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The Role of Bystander T Cells in CNS Pathology and Pathogen Clearance

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Abstract

It is generally accepted that both self- and pathogen-specific T lymphocytes have the potential to mediate immunopathogenesis and contribute to a variety of human ailments. Despite this unfortunate tendency to induce tissue injury, these cells are guided by interactions with peptide-loaded major histocompatibility complexes (MHC) and adhere appropriately to a vital evolutionary constraint imposed by the host: specificity. More recently, a series of studies have demonstrated that bystander T cells of an irrelevant specificity can bypass peptide/MHC restriction and become active participants in immunopathology. This review critically evaluates the role of bystander T cells in immunopathogenesis and pathogen clearance in the periphery as well as the central nervous system and attempts to establish the likelihood of their participation in human disease.

Keywords

brain; activation; virus; LCMV; autoimmunity; herpes stromal keratitis

I. INTRODUCTION

An important evolutionary constraint imposed by the host on the adaptive immune system is that of specificity. The pioneering studies of Zinkernagel and Doherty^{1–4} through the 1970s led to the discovery of major histocompatibility complex restriction—a discovery worthy of the Nobel Prize.⁵ The fate of T lymphocytes is routinely determined by the impositions of major histocompatibility complexes (MHC). From their education in the thymus to their exquisite ability to distinguish an invading pathogen from self, T lymphocytes rely heavily on guidance from MHC displaying foreign and self-derived peptides. In fact, the responsiveness of T lymphocytes is usually dictated by peptide/MHC complexes. The present dogma indicates that it is the cellular context within which these peptide/MHC complexes are displayed that enables T lymphocytes to make the vital distinction between what is self and what is foreign. This concept has been popularized and elegantly captured in a single word—*danger*.⁶ Host antigen-presenting cells possess the ability to innately sense foreign (or “dangerous”) material through pattern recognition receptors.⁷ This ancient machinery has likely evolved to allow immediate responsiveness to common elements of

invading pathogens. Professional antigen-presenting cells (e.g., dendritic cells) respond dramatically to danger signals by changing their surface expression of a variety of molecules involved in adhesion, costimulation, and signaling. This rapid alteration in cellular display in turn modifies the overall context in which peptide/MHC complexes are presented. T lymphocytes respond to cognate peptide/MHC complexes displayed in this manner through activation, clonal expansion, and acquisition of effector function.⁸ These activated T lymphocytes are also licensed with the ability to migrate throughout the host in an attempt to purge pathogens from other cells displaying similar peptide/MHC complexes.

It is likely that the intricate reliance on specificity through peptide/MHC recognition serves to maintain our livelihood by minimizing the amount of tissue injury that occurs when warding off an invading pathogen. T lymphocytes are equipped with a vast array of biological machinery that, when necessary, enables rapid elimination of infected host cells through the induction of cellular apoptosis.^{9–12} Despite the inherent ability of T lymphocytes also to cleanse infected cells in a noncytopathic manner,^{13–15} the maintenance of cytopathic strategies for pathogen clearance through the evolutionary selection process underscores the importance of these strategies in host defense. However, use of such noxious mechanisms does not come without a price. It is well known that T lymphocytes can mediate a significant (and sometimes fatal) amount of immunopathology while attempting to eliminate an infectious agent.¹⁶ This scenario, which is a major cause of human disease, is an unfortunate consequence of the design of the adaptive immune response. T lymphocytes are indeed responsible for these transgressions, yet it is clear that the immunopathology results from nothing more than a biologically ingrained response. T lymphocytes under these circumstances are continually guided by peptide/MHC restrictions and appear to remain loyal to the concept of specificity. It is rather the localization or nature of the pathogen that tips the balance in favor of overt disease. For example, replication of a pathogen in a sensitive anatomical region such as meningeal cells (which make up the lining of the central nervous system) can result in fatal immunopathological consequences (Fig. 1).¹⁶ Meningeal cells readily express antigen-presenting machinery and thus can be engaged directly by cytotoxic lymphocytes (CTL).¹⁷ In an attempt to eliminate pathogens from this sensitive anatomical region, CTL employ cytopathic mechanisms and induce cellular apoptosis of meningeal cells (Fig. 1). Unfortunately, this can result in alterations in intracranial pressure, which can be fatal in animal models¹⁶ as well as humans.¹⁸

