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Brain Behavior and Immunity: twenty years of T cells

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Abstract

During the period from 1987–2007, our understanding of nervous system-T lymphocyte bidirectional communication advanced exponentially. Progress in exploring these relationships was aided by the constant development of new, cutting-edge technologies, and by a steady growth in interest, and number, of scientists who recognized the need to conduct cross-disciplinary research. In this brief review of 20 years of *Brain, Behavior, and Immunity (BBI)*, we highlight just a small number of the important studies published in the journal that collectively have provided the foundation for our current understanding of brain, behavior, and, specifically, T cells.

Introduction

In the inaugural issue, T lymphocyte function was established as an important topic for manuscripts to be published in *Brain, Behavior, and Immunity (BBI)*. Volume 1, Issue 1, published in March of 1987, included a paper from Glaser et al. (Glaser et al., 1987) documenting that killing of Epstein-Barr virus (EBV) infected B lymphocytes by mononuclear cells (likely cytotoxic T cells) from EBV seropositive medical students was significantly diminished during periods of school examinations compared to baseline (non-examination) periods of time. Significant increases in psychological distress (as measured by the General Severity Index of the Brief Symptom Inventory) were also observed during periods of examination compared to non-examination.

Also in this first issue of *BBI* were manuscripts from Bovbjerg et al. (Bovbjerg et al., 1987a; Bovbjerg et al., 1987b) on conditioned enhancement of T cell-mediated delayed type hypersensitivity in mice, and from Kusnecov et al. (Kusnecov et al., 1987) examining the effects of β -endorphin on lymphocyte proliferation and interleukin-2 (IL-2) production. The authors reported that *in vivo* infusion of rats with β -endorphin was associated with an enhancement of the *in vitro* proliferative response of spleen cells to the potent, but non-specific, T cell mitogen concanavalin A (Con A). There was no effect of treatment on levels of the T cell cytokine IL-2 in culture supernatants, leading the investigators to conclude that changes in IL-2 production do not occur in association with the increase in proliferation induced by β -endorphin.

These three papers—among the very first in the 20-year history of *BBI*—set a standard of excellence for all of the manuscripts that have followed. It is certainly worth noting (happily)

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that Drs. Glaser, Bovbjerg, and Kusnecov, as well as many of their collaborators in 1987, remain important contributors to the journal and the field of psychoneuroimmunology in 2007. Importantly, their papers highlight some of the powerful tools we have used, and still use, to investigate brain to immune system modulation: psychological stress, Pavlovian conditioning, and *in vivo or in vitro* exogenous neurohormone administration. They also utilized state-of-the-art, circa 1987, tools available for probing immune function. Although in 2007, we have advanced to using more sophisticated methods for examining T cell specific cytokine and effector responses, as well understanding T cell signaling pathways at the molecular level, we can still appreciate the value in a simple assay that examines proliferation in response to a nonspecific T cell mitogen. In 2007, we also appreciate that our conclusions in 1987 may not have been entirely correct. We now understand that cytokines secreted into culture can be consumed by cells, affecting the measurement of cytokine production (Ewen et al., 2001). Newer methods for examining cytokine production, such as intracellular cytokine assay using flow cytometry, or enzyme linked immunospot assay (ELISPOT) can provide more accurate information about cytokine production at the single cell level. Certainly, the ability to pair such assays with analysis of messenger RNA levels increases our confidence in studies examining, for example, stress effects on T cell function.

1987: *BBI*'s T cell themes emerge

During 1987, a number of enduring themes involving T lymphocytes emerged from the pages of *BBI*. Studies conducted at the University of Rochester beginning in the mid 1970's by Robert Ader (Editor-in-Chief of *BBI* from 1987–2002) and Nicholas Cohen, PhD (Associate Editor during the same period) (Ader et al., 1975), likely gave rise to behavioral conditioning of T cell responses as the number one theme in the hearts of at least 2/3 of the editors! The second major theme, reflecting the research interests of David Felten, MD, PhD (Williams et al., 1980; Williams et al., 1981) (Associate Editor during the same period), involved examining the relationship between the sympathetic nervous system and primary and secondary lymphoid organs. The first of these papers to be published in *BBI* was from Nance et al. (Nance et al., 1987); these investigators described the patterns of sympathetic innervation of the thymus. Further, Besedovsky et al. (Besedovsky et al., 1987) demonstrated that T cells play a role in the degree to which mouse spleen is innervated; athymic nude (nu/nu) mice had significantly increased splenic norepinephrine (NE) levels compared to nu/+ animals, or nu/nu mice that received thymus transplants.

