumocystis* infection remains a health concern, especially for HIV+ individuals with low CD4+ T cell counts, the authors propose that Treg dysfunction may partially underlie the observed pathogenic responses of IRIS.⁶² Schulze *et al.* report a more dramatic phenotype of Treg ablation in mice infected with *Cryptococcus neoformans*.⁶³ Tregs expand and accumulate in the lung during *C. neoformans* infection to control T_H2-mediated inflammation, and in their absence, eosinophilia, overt mucus production, T_H2-skewed cytokine production, and fungal burden are exacerbated.⁶³ In contrast, the early depletion of Tregs during *Para-coccidioides brasiliensis* infection reduced tissue pathology and fungal burden in the lungs, liver, and spleen of two genetically diverse strains of mice, representing variation in resistance to *P. brasiliensis* infection.⁶⁴ Finally, Tregs have been shown to promote acute T_H17 cell responses to *Candida albicans*, allowing for enhanced fungal clearance, while at later timepoints they had suppressive roles.⁶⁵ These functions were mediated by IL-2 competition, especially at early timepoints, again indicating the importance of appropriate timing in response profiles.

2.2 The Brain

2.2.1 West Nile Virus—Studies which have interrogated the role for Tregs in clearing neurotropic viruses from the central nervous system (CNS) and brain similarly highlight the necessity for a regulated balance of effector and regulatory T cell responses. For example,

sterilizing immunity against West Nile virus (WNV) requires the recruitment of CD8⁺ T cells into the CNS,⁶⁶ although these responses must be regulated to avoid excessive damage to nonrenewable neurons. In agreement with this hypothesis, Lanteri *et al.* demonstrate that individuals who remain asymptomatic during West Nile infection have an increased frequency of Treg cells in peripheral blood, as compared to individuals with more severe disease.⁶⁷ Their findings are further corroborated in a mouse model of Treg ablation in which case Treg-deficient mice similarly suffer an increase in disease severity, weight loss, and lethality.⁶⁷ A link between these complex and pendulous mechanisms has been recently reported by Graham *et al.* who show that Tregs work to limit both the proliferation and inflammatory cytokine secretion of T cells in the CNS, while simultaneously encouraging the retention and maintenance of protective resident memory T cells in the brain.⁶⁸ Moreover, an early Treg presence in the brain is important to this protection.⁶⁸ Such a dual role for Tregs during infectious disease is thus becoming a repetitive theme where both the timing and the calibration of Treg responses are essential to a positive outcome, particularly in tissues with unique requirements or delicate architecture.

3. ACUTE SYSTEMIC VIRAL INFECTIONS

3.1 Dengue Virus

Dengue virus, similar to WNV, is another example of a vector-borne acute flaviviral infection which can present with a spectrum of symptom severity and pathology. Instances of extreme disease severity are characterized by cytokine storm, life-threatening vascular leakage syndrome, and hemorrhagic fever or shock.⁶⁹ Immune control of dengue virus, particularly in the context of a heterologous strain challenge, can be enigmatic as cross-strain reactive antibody and memory T cell responses often amplify viral replication and associated pathology.⁶⁹ Interestingly, children suffering more severe outcomes during acute dengue virus infection harbor reduced frequencies of Treg cells in peripheral blood.⁷⁰ However, Treg function in terms of cytokine secretion and suppressive abilities remain unaltered despite the infection course.⁷⁰ Immunohistological analysis of liver biopsies from adults with severe dengue hemorrhagic fever similarly shows high expression of proinflammatory markers, TLR2 and TLR3, iNOS, IL-6, IL-18, TGF- β , and granzyme B, whereas Foxp3 was notably undetectable.⁷¹ Along these lines, mouse models have shown a lack of Treg expansion in the spleen in response to dengue viral challenge.⁷² A conclusive role for Tregs in response to dengue virus infection remains elusive, but again appears to depend on appropriate effector timing. This may be particularly important in the context of reinfection with a disparate strain of dengue virus.