Another scenario that can favor immunopathological consequences relates to the nature of the pathogen. A broad assortment of pathogens, including hepatitis B and C virus,¹⁹ cytomegalovirus (CMV),²⁰ and human immunodeficiency virus (HIV),²¹ have acquired the ability to establish persistence in the human population. This adaptation favors propagation of the pathogen and, unfortunately, can be associated with long-term tissue injury or transformation. In fact, a chronic pathogen-specific immune response is rarely associated with a benign outcome, and thus it comes as no surprise that incessant activation of T lymphocytes can be associated with severe human disease. It is also reasonable to theorize that the host responds to this unfavorable situation by inactivating pathogen-specific T lymphocytes in an attempt to avert fatal immunopathology, a concept referred to as *exhaustion*.^{22, 23} When exposed to a chronic source of antigen, pathogen-specific T lymphocytes often become incrementally relieved of their functional responsiveness, which

includes their ability to lyse target cells as well as the ability to produce anti-pathogen cytokines.²⁴ This might at first glance appear to be counterproductive to the attempt to ward off a persistent infection. In fact, it could be argued that it would further ensure the persistence of the pathogen. However, from the standpoint of the host, it seems intuitive to envision how the preservation of tissue integrity and survival might outweigh pathogen clearance. In other words, under certain circumstances peptide/MHC-restricted T lymphocytes may need to be relieved of their duties (or at least functionally dampened) in order to ensure survival of the host.

Whereas pathogen-specific T lymphocyte responses have the potential to be detrimental to the host, it should be noted in fairness to the adaptive immune system that a multitude of pathogens are routinely cleared in a highly specific manner without incident. The host makes every attempt to overwhelm an invading pathogen by mobilizing a sizeable number of pathogen-specific T lymphocytes. Based on the use of limiting dilution analyses, it was previously assumed that the majority of T lymphocytes observed in various tissue compartments following infection were of an irrelevant specificity.²⁷ In other words, these cells were thought to have no capacity to recognize the invading pathogen and thus were not active participants in pathogen clearance. However, with the advent of MHC tetramers,²⁸ the immunologic community was equipped with a more sensitive reagent to reanalyze the issue of T-lymphocyte specificity, and it was subsequently realized that the degree of clonal expansion following infection was far greater than previously appreciated.^{26,29} In fact, in a variety of infectious models, T lymphocytes of an irrelevant specificity now represent the minor, rather than the major, fraction of the infiltrate. These data meshed nicely with our conceptual understanding of immunologic specificity and suggested that T lymphocytes of an irrelevant specificity were nothing more than innocent bystanders, moving into and out of tissue compartments without consequence. More recently, studies focusing on viral infection of the central nervous system (CNS)^{30–32} and eye^{33–38} have demonstrated that bystander T lymphocytes of an irrelevant specificity can be active and unwitting participants in immunopathology. These studies appear to be in direct opposition to the evolutionary elegance of the adaptive immune system, which appears to control T lymphocytes stringently through specificity^{1–4,39} and regulatory mechanisms.^{40–42}

Because the participation of T lymphocytes of an irrelevant specificity in immunopathological injury would undoubtedly alter our understanding of pathogenesis in a variety of human ailments, it is important to understand fully both the nature of this injurious process and whether it is likely to occur in an immunologically competent host without any genetic deficiencies in T-lymphocyte regulation. In the context of this review, T lymphocytes of an irrelevant specificity will be considered “bystander” T cells. It is important to note that these cells are not specific to the invading infectious agent, nor are they a population of clonally expanded self-reactive T lymphocytes, which are thought to serve as the driving force behind a variety of autoimmune disorders. In other words, bystander T cells are not restricted by specificity (i.e., peptide/MHC interactions). This review is constructed to critically evaluate the existing body of literature that relates to the role of bystander T cells in immunopathological damage, with a special emphasis on CNS pathology. However, it should be noted at the outset that our present understanding of bystander T cells stems largely from studies conducted in peripheral tissue compartments. Therefore, it is important

to review these studies in concert with the research conducted in the CNS, especially given that most immune responses are thought to originate in peripheral lymphoid tissues. Moreover, it is likely that the immunologically specialized CNS⁴³ could impose an additional set of constraints on bystander T cells. It is important to point out that the CNS resides behind the blood–brain barrier, does not have a traditional lymphatic drainage system, heavily regulates MHC expression, and employs a collection of immune dampening mechanisms.⁴³ Thus, we surmise that bystander T-cell–mediated injury (if it occurs) is most likely to be maximal in the periphery rather than in the CNS.