The third theme, as discussed in the Introduction, explored regulation of T cell function by hormones, including pro-opiomelanocortin (POMC)-derived peptides (β -endorphin and adrenocorticotrophic hormone (ACTH)). Interestingly, responses of Con-A stimulated human peripheral blood leukocytes (PBL) were either enhanced or suppressed by these hormones, depending upon the PBL donor (Heijnen et al., 1987). This paper by Heijnen et al. also is the earliest journal illustration of individual differences, presumably driven by genetics or environmental factors, in T cell responses to CNS stimuli, a fourth theme. In another early study, the opioid peptide met-enkephalin was unable to affect the splenic plaque forming cell (PFC) response to 1% sheep red blood cells (SRBC), but could reverse the suppression observed following culture with high levels of antigen, 10–20% SRBC (Rowland et al., 1987); this paper also is the first to highlight the dose dependent (either hormone or antigen) nature of the relationships in neural-immune interactions—the fifth theme. It should be noted that antibody production is the outcome measured in the PFC response; however, immunoglobulin-producing B cells require help from T cells, so it isn't clear which cell type (s) is affected by met-enkephalin.

The sixth early theme addressed how these hormones might exert their regulatory effects, resulting in studies examining specific hormone receptor expression by T cells and hormone

binding to T cells. The first such elegant paper was published in June 1987 by Scicchitano et al. (Scicchitano et al., 1987), using both fluorescent binding and radioligand binding assays. The authors determined that somatostatin, an inhibitory peptide produced in the hypothalamus and other tissues such as the intestines and pancreas, could bind to the majority of Peyer's patch T cells (Thy 1.2+ cells), but to only 30% of T cells in the spleen from mice. The authors had previously demonstrated that somatostatin inhibited Con A-induced lymphocyte proliferation (Stanisz et al., 1986).

In addition to these early studies that described the mechanisms of CNS regulation of T cell function, it was already becoming clear that the communication between the brain and the immune system was bidirectional (theme seven). In Volume 1 Issue 2 of *BBI*, Dunn and Hall (Dunn et al., 1987) reported that supernatant from Con A-stimulated rat spleen cells (containing a mixture of cytokines, including IL-2), or thymosin factor 5 (TF5, a collection of small molecular weight proteins extracted from the T cell-rich thymus) increased corticosterone levels following injection into mice; the authors concluded that activation probably was at the level of the pituitary, and not the central nervous system (CNS).

During the first year of *BBI*, several papers highlighted that an array of physical or psychological manipulations of a variety of species could result in altered T cell responses (theme eight). These include the medical student exam stress study described above (Glaser et al., 1987). Rabin and Salvin (Rabin et al., 1987) noted that housing mice 1/cage resulted in increased Con A proliferative responses compared to mice housed 5/cage, and that this effect was dependent upon the time spent in these housing situations. Levine and Saltzman (Levine et al., 1987) reported that the rate of relapse in experimental allergic encephalomyelitis (EAE, a T cell-mediated disease) in rats was significantly decreased by 5 days of restraint stress for 7 hr/day, as well as by injection of corticosterone or dexamethasone.

Finally, in 1987, we as readers of *BBI* were introduced to the enduring field of developmental psychoneuroimmunology (theme nine). The first *BBI* manuscript to examine the effects of early manipulation of mice on immune function in young adulthood (day 60) was authored by Lown and Dutka (Lown et al., 1987). Mice that were handled from birth to weaning had higher splenic proliferative responses to the T cell mitogen Con A than mice that were not handled during that same period.

1987–1997: The first 10 years of *BBI*

By 1997, the pages of *BBI* documented advances in all of the themes introduced in 1987.