3.2 Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis virus (LCMV) of mice is a widely used model for both acute and chronic systemic viral infection. Adult mice infected with LCMV strain Armstrong (Arm) undergo acute infection, which is resolved by robust T cell responses roughly 8–10 days after challenge, whereas mice infected with the strain clone 13 (Cl13) remain chronically infected.^{73,74} Akin to other viral infections, chronic LCMV infection results in the exhaustion of effector T cells which upregulate PD-1 and further exhibit impairment in cytokine secretion and killing.⁷⁵ Moreover, mice chronically infected with LCMV elicit an

increased frequency of splenic Tregs bearing a heightened activation marker expression profile.⁷⁶ Thus, it has been suggested that Tregs actively constrain CD8⁺ T cell responses during chronic infection, allowing for viral persistence. Upon Treg ablation of chronically LCMV-infected mice, CD8⁺ T cells rapidly rebound in both number and function including proliferation, production of IFN- γ , TNF- α , CD107a/b and granzyme B, and target cell killing after antigen-specific *ex vivo* stimulation.⁷⁶ Interestingly, whereas rescued CD8⁺ T cells from Treg-depleted mice showed restored functionality *ex vivo*, these responses have no effect on controlling the LCMV viral burden. The authors go on to show that Treg depletion elicited an upregulation of PD-L1, in particular by LCMV-infected cells, providing an additional link between T cell exhaustion and Treg function. Viral control could be restored only when chronically infected mice were both Treg-depleted and dosed with PD-L1 blocking antibody.⁷⁶

Early type one interferon (IFN) signaling is pivotal to infection outcome for both acute and chronic LCMV infections. As acute infection (with strain Arm) is resolved, IFN- α/β dissipates, whereas during chronic infection (with Clone 13) IFN- α/β remains elevated, as do other indicators of immune activation.⁷⁷ Systemically depleting IFN- α/β after chronic infection can improve disease outcome by ameliorating chronic immune activation and viral burden, and restoring lymphoid architecture.^{77,78} Along these lines, within 2 days postacute infection with LCMV Arm, IFN- α/β is known to act directly on activated Treg cells to provide inhibitory signals.⁷⁹ Downstream, the effect of early IFN- α/β -mediated Treg inhibition is the allowance of optimal CD4⁺ and CD8⁺ effector T cell expansion, which act to clear the virus. Tellingly, Foxp3-DTR mice which had undergone DT-mediated Treg ablation, and were reconstituted with *Ifnar1*^{-/-} Tregs, were more susceptible to LCMV infection severity due to poor production of IFN- γ , reduced killing capacity of CD8⁺ T cells, and an increased viral load at day 7 postinfection.⁷⁹ In this regard, the transient, early inhibition of Tregs appears to promote protection by encouraging the generation of effector immunity. Complete Treg ablation during early acute infection, however, is highly detrimental to viral clearance.⁸⁰ Treg-ablated mice harbor an increased liver viral load at day 5 post-LCMV Arm challenge as compared to intact mice.⁸⁰ Taken together, viral resolution appears to rely on the generation of timely effector T cell responses, which are allowed by transient IFN- α/β interference of Tregs. Paradoxically, Treg presence is necessary for viral clearance, implying additional functions of Tregs during LCMV infection.

4. GASTROINTESTINAL INFECTIONS

The gut and intestines are constantly challenged by a dense array of food antigens, normal microbiota, and potential pathogens, implicating Tregs as a prevalent mechanism of controlling tolerance and pathogen breach in these sites. During oral rotavirus challenge of mice, Tregs expand significantly in the spleen, mesenteric lymph nodes, and gut-associated lymphoid tissue.^{81,82} However, the ablation of Tregs has no effect on measurements of disease severity including diarrheal incidence, viral shedding, or early IgA responses, despite an expansion of T and B cells, upregulation of activation markers such as CD69, and increased IFN- γ production, with concurrent loss of IL-10.^{81,82} Similarly, during *Listeria monocytogenes* infection, the depletion of Tregs prior to challenge with rLM-OVA does not affect the ability of the mice to clear bacteria during primary challenge.⁸³ However, Treg

ablation does impair antigen-specific CD8⁺ T cell responses in terms of number and avidity during the peak of the primary immune response. Such primary impairment may have grave effects on memory responses, and indeed, during secondary bacterial challenge, those mice which had been depleted of Tregs prior to primary infection were significantly less efficient at clearing bacteria. This could be attributed to a reduction in the overall numbers and affinity of antigen-specific T cells, as well as their ability to dually produce IFN- γ and granzyme B upon *ex vivo* stimulation.⁸³