II. BYSTANDER T-CELL ACTIVATION AND RECRUITMENT

It is now generally accepted that infection can trigger a massive and highly specific expansion of T lymphocytes that are restricted by peptide/MHC complexes.^{25,26,29,44} However, there is also a considerable body of literature focusing on the activation^{45–56} and recruitment^{57–64} of bystander T cells following infection. Infectious agents are often potent stimuli of our innate defense system and can consequently trigger an immediate elevation of cytokines. We especially note that bacteria are particularly adept at triggering our innate defenses and cytokine release because they possess molecular components that are recognized by pattern recognition receptors.⁷ The cytokines released following infection have been shown to induce a variety of responses (e.g., activation, proliferation, acquisition of effector function) primarily in effector/memory T lymphocytes through a process referred to as bystander activation. For example, it was shown recently that a primary HIV infection in humans results in the activation of Epstein-Barr virus (EBV), CMV, and influenza-virus–specific CD8⁺ T cells.⁵⁴ MHC class I tetramers were used to identify the virus-specific T-cell populations, and activation was evaluated by the expression of CD38 and HLA-DR. CD8⁺ T cells specific for viruses other than HIV showed a significant degree of activation during the acute phase of HIV infection, and this activation profile returned to baseline levels following 1 year of treatment with highly active antiretroviral therapy. Whereas the significance of this observation in HIV pathogenesis remains unclear, the authors suggested that it is likely to be of no major biological significance. The rationale underlying this statement is that no clinical manifestations are observed during the initial year of HIV infection.

It is conceivable that bystander activation of T lymphocytes following HIV infection has no impact on disease pathogenesis; however, the fact that bystander activation has been observed in a variety of systems^{45–47,49–56} suggests that it is of some biological importance and thus warrants further discussion. The authors of a recent study surmised that the *raison d'être* for bystander activation is three-fold⁵¹: early host defense, maintenance of the memory T-cell pool, and expansion of effector T cells. Bystander activation might afford nonspecific T lymphocytes the ability to participate in the first line of defense against an invading pathogen during the acute phase of an infection. An extension of this supposition not proposed by these authors is that if nonspecific (or bystander) T lymphocytes are licensed with the ability to release antiviral cytokines and contribute to host defense, then it is also probable that they could contribute to tissue injury. One interesting observation *in vitro* was that stimulation of splenocytes from uninfected mice with the bacterium *Burkholderia pseudomallei* resulted in IFN- γ production by CD8⁺ T cells within 15 hours.⁵¹

The production of IFN- γ depended on the production of IL-12 and IL-18—two cytokines known to be important for the activation of T lymphocytes in an antigen-independent manner.⁶⁵ However, the significance of this observation following *B. pseudomallei* infection *in vivo* remains unknown. In fact, it was demonstrated more recently that infection with another intracellular bacterium (*Listeria monocytogenes*) resulted in minimal destruction of bystander bacteria.⁶⁶ The authors set up an elegant coinfection model in which they evaluated the ability of epitope-specific CD8⁺ memory T cells to eliminate two isogenic strains of *L. monocytogenes*—one that contained the epitope to which the memory cells were specific and another that did not. The authors theorized that if clearance largely depended on cytokines, then pathogen-specific T cells might possess the capacity to relieve adjacent cells of epitope-deleted bacteria nonspecifically. However, the results of this line of experimentation further underscore the exquisite specificity of the adaptive immune response by demonstrating that the CD8⁺ memory T cells possessed the ability to interfere only with the growth of the bacteria harboring the specific epitope. The growth of epitope-deleted bacteria was not impeded in any way. Although this study does not rule out the possibility of indirect, bystander mechanisms participating in the clearance of other pathogens, it does suggest that cytokine release following bystander activation may not contribute to early host defense by interfering with pathogen growth.

Because there is ample evidence to support the contention that cytokine-mediated bystander activation does occur following pathogen infection,^{46,50,51,53,54,56,61,63} it is important to establish the reason for this activation as well as its biological consequences. It seems unlikely that the immune system would have evolved to allow T lymphocytes fitted with an array of noxious compounds to operate in an unregulated, nonspecific manner. In fact, the consequences of unregulated cytokine production can be quite dire. An important example of this can be found in the literature pertaining to endotoxic shock.^{56,67–69} IL-12 and IL-18 are produced by antigen-presenting cells,⁷ and as mentioned above can be potent stimuli of IFN- γ production by activated T cells.^{51,65} For example, effector and memory CD8⁺ T cells specific to lymphocytic choriomeningitis virus (LCMV) will produce IFN- γ in the absence of cognate antigen recognition following exposure to a combination of IL-12 and IL-18.⁵⁶ This translates into a dramatic impact on host survival when viral infections are complicated by bacterial infections^{56,70}—a scenario that results in human septic shock. When LCMV-infected mice are exposed to LPS (a microbial product) at the peak of the effector T-lymphocyte response (i.e., day 8), death is observed in nearly 80% of the mice.⁵⁶ It is interesting that mice harboring memory T lymphocytes to LCMV have no greater risk than uninfected mice to endotoxic shock, suggesting that memory T lymphocytes are less susceptible to this type of innate cytokine stimulation. The reduced susceptibility to innate cytokine stimulation is probably enforced by the host to limit the potential for severe immunopathological consequences.⁵⁶