Theme 1: T cell function can be conditioned

By the end of 1997, roughly a dozen papers had been published in *BBI* documenting that numerous T cell functions could be behaviorally conditioned. In 1989, Solvason et al. (Solvason et al., 1989) authored *BBI*'s first manuscript to explore a possible opioid-mediated mechanism for conditioned elevation of natural killer cell activity. In 1992, Lysle et al. (Lysle et al., 1992) extended this observation to T lymphocyte proliferation, and convincingly demonstrated that suppression of rat splenic T cell proliferative responses to either Con A or ionomycin plus phorbol myristate acetate (PMA) following conditioned aversive stimulus presentation (the cues associated with footshock) was abrogated by administration of the opioid receptor antagonist naltrexone.

The conditioning papers published between 1987–1997 largely used aversive stimuli such as shock or immunosuppressive agents such as cyclophosphamide as the unconditioned stimulus (US). However, an intriguing set of findings on conditioned immune responses (although we do not know if we were conditioning T cell and/or B cell responses) was published in 1993 by

Ader and colleagues (Ader et al., 1993). The findings of our paper supported the hypothesis that a subthreshold concentration of an antigen itself—too low to induce detectable antibody increases alone—when paired with a conditioned stimulus (CS) could induce a significant increase in antibody titer. This finding that antigen can function as a US raises some interesting questions, including the possible role of contextual cues that might be present when the immune system is either deliberately (vaccination in the doctor's office) or naturally (infection that inevitably occurs during times of stress) challenged.

Theme 2. Sympathetic nervous system regulation of T cell function

The studies conducted during the first 10 years of *BBI* used a variety of strategies to explore sympathetic involvement with T cell function, including immunocytochemistry and histofluorescence to map fibers and T cells in lymphoid organs, receptor binding studies, administration of exogenous agonists/antagonists, and ablation of sympathetic nerve fibers by injection of the neurotoxin 6-hydroxydopamine. A paper by Heilig and colleagues (Heilig et al., 1993) in 1993 showed that catecholamines (induced by injection of amphetamine) have a robust effect on the CD4+ T helper cell proliferative response to a specific antigen, but that the effects were indirect, resulting from an inhibition of antigen processing by antigen presenting cells. Further *in vitro* studies implicated α -, rather than β -adrenergic receptor-mediated suppression. It should be noted that these investigators examined proliferative responses of popliteal lymph node cells in culture, adding the same number of cells from treated and control mice into the wells. Therefore, they did not determine if elevated epinephrine and NE levels affected lymph node cellularity. Acute elevations of catecholamines are known to affect lymphocyte migration, inducing egress from lymphoid organs into the circulation. Carlson et al. (Carlson et al., 1996) determined that epinephrine or the β -adrenergic agonist isoproterenol may facilitate this migration by decreasing T lymphocyte adhesion to endothelial cells.

Theme 3. Regulation of T cell function by hormones

By 1997, well over a dozen hormones and transmitters traditionally thought of as being neuroendocrine in function had been shown to affect T cell proliferation, cytokine production, and a host of T lymphocyte effector functions. In addition to the plethora of studies indicating that these hormones can regulate T cell function, and demonstrating that this regulation could occur in a receptor-mediated fashion, an important new concept emerged in 1990. Although corticotrophin releasing hormone (CRF), a key component in the stress response, was long-known to be produced and secreted in the hypothalamus, Stephanou et al. (Stephanou et al., 1990) provided the first report in *BBI* that CRF mRNA was present in both human peripheral blood lymphocytes as well as a T cell clone and that a peptide reactive with antibody to human CRF-41 was detectable in lymphocytes. In this paper, the authors speculated that T cells and the CNS might use some of the same peptides or hormones to regulate their individual functions. Over the next seven years, other hormone mRNA and peptides were found in T cells (and B cells)—prompting Keith Kelley (current Editor-in-Chief of *BBI*) and colleagues to consider that “old, well-recognized players,” including growth hormone, prolactin, and insulin-like growth factor-1, serve as multi-purpose hormones that can be synthesized by lymphocytes themselves and can regulate immunological networks (Kelley et al., 1992).