Further evidence for Treg potentiation of effector immunity during intestinal infection comes from investigations of *Citrobacter rodentium*. *C. rodentium* notoriously drives proinflammatory T_H17 responses for effective bacterial clearance, which must be tightly controlled to limit damage. T_H17 cells share a unique “reciprocal” and plastic¹⁷ relationship with intestinal Tregs through their shared expression of ROR- γ t and responsiveness to TGF- β ,^{84,85} indicating their cooperative balance. Wang *et al.* show that Treg depletion prior to and during acute *C. rodentium* infection significantly exacerbates morbidity, mortality, and systemic bacterial dissemination.⁸⁶ The mechanisms underlying exacerbated disease appear to be mediated by a loss of T_H17 cell and neutrophil frequency and function, specifically at the site of infection. Moreover, neutralizing IL-2 counteracted the exacerbated disease in Treg-deficient animals, indicating that Treg-mediated control of IL-2 availability in the mucosa promotes appropriate T_H17 cell responses. Conversely, during *Salmonella* infection, Treg ablation was beneficial to the host in accelerating bacterial clearance, as well as leading to timely activation of effector T cells.⁸⁷ Again, the role of Tregs in infection, with gastrointestinal infections highlighted here, proves to be dynamic in nature, with Treg responses and cooperation with effector cells in a fluid state of change, a theme in common for many infectious disease scenarios.

5. CHRONIC INFECTIONS

5.1 Herpes Simplex Virus

Infection with herpes simplex virus (HSV) type-1 and type-2 elicits a dominant T cell-mediated response, in which successful resolution of primary disease requires a carefully orchestrated balance of IFN and toll-like receptor signaling, and antigen-presenting cell and T cell trafficking.^{88,89} Importantly, Tregs serve an indispensable function in orchestrating these effector responses. Upon intravaginal challenge of mice with HSV-2, CD11b⁺ submucosal DCs and plasmacytoid DCs provide early antigen presentation,⁹⁰ and IFN,⁹¹ respectively. T cell responses subsequently follow, specifically leading to both effector T cell and Treg activation, expansion, production of effector cytokines, and trafficking to the vagina and dLN.⁸⁰ In the absence of Tregs, HSV-2-infected mice succumb to infection rapidly, whereas Treg-sufficient mice succumb more slowly to fatal infection. Specifically, following Treg ablation, viral replication, and dissemination into spinal cord tissue is augmented, concurrent with clinical disease severity and mortality.⁸⁰ In the absence of Tregs, strong effector responses and the production of proinflammatory cytokines (TNF- α , IL-2, and IFN- α/β and IFN- γ) ensue in the dLN. However, early local cytokine signaling and T cell trafficking into the vagina are notably impaired, due to dysregulated retention of effector lymphocytes in the dLN.⁸⁰ Cellular egress into the vagina relies on correct and

complex chemokine gradients, which were found to be altered upon Treg ablation, and therefore linked to the impairment of cellular trafficking in the absence of Tregs.⁸⁰ Thus, Tregs coordinate the timely balance of effector cell recruitment and trafficking into the vaginal mucosa, control the chemokine gradient, and suppress overly exuberant proinflammatory responses to the great benefit of the host.^{80,92}

During ocular infection with HSV-1, adept viral control similarly relies on strong effector immunity, and again, associated collateral damage can be highly detrimental in the eye. Specifically, recurrent effector immune responses to viral recrudescence are a prevalent cause of blindness.⁸⁸ In this regard, Tregs act to ameliorate disease pathology and severity by limiting neutrophil and T cell migration into the delicate site, and secreting IL-10 and TGF- β .^{93,94} Conversely, when HSV-1 is delivered as a subcutaneous challenge, where collateral damage may not be as costly, Treg-mediated suppression of effector T cell responses is arguably detrimental to the intensity of the response.^{95,96} In this scenario, Treg ablation enhances both primary and memory vaccine-amnestic T cell functions after CD8+ T cell epitope immunization, and as a result, primed effector T cells in Treg-ablated mice are more successful in clearing virus.^{97,98} Both of these scenarios implicate Treg function as pivotal to well-balanced immunological outcomes where viral clearance and the preservation of tissue architecture and function may both be attained.