If one assumes that bystander-T-cell activation is not a major component of host defense early after infection, then it is likely that this mode of activation serves another purpose. One very distinct possibility is that innate cytokine stimulation contributes to the maintenance of the memory T-lymphocyte pool. There is ample evidence in the literature to support the assertion that cytokines released following infection can induce bystander proliferation of memory T lymphocytes *in vivo*.^{46,50,55,71,72} Proliferation of CD44^{hi} CD8⁺ memory

phenotype T cells can be induced by the injection of IFN- $\alpha\beta$,⁴⁶ poly(I:C),⁴⁶ LPS,⁷¹ and CpG DNA.⁷² The induction of proliferation in each case results from the IL-15 induced by IFN- $\alpha\beta$.⁷³ More recently, it was shown that CD40 ligation *in vivo* can also induce bystander proliferation of memory CD8⁺ T cells, which depends in part on IL-15 production.⁵⁵ Thus, it seems that both IFN- $\alpha\beta$ and CD40 ligation can contribute to the maintenance of memory T lymphocytes in an antigen-independent manner through the release of IL-15.

On the basis of the aforementioned studies, it appears that bystander T-lymphocyte activation observed following infection is of some biological significance *in vivo*. However, no studies presented thus far in this review support a role for bystander T lymphocytes of an irrelevant specificity in pathogen clearance or disease pathogenesis (other than the extreme case of endotoxic shock). It is clear that cytokines released during infection have the capacity to initiate activation, proliferation, and acquisition of effector function in bystander T cells; however, these cells must also gain access to target tissues before they can be considered potential participants in immunological processes. There is evidence that bystander T cells have the capacity to migrate into inflamed peripheral tissue compartments^{57–60} as well as the CNS.^{30,62–64,74,75} Several studies have shown that antigen specificity does not influence the ability of T lymphocytes to enter an inflamed CNS.^{30,62–64,74,76} In fact, it was demonstrated recently that virus-specific and bystander CD8⁺ T cells peaked concurrently within the CNS; however, only the antigen-specific T lymphocytes were retained in this compartment over time.⁶⁴ This finding is supported by several studies showing that T-lymphocyte retention within the CNS depends on antigen recognition.^{74–76}

Although it is accepted that bystander T cells can gain access to inflamed tissues, there is no evidence in either the periphery or the CNS that these cells can positively impact pathogen clearance. The aforementioned study focusing on the migration of antigen-specific and bystander memory T cells into the CNS concluded that the bystander cells had no impact (either positive or negative) on virus clearance from the CNS.⁶⁴ Another recent study demonstrated that bystander recruitment of systemic memory CD8⁺ T cells into the lung could actually interfere with the clearance of a secondary, unrelated pathogen.⁶¹ In this study, mice were infected intravenously with LCMV to generate a systemic antiviral immune response, which purges LCMV in approximately 1 week. Three weeks after the infection with LCMV, mice were challenged intranasally with respiratory syncytial virus (RSV). This resulted in the recruitment of LCMV-specific memory T cells into the lung, where they underwent a single round of cell division (consistent with the cytokine-mediated bystander proliferation described above) but did not upregulate activation markers or acquire effector function. Moreover, these bystander memory T cells did not enhance RSV clearance from the lungs but rather interfered with the recruitment of RSV-specific primary effector cells into this compartment, which delayed elimination of the virus. Collectively, these data suggest that bystander T lymphocytes of an irrelevant specificity are not likely participants in pathogen clearance. In fact, in some cases these cells can actually interfere with the primary immune response to an unrelated secondary pathogen.

III. BYSTANDER T CELLS AND AUTOIMMUNE DISEASE

The fact that bystander T cells do not appear to be active participants in pathogen clearance does not exclude their possible involvement in the pathogenesis of T-cell-mediated disorders that adversely impact the human population. T lymphocytes are thought to contribute to autoimmunity as well as immunopathological conditions associated with infectious agents. Therefore, the remainder of this review will focus on existing literature that pertains to the role of bystander T cells of an irrelevant specificity in immune-mediated disorders of the periphery and CNS.