Theme 4. Individual differences play a role in T cell responses to CNS stimuli

Numerous interesting papers examining behavioral differences, dominance status, and defined CNS genetic mutations or gene knockouts and associated changes in immunity have been published in *BBI*. One good example of a behavior that, arguably, should not itself confer any immunological advantage or disadvantage to an individual, is paw preference. While paw preference in mice had previously been associated with onset of autoimmune disease

(Geschwind N et al., 1987), a paper by Fride and her colleagues (Fride et al., 1990) was published in *BBJ* in 1990 demonstrating that extremes of either right or left paw preference, and not paw preference itself, were correlated with decreased mixed lymphocyte reactions (MLR) and cytotoxic T cell (CTL) activity.

Fride et al. went on to explore other individual differences and their association with altered T cell function. In 1993, the group characterized T helper and CTL responses in mouse lines selectively bred for long sleep (LS) versus short sleep (SS) reactivity to ethanol administration (Fride et al., 1993). Interestingly, the proliferative response to T cell mitogens, MLR, and CTL in spleen cells from unprimed LS mice were lower than responses in SS mice. In contrast, following priming of mice with allogeneic spleen cells, the CTL response in spleen cell cultures from LS was significantly enhanced compared to cultures from primed or unprimed SS spleens, as well as unprimed LS spleens; responses of spleen cells from primed and unprimed SS mice did not differ. Since the response to ethanol is considered to occur through the γ -aminobutyric acid (GABA) receptor complex, these data suggest that GABA may also regulate immune function.

Theme 5. Dose-dependent neural-immune interactions

In 1993, our group showed that housing C57Bl/6 mice individually resulted in higher secondary IgM antibody responses following initial priming with 150, but not 50 or 5 μ g, of the T cell dependent antigen keyhole limpet hemocyanin (KLH) compared to mice housed in groups (Karp et al., 1993). Why we choose this paper to illustrate dose-dependent effects is because the results of this study were surprising for two reasons. First, we have always assumed that the brain is likely to affect immunity in subtle ways, much like fine-tuning; therefore, it was surprising to us that differential housing effects were observed only at the highest (optimal) priming dose of antigen and not lower (suboptimal) doses. Second, numerous investigators have observed that mice housed individually can be immunosuppressed due to “social isolation;” but we did not find this to be the case.

In addition to antigen dose-dependent interactions, there are many reports of interactions between the frequency/intensity of stressor administration and changes in immunity. For example, Shurin et al. reported in 1994 that T cell mitogen responses of spleen or peripheral blood lymphocytes were suppressed in general in a dose-dependent manner by 3, 8, or 16 discrete footshocks in a single session. Interestingly, 1 footshock significantly suppressed the PHA response by spleen cells, but significantly enhanced the PHA response of peripheral blood lymphocytes compared to home cage control rats.

Theme 6. Hormone receptor expression by lymphocytes

During the period of 1987–1997, a few papers published in *BBJ* further described receptor-mediated binding of hormones to receptors on lymphocytes. In addition to the work of Scicchitano et al. (Scicchitano et al., 1987) on somatostatin binding, binding studies showed that vasopressin receptors were present on human peripheral blood mononuclear cells (Elands et al., 1990) and that cells from female donors had a seven-fold higher receptor density compared to cells from male donors.

One of the first papers to attempt to consider adrenergic receptors on lymphocytes and altered immune function was authored by Carr et al. (Carr et al., 1993). Genetically epilepsy-prone rats (GEPR-9) had been previously shown to have a decreased PFC response to SRBC (Rowland et al., 1991). Here, the authors demonstrated higher splenic NE levels in GEPR-9 compared to control animals but a significant reduction in β -adrenergic receptor binding. These results suggested that chronic, elevated NE levels in the spleen might down-regulate adrenergic

receptor expression and lead to a subsequent inability of NE, which is elevated following an immune challenge, to regulate immune responses.

Theme 7. Bidirectional communication between the central nervous and immune systems

By the early 1990's, it was becoming quite clear that proinflammatory cytokines, such as IL-1 produced by monocyte/macrophages and other cells, could have potent effects on the brain, inducing, for example, hypothalamic activation and sickness behaviors. Some T cell-derived cytokines, as well, were shown to induce activation of CNS pathways.