5.2 Human Immunodeficiency Virus

Human immunodeficiency virus (HIV) uniquely infects and is maintained in CD4+ T cells and susceptible APCs, posing a major hurdle for the generation of effective antiviral immunity. HIV-infected individuals remain infected for life, and most suffer consequent health complications including chronic immune activation and increased susceptibility to unrelated pathogens, cancers, and inflammatory disorders.⁹⁹ Correlates of protection against HIV acquisition and cure remain unknown, though some hints derived from rare individuals who are refractory to HIV acquisition or progress significantly more slowly than average remain an intensive area of interest.^{100–104} In this regard, a role for Tregs in HIV acquisition and disease progression is multifaceted. Akin to other infections reviewed here, the timing and phase of immune responses to HIV pivotally impact disease outcome.

Highly HIV-exposed seronegative (HESN) individuals provide a unique opportunity to investigate natural resistance to HIV acquisition, which has been proposed to be partially facilitated by unique Treg responses. For example, commercial sex workers that have remained uninfected for at least 7 years, despite high HIV exposure, uniformly show heightened frequencies of Tregs, and a concurrent reduction in the frequencies CD69+-activated T cells.¹⁰⁵ Similar results have been shown in terms of reduction in immune activation for other cohorts of HESN,^{102,106,107} potentially implying a contributory role for Tregs in protection from HIV infection. Along these lines, seronegative infants born to HIV-positive mothers, who were not receiving antiretroviral treatment during pregnancy, were found to have enhanced frequencies of Tregs and a reduction in the frequencies of activated effector T cells in cord blood.¹⁰⁸ Thematically, these studies implicate a state of low immune activation, expanded Treg numbers, and overall immune quiescence in resistance to HIV acquisition. Conversely, Pattacini *et al.* demonstrate that a subset of HESN individuals

from a cohort of heterosexual serodiscordant couples have impaired Treg-mediated suppression of HIV-specific CD4⁺ T cell responses.¹⁰⁹ Interestingly, MIP-1 β was found to be upregulated in these individuals, and since the autocrine signaling of MIP-1 β is known to block the dominant HIV coreceptor, CCR5, Treg allowance of enhanced MIP-1 β signaling could play a role in protection from HIV acquisition.¹⁰⁹

HIV disease progression is also impacted by Treg frequency and function. Studies examining the frequencies of Tregs in long-term nonprogressors and elite controllers, who have significant delay in disease progression and/or exert some control over HIV infection, have found that Treg frequencies are similar to, or less than, those of uninfected control individuals.^{110,111} Importantly, whereas a reduction in the frequency of Tregs may allow for better viral control, immune activation and associated comorbidities were found to be heightened in these cohorts.^{110–112} Moreover, others have shown that a dominant Treg presence during HIV infection may better allow viral persistence by detrimentally inhibiting effector T cell responses.^{113,114} Taken together, the role for Tregs during HIV infection is central, and extremely context dependent, reliant upon virus–host interaction, and disease phase.

5.3 Hepatitis B and C Viruses

Hepatitis B (HBV) and hepatitis C viruses (HCV) are members of disparate viral families, Hepadnaviridae and Flaviviridae, respectively; however both are hepatotropic and require similar T_H1 and CTL-dominated immunity for successful disease resolution.¹¹⁵ Infection with HBV or HCV results in one of two outcomes—viral clearance or chronicity, where the relative vigor of the acute immune response to infection controls disease outcome. A robust and early T_H1 response is thought to be sufficient to clear acute infection; however decreases in T_H1 intensity or T_H2 skewing tend to result in chronicity.^{116,117} Interestingly, 95% of HBV infections spontaneously resolve, whereas less than 30% of individuals are able to acutely control HCV infection.¹¹⁵ Some have hypothesized that CD8⁺ T cell exhaustion, and the loss of CD4⁺ T cell help may together be responsible for the inability to clear virus,¹¹⁷ although a second body of evidence suggests that a shift to favor Treg induction promotes viral persistence for both HBV and HCV.^{118–123} In support of this argument is the observation that the proportion of Tregs is increased in chronically infected individuals in both the liver and periphery.^{117–127}