A defining feature of autoimmunity is a collapse in the foundation of self-tolerance. Because the immune system does not always clearly distinguish self from non-self, autoimmunity is an unfortunate side effect associated with the presence of this protective compartment in vertebrate animals.⁷⁷ In fact, benign autoimmunity is a common occurrence resulting from the existence of self-reactive T cells and antibodies in all individuals. Thus, we all appear to be teetering on the edge of autoimmunity, and yet the vast majority of us will never present with clinical manifestations. So what are the factors that tip the balance in favor of overt disease? Genetics can clearly enhance the risk of developing an autoimmune disease. It is estimated that genetics contribute from one-third to one-half of the risk for most autoimmune diseases.⁷⁷ This leaves the majority of the risk factors at the mercy of environmental influences, which likely participate in most human autoimmune diseases. Environmental influences can range from infectious to noninfectious and include viruses, bacteria, pollutants, ultraviolet light, medicinal drugs, and anesthetic agents. Ascertaining how these factors trigger autoimmune disorders represents a fundamental challenge in the field of biomedical research.

Burnet⁷⁸ originally stated that unfavorable circumstances permit a “forbidden clone” to slip through thymic education and drive an autoimmune process. Although these unfavorable circumstances remain poorly defined, it is clear that infectious agents can serve as powerful stimuli to misdirect the immune system and provoke a self-reactive response. At one point or another, infectious agents have been suggested as an environmental trigger for virtually all autoimmune disorders,^{79,80} although definitively proving their involvement remains an arduous challenge. The mechanisms by which infectious agents can trigger an autoimmune process include bystander activation,⁴⁹ epitope spreading,^{81,82} and molecular mimicry.^{83–91} In each of these instances, the element of specificity is maintained at the level of the pathogenic T-cell response, which fits appropriately within the peptide/MHC framework imposed by the host. In other words, a clonally expanded and misdirected population of self-reactive T cells should not be considered bystander T cells because they are of a relevant (rather than an irrelevant) specificity. Autoreactive T cells are specific to self-antigens and, consequently, must still conform to the constraints of peptide/MHC restriction.

Presently, there is no convincing evidence to support a role for bystander T cells in the pathogenesis of autoimmune disease, either in the periphery or the CNS. It is known that inflammatory stimuli derived from viruses and bacteria have the potential to interfere with the ongoing process of peripheral tolerance and trigger autoimmune pathology.^{92,93} In fact, studies have demonstrated that pathogens lacking homology to self-proteins can generate an

inflammatory milieu conducive to the development of autoimmune disease.⁹³ However, it should be emphasized once again that this occurs through the bystander activation of a self-reactive T-cell response. Bystander T cells of an irrelevant specificity are not thought to be participants in this process.

The dearth of evidence supporting a role of bystander T cells in autoimmune pathology should not be interpreted to signify that these cells could not contribute to a chronic condition such as human multiple sclerosis (MS),⁹⁴ which spans the course of several decades. It is conceivable that chronic exposure to a proinflammatory cytokine milieu could disengage T lymphocytes from their reliance on peptide/MHC restriction, resulting in antigen-independent release of injurious effector molecules. In fact, a combination of cytokines (i.e., IL-2, TNF- α , IL-6) known to activate naïve and memory T lymphocytes in an antigen-independent manner⁴⁵ are found in the plaques of MS patients.⁹⁴ Sustained exposure of T lymphocytes to this cytokine combination has been postulated to enhance effector functions in the liver of individuals with chronic viral hepatitis.⁹⁵ However, it remains to be determined whether antigen-independent stimulation of bystander T cells is a likely (and efficient) pathway to lesion development in MS patients. It seems more probable that antigen-independent cytokine stimulation would serve to further aggravate an existing population of self- or pathogen-specific T lymphocytes that would then operate through peptide/MHC interactions to induce immunopathology.

IV. HERPES STROMAL KERATITIS

Some of the most compelling data supporting a role for bystander T cells in immunopathogenesis can be found in the herpes stromal keratitis (HSK) literature.⁹⁶ HSK is an immune-mediated disorder that is a common cause of blindness in humans.^{96,97} Ocular immunopathology results from a herpes simplex virus (HSV) infection, which routinely establishes persistence in the trigeminal ganglion. However, during reactivation periods the virus can gain access to the cornea and give rise to a severe immunopathological condition that is orchestrated by CD4⁺ T cells.^{98,99} Studies in a murine model of the disorder suggest that multiple mechanisms might give rise to the immunopathogenesis of HSK.⁹⁷ One possibility that has received some experimental support is that HSV possesses a peptide in its UL6 protein that is a molecular mimic of a self-protein-derived epitope in the eye.⁸⁹ Molecular mimicry is a very efficient means to trigger an immunopathological disorder following a viral infection,⁸⁷⁻⁹¹ and thus if HSV shares sequence similarity with a corneal protein, it seems quite probable that this would favor the development of severe T-cell-mediated pathology.

Rouse and colleagues,³³⁻³⁸ on the other hand, favor a role for bystander T cells (that are specific neither to the HSV nor to a self-protein) in the immunopathogenesis of HSK and have published a collection of studies over the years that lend support to this theory. The observation that lies at the heart of this supposition is that CD4⁺ T-cell-receptor (TCR)-transgenic (tg) mice on a recombina-activating gene (RAG) or severe combined immunodeficiency (SCID) background develop corneal lesions following ocular HSV infection that are similar to those observed in immunocompetent wild-type mice.³³⁻³⁶ More recently, Rouse and colleagues³⁷ have demonstrated that similar lesions are also observed in

a variety of CD8⁺ TCR-tg mice. Therefore, bystander CD8⁺ and CD4⁺ T cells appear to possess an equivalent capacity for corneal lesion development following an HSV infection. Because the TCR-tg mice used in these studies harbored a monoclonal repertoire of T cells that were specific to an irrelevant antigen and were not cross-reactive to HSV, it is fair to conclude that the lesions were generated in a peptide/MHC independent manner. In support of this conclusion, Rouse and colleagues³⁶ have demonstrated that lesion development in the TCR-tg mice does not appear to depend on TCR signaling.

The precise mechanism of pathogenesis in this model system is not known; however, the authors favor the induction of a “cytokine storm” by HSV that activates corneal T lymphocytes in a peptide/MHC-independent manner. Following activation, it is surmised that the T lymphocytes would release effector molecules and damage the cornea in a manner not dependent on their specificity. Although this is a plausible scenario that warrants further investigation, one cautionary note is that pathology mediated by bystander T cells following an HSV infection might be associated with the use of TCR-tg mice, which are known to possess deficiencies in immune regulation, especially when crossed onto the RAG^{-/-} and SCID backgrounds.^{100–102} Thus, a monoclonal population of bystander T cells might not conform to routine host constraints when there is a deficiency in immune regulation. In the HSV model, we know that bystander T cells traffic to the eye in wild type mice following infection.³⁸ In future studies, it will be important to determine whether these cells perform similarly in an immunocompetent environment without any deficiencies in regulation. Proof of bystander T-cell injury in an immunocompetent environment would broaden the relevance of these findings and establish the likelihood that the event would occur in human disorders that depend on T lymphocytes.

V. BYSTANDER T-CELL INJURY IN THE CNS

As in the HSK model system, support for bystander T-cell injury in the CNS is dependent on the use of TCR-tg mice on the RAG^{-/-} background. Recently, Perlman and colleagues^{30–32} have focused their efforts on the role of bystander T cells in development of CNS pathology observed following an intracerebral infection with mouse hepatitis virus (MHV). Immunocompetent mice infected intracerebrally with MHV develop demyelinating lesions pathologically similar to the white matter plaques observed in MS patients.¹⁰³ A large proportion of the CNS-infiltrating T lymphocytes observed in MHV-infected mice were virus specific^{104–106}; however, studies have also shown that bystander T cells of unknown specificity traffic into the CNS in this model system.^{64,106,107} This led Perlman and colleagues to evaluate whether bystander T cells could indeed contribute to lesion development in MHV-infected mice. RAG-deficient TCR-tg mice that harbored CD8⁺ T cells specific to either LCMV or vesicular stomatitis virus (VSV) were immunized with their cognate peptide in complete Freund’s adjuvant (CFA). This aggressive immunization strategy was performed specifically to activate the bystander T cells and circumvent the requirement for bystander activation observed in the HSK model system. Moreover, the administration of CFA might have also facilitated the recruitment of antigen-presenting cells and innate cytokine release within the CNS. Three days following the immunization, mice were infected intracerebrally with MHV and analyzed at day 12 postinfection for the presence of demyelination in the CNS. TCR-tg mice not immunized before MHV infection

were devoid of CNS lesions. This indicates that MHV infection of the CNS is not able to induce the same bystander T-cell activation and subsequent damage that is observed in the cornea following an ocular HSV infection.^{33–38} In contrast, immunized TCR-tg mice showed clear evidence of demyelination in the CNS; however, the demyelination was not as severe as that observed in immunocompetent mice. These data indicate that activated, but not naïve, bystander CD8⁺ T cells have the capacity to induce small demyelinating lesions in the CNS following an MHV infection. However, in contrast to the HSK model, CD4⁺ T cells did not possess a similar capacity to induce CNS lesions.³¹ Although the precise mechanism by which the bystander T cells in the MHV model cause demyelination is unknown, Perlman and colleagues³² revealed through the generation of bone marrow chimeras that lesion development depended on the cytokine IFN- γ . Because the expression of IFN- γ alone in the CNS can result in demyelination,¹⁰⁸ it is conceivable that the release of IFN- γ from bystander T cells is directly responsible for oligodendrocyte injury and subsequent demyelination.