Functionally, CD4⁺CD25⁺ Tregs isolated from chronically infected HCV patients inhibit peptide-specific CD8⁺ T cell proliferation, IFN- γ production, and perforin expression reliant on cell-to-cell contact.^{125,126,128} Li and colleagues further show that CD25 and Foxp3 are both upregulated upon antigenic restimulation *in vitro*, suggesting that these cells are HCV specific, and responsive.¹²⁹ IL-10-producing CD4⁺ (Tr1) T cells are additionally found to be upregulated during chronic HCV infection and some groups suggest that regulatory Tr1 cells are the subset responsible for effector T cell interference during chronic HCV infection.^{119,130,131} Along these lines, Eckels *et al.* noted that a lack of IL-2, and low levels of IFN- γ , in combination with high levels of IL-10, corresponds to HCV viral chronicity, whereas the production of IFN- γ with little to no IL-10 corresponds to a self-limited infection.¹³²

Viral factors, such as an HCV-associated promotion signal, may engage Treg expansion, thus encouraging immune tolerance to the virus and allowing a prolonged infection.^{133,134} Alternatively, the body may induce the shift to Treg cell production in an attempt to mitigate cellular damage caused by the caustic primary antiviral immune response. The liver is an inherently tolerogenic environment where DCs secrete high levels of IL-10, and the surrounding hepatocytes naturally produce TGF- β in order to sustain these liver DCs. Moreover, recent evidence additionally implicates upregulation of the healing and T cell-suppressive factor amphiregulin in response to chronic HBV infection.¹³⁵ Thus, environmental cues in the liver may additionally contribute to the propensity for a host-mediated induction of Tregs during infection.^{119,131} Taken together, Tregs arguably provide the host both benefit and detriment, by permitting long-term chronicity, but also protecting from excessive liver damage and hepatocellular carcinoma.¹¹⁹

5.4 Parasitic Infections

Parasitic disease is a major global health concern, especially in areas where climate and poverty synergize to allow for transmission. The complex lifecycle of parasites combined with vector involvement adds delay and difficulty in generating sterilizing immunity and durable memory responses.¹³⁶ Moreover, immunity to these types of infections is additionally convoluted in that natural infection often induces T_H2-driven tolerance rather than clearance. A role for Tregs is equally enigmatic. For instance, the nematode *Heligmosomoides polygyrus* can drive the differentiation of Tregs from naïve CD4⁺ T cells through the engagement TGF- β ,¹³⁷ and *Litomosoides sigmodontis* requires Treg induction to establish long-lived infection.¹³⁸ In contrast, for African trypanosomiasis, Tregs are known to limit host and parasite-derived pathology, without compromising parasite clearance,¹³⁹ and the transfer of Tregs improves pregnancy outcomes in mice infected with *Toxoplasma gondii*.¹⁴⁰ Liver disease attributed to *Schistosoma mansoni* is limited by Tregs, and upon their removal, liver granulomas are augmented.¹⁴¹ Intriguingly, *Leishmania major* infection elicits an expansion of Tregs to establish chronic infection, yet the long-term exposure of antigen paradoxically allows for the generation of strong protective memory responses upon subsequent reexposure.¹⁴² In these studies, mice which generated complete sterilizing immunity during primary infection were not protected against secondary challenge, implicating a role for host–pathogen coevolution.¹⁴²