Because activated bystander CD8⁺ T cells are capable of inducing demyelination in the CNS of MHV-infected mice, Perlman and colleagues¹⁰⁹ postulated that this mechanism of pathogenesis might explain the established link between disease exacerbation and infection in MS patients. However, before this generalization can be realized, it is important that a role for bystander T cells be demonstrated convincingly in immunocompetent mice. The established link between infection and disease exacerbation in MS patients can also be explained by molecular mimicry^{83–91} or activation of a pre-existing population of self-reactive T cells.^{49,92,93} Both of these scenarios would operate within the framework of specific peptide/MHC interactions and thus are predicted to provide a far more efficient channel to lesion development. Presently, there are no data to support a role for bystander T cells in CNS disease pathogenesis of immunocompetent mice. Bergmann and colleagues⁶⁴ recently made an elegant attempt to address this issue in the MHV model system. Immunocompetent mice were first infected with a recombinant vaccinia virus (VV) that expressed an HIV epitope referred to as p18. Forty-five days following the VV infection, mice were challenged intracerebrally with MHV, and the impact of the p18-specific CD8⁺ memory T cells on the primary MHV response was determined. Because the p18-specific T cells did not crossreact with MHV, they were considered to be bystander T cells in this model system. This enabled Bergmann and colleagues to evaluate the impact of a bystander T-cell population on CNS pathogen clearance in an immunocompetent host. MHV-specific and bystander T cells peaked concurrently within the CNS following MHV infection; however, only the MHV-specific T cells were retained in this compartment over time. Moreover, the bystander T cells that trafficked to the CNS showed no signs of activation or acquisition of effector function, and, most importantly, these cells did not have any impact on viral clearance. These data suggest that bystander T cells in an immunocompetent host⁶⁴ may be more strictly regulated than those operating within an immunodeficient environment.^{30–32}

Working under the assumption that bystander T cells could indeed contribute to CNS lesion development, we recently conducted a series of studies to define the relative pathogenicity of antigen-specific versus bystander T cells in the LCMV model system.⁶³ Immunocompetent mice infected intracerebrally with LCMV develop a fatal choriomeningitis (the disease from

which the virus is named) within 6–7 days.¹⁶ Following an intracerebral infection, LCMV replicates in the meninges, ependyma, and choroid plexus as well as peripheral tissues (Fig. 1). This initiates a systemic antiviral T lymphocyte response that eventually traffics into the CNS and induces a fatal immunopathology. The disorder bears all the hallmarks of specificity: disease is not observed in the absence of CD8⁺ T cells¹¹⁰ or the effector molecule perforin.¹⁰ However, we became intrigued by the fact that no more than 20% of the CNS-infiltrating CD8⁺ T cells could be classified as LCMV specific at the time point just before the mice succumbed to the meningitis. This led us to surmise, based on the results of Perlman and colleagues, that perhaps bystander T cells were participants in this disease process. To address this issue, we infected immunocompetent OT-I TCR-tg mice^{111,112} intracerebrally with LCMV. OT-I mice contain a population of CD8⁺ T cells that recognize a peptide residing in ovalbumin, and these cells do not crossreact with LCMV antigens. For our studies we did not breed the OT-I mice onto the RAG^{-/-} or SCID backgrounds in order to maintain at least some assemblage of normalcy in the immunologic compartment. Curiously, OT-I mice did not mount an LCMV-specific CD8⁺ or CD4⁺ T-cell response following infection and, as expected, did not succumb to a meningitis. Bystander CD8⁺ T cells had the capacity to traffic to the CNS but did not induce any CNS pathology, even when specifically activated by the very same immunization protocol employed by Perlman and colleagues.^{30–32} Over time, bystander T cells were retained within the CNS and acquired a limited capacity to produce the cytokine IFN- γ ; however, this did not result in CNS pathology. Finally, to demonstrate the relative potency of the LCMV-specific T cells in mediating CNS immunopathology, we reconstituted OT-I mice with log serial dilutions of naïve TCR-tg CD8⁺ T cells specific to the glycoprotein of LCMV. We observed that adoptive transfer of as few as 1000 naïve glycoprotein-specific CD8⁺ T-cell precursors was sufficient to completely reconstitute a repertoire filled with bystander T cells. Following clonal expansion, ~50,000 glycoprotein-specific effectors were found in the CNS and were capable of inducing a fatal immunopathology (Fig. 2). These data underscore the pathogenic potential of antigen-specific T cells when compared to bystanders of an irrelevant specificity and suggest that even if bystander T cells can mediate tissue injury, their contribution may be inconsequential in relation to T cells restricted by peptide/MHC interactions.

VI. CONCLUDING REMARKS

Given that both environmental and genetic factors are intertwined as contributors to a number of human disorders, a precise determination of the individuals that will ultimately suffer from one of several ailments is incredibly difficult (and perhaps impossible) to achieve. Thus, investigators must conduct meticulous analyses to unlock the underlying mechanisms of pathogenesis. Because T lymphocytes contribute to the pathogenesis of myriad CNS and peripheral disorders, it is important to define their topography, interactions, and effector mechanisms during the onset and perpetuation of disease. Attaining this information requires experimental tools to distinguish the peptide/MHC-restricted T cells from the bystanders of an irrelevant specificity. Fortunately, with the latest methodological advances,^{17,113–117} the particular localization and function of antigen-specific T cells can now be investigated in a variety of *in vivo* systems that rely on identifiable or traceable populations of T cells. It is important to note that these recent advances will undoubtedly

refine our understanding of T-cell biology in physiological microenvironments and facilitate the identification of novel therapeutic targets. For example, encephalitogenic CD4⁺ T cells were recently transduced with a retrovirus genetically engineered to express green fluorescent protein (GFP).¹¹⁸ Following adoptive transfer, the migration and phenotype of GFP⁺ myelin-specific T cells were analyzed during the development of experimental autoimmune encephalomyelitis. In two additional studies of note, antigen-specific lymphocyte expansion and migration were evaluated by adoptively transferring ovalbumin (OVA)-specific¹¹⁴ or salmonella-specific¹¹⁶ CD4⁺ Thy1.1⁺ T cells into congenic Thy1.2⁺ recipients. After challenging mice with OVA¹¹⁴ or salmonella,¹¹⁶ the whole body distribution of antigen-specific CD4⁺ T cells was determined using an antibody directed against the congenic Thy1.1 marker. Each of these studies enhanced our understanding of antigen-specific T-cell immunity *in vivo*.

Given that the methodologies are now in hand to readily distinguish specific T cells from those of an irrelevant specificity, it is only a matter of time before the role of bystander T cells in a variety of inflammatory microenvironments will be defined. Barring the extreme example of endotoxic shock, there is presently a shortage of compelling evidence to implicate bystander T cells in pathogen clearance or immunopathogenesis occurring in the periphery or the CNS of an immunocompetent host. Thus, for the time being, bystander T cells should retain their “innocent” status, and our attention should remain focused on their pathogenic brethren guided by peptide/MHC interactions. Specific T cells possess an enormous potential to contribute to disease pathogenesis and can be misguided by several well supported scenarios, which include bystander activation,^{45–56} epitope spreading,^{81, 82} molecular mimicry,^{83–91} and heterologous immunity.¹¹⁹

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FIGURE 1.

Immunopathology in the meninges 6 days following an intracerebral infection with LCMV. Following intracerebral inoculation, LCMV replicates in the meninges, ependyma, and choroid plexus of the brain. Approximately 5–6 days after infection, LCMV-specific CD8⁺ T cells arrive in the CNS and mediate several immunopathology by engaging infected cells. Note the abundance of cellular apoptosis (green) around the lining of the brain. Nuclei are shown in red for anatomical purposes.

FIGURE 2.

Localization of glycoprotein (GP)-specific CD8⁺ T cells in the brain following reconstitution of disease in OT-I mice. OT-I TCR-tg mice received 10^3 naïve green fluorescent protein (GFP)-tagged D^bGP₃₃₋₄₁-specific CD8⁺ T cells. One day later the mice were infected intracerebrally with LCMV. Adoptive transfer of 10^3 virus-specific CD8⁺ T cells was sufficient to reconstitute the fatal meningitis routinely observed in wild type B6 mice. Two-color coronal brain reconstructions were performed on symptomatic mice to analyze the distribution of GFP⁺D^bGP₃₃₋₄₁-specific CD8⁺ T cells (green) in reconstituted mice. Note the presence of GP-specific effector cells around the meninges and ependyma. Nuclei are shown in blue for anatomical purposes.