Efficacious immunity to malaria infection is thematically different from other parasitic infections in that T_H1-dominated responses prevail, and protect, with the caveat that reliable memory responses often remain absent. Upon acute malaria infection, IFN- γ -production and antibody responses are essential to parasite control.¹⁴³ Perhaps not surprisingly, *Plasmodium* infection (of various species) in both mice and humans elicits the expansion of Tregs (reviewed extensively in Ref. 144). Importantly, the ratio of effector T cells to Tregs seems to be most indicative of outcome, with increased frequencies of Tregs correlating to an increased parasite burden, although these ratios also appear to be a “moving target,” in that T cell frequencies react fluidly to disease severity and stage. Moreover, adults and children show conflicting results in that Gambian children with both uncomplicated and severe malaria disease show increased Treg frequencies, which are correlated with high parasite burden, whereas increased Treg frequencies in Papuan adults could only be correlated with

an increased parasite burden during severe disease.^{144–146} Importantly, in the cohort of children, parasite loads exceeded 10^5 parasites per microliter of blood in both severe and uncomplicated disease, whereas infection of adults only reached such extreme concentrations of parasitemia upon complicated infections, potentially indicating a parasite burden threshold for Treg expansion.^{144–146} Along these lines, using a mouse model of cerebral malaria, Amante *et al.* show that delivery of therapeutic anti-CD25 protects against neurologic disease and parasite burden in the brain, even after the establishment of blood-stage infection.¹⁴⁷ Interestingly, these mice did not show increased neurologic damage. In this regard, the distillation of Treg biology during parasite infection is convoluted, where discrepancies in consensus details appear to rely on the model, ethnicity, age, and timing and burden of parasitemia in relation to effector and regulatory immune responses. However, it seems reasonable to propose that similar to many other infectious diseases, an appropriate and well-timed Treg response provides important protection by limiting pathology, and in certain scenarios, potentiating protective memory.

6. CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, Tregs are an indispensable subset of T cells that not only provide governance of self-tolerance but also critically impact disease outcomes during infection. As we have extensively discussed, depending on the timing, the pathogen, and the severity of disease, Tregs may potentiate protective responses via recruiting mechanisms, dampen overexuberant responses, diminish tissue pathology, and aid in resolution and memory responses upon secondary challenge. Alternatively, various published reports ascribe a more villainous component to Treg cell function, as effector T cell responses may be pathologically diminished by Tregs, negating protective effects for T cell responses.

Whereas Treg interactions controlling effector T cell responses have been explored in many infection models, a role for Treg control of B cells and humoral responses is less well defined. It is known that the loss of function of Foxp3 in mice and humans causes dysfunctional humoral immunity with spontaneous GC formation, recruitment of T_{FH} cells, and the overproduction of IgE, implicating an undeniable role for Tregs in management of B cell responses.^{148–151} In line with these findings, the short-term depletion of Tregs increases the frequency of B cells of several phenotypes and states of maturation.¹⁵² Phenotypic data additionally shows that Foxp3⁺ Tregs are known to upregulate the expression of the T_{FH} markers CXCR5 and Bcl6, allowing them to enter the GC and exert control over GC B cell expansion.^{150,153,154} It has been suggested that CTLA-4-dependent suppression is important for these results.¹⁵² However, contrasting evidence from León *et al.*, who utilized an influenza model in mice, shows that Treg depletion prevents the formation of GCs.³⁷ Thus, a role for Treg-mediated governance of B cell responses in terms of autoimmunity, innocuous antigen exposure, and infectious disease is likely disparate and will necessitate more in-depth analysis.

Finally, we have described several examples of antigen-specific Tregs presence and function during infectious disease.^{36,51} However, it remains unknown whether Tregs are always antigen specific, or if global bystander functions may also be in play, and the circumstance for each response. Moreover, it remains unknown whether infection drives the conversion of

conventional CD4⁺ T cells into pTregs. Gagliani *et al.* have begun to elucidate some of these mechanisms by fate tracking of peripherally induced Tregs, which they report to have transdifferentiated from T_H17 cells.¹⁷ However, very few transdifferentiated cells expressed Foxp3, despite abundant detection of IL-10-producing Tr1 cells. These data are exciting, and such models could be pursued for a variety of infections and lineages beyond T_H17, as to date the accurate distinction of thymically derived Tregs from peripherally induced Tregs has been hindered by overlapping phenotypic signatures in both subsets.

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