Harbuz et al. (Harbuz et al., 1992) administered exogenous IL-2 or IL-4 peripherally to rats and examined POMC and CRF mRNA expression using *in situ* hybridization histochemistry. IL-2 injection resulted in a significant dose-dependent increase in POMC mRNA expression, whereas at the highest concentration of IL-4, a significant decrease was observed. No changes in CRF mRNA were observed. In support of this, Bartholomew and Hoffman (Bartholomew et al., 1993) noted that following injection via the tail vein in mice, IL-2 decreased the activity of neurons in the lateral margin of the hypothalamus.

These early data are particularly interesting because of the dichotomy between T helper 1 (Th1) cells that produce proinflammatory cytokines, including IL-2 and interferon (IFN)- γ (important cytokines for the generation of cell-mediated immunity), and Th2 cells (which produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, cytokines that promote humoral immune responses (antibody responses)). Th1 and Th2 cells can be cross-inhibitory, and some evidence suggests that the HPA axis itself can regulate the balance between the two types of Th cells. These early publications in *BBI* were also important because they provided some of the foundation for the current hypothesis that proinflammatory cytokines, including IL-2 and IFN- γ , play a role in major depression (reviewed by Schiepers et al. (Schiepers et al., 2005)).

Theme 8. Differential effects of acute versus chronic stressors

Investigators who have spent any time examining stress effects on immune function have adopted the philosophy that a number of variables interact to determine the magnitude *and* direction of stress effects on an immune outcome, including the history of the experimental subject, the intensity of the stressor, and the chronicity of stress. In 1997, Dhabhar and McEwen (Dhabhar et al., 1997) addressed the question of differential effects of acute versus chronic stress on the same immune effector function, that is, the delayed type hypersensitivity (DTH) response to 2,4-dinitro-1-fluorobenzene (DNFB). This classic T cell mediated response was assessed by sensitizing rats to the antigen, followed by challenge (administering DNFB to ear pinnae) and measurement of ear swelling. The authors demonstrated that a single administration of 2 hr of restraint led to a very convincing increase in the DTH response. In addition, they determined that a more intense stressor, restraint plus shaking for 2 hr, led to a significant increase in DTH compared to both control and 2 hr restrained rats. In contrast, when stress became chronic (5 weeks of a daily randomized sequence of restraint, shaking, and both for 6 hr/day), the DTH response was significantly attenuated. These findings were put within the context of the then-developing concept of "allostatic load," characterized by initial preparatory attempts to deal with apparent or perceived threat or stress, which over time lead to physiological and immunological exhaustion (McEwen, 1998).

Theme 9. Developmental PNI

During the first 10 years of the journal, many pages were devoted to discussions of early life experiences and subsequent changes in behavioral, physiological and T cell-driven immunological responses. The special issue on this subject published in September 1996 (, 1996) highlighted two important points: 1) that there is an important relationship between early life events and immunity across the lifespan, reflecting what we now know to be, at least in

part, driven by epigenetic programming (e.g., (Weaver et al., 2004)); and 2) that this relationship is present across species—from rodents to primates to humans. These early life events affect the developing brain, but it is still unclear whether downstream changes in immune function are an indirect effect of those brain changes, or result from epigenetic changes in cells of the developing immune system as well. Thus, the paper published in this special issue written by Kavelaars et al. (Kavelaars et al., 1996) remains especially interesting. The authors observed that sensitivity to the synthetic glucocorticoid dexamethasone was markedly enhanced in T cells from either full-term or premature neonates, such that proliferation to the mitogen PHA was significantly more inhibited in a dose-dependent manner in T cells from neonates compared to adult T cell responses. Of further interest was the finding that the pattern of adult dexamethasone sensitivity developed over the first year of life. The significance of this finding in terms of normal T cell development, as well as in adverse early environments in which glucocorticoid levels may be elevated, remains to be determined.

1997–2007: The next 10 years of *BBI*

From 1997–2007, another astonishing amount of data reflecting the interactions between the neuroendocrine system and T lymphocyte biology was amassed. As a result of our understanding that the interactions modulating T cell functionality are highly complex, more ambitious research strategies were employed to further understanding of the functional consequences of neural-immune interactions, as well as the underlying mechanisms. In addition to the standard fare of assays to measure T lymphocyte proliferation and cytokine production, new tools and techniques, such as multiple color flow cytometry and use of genetic knockout mice, have been introduced into the PNI literature, which have allowed us to better-define T cell subsets. The growing interest in functional and mechanistic information has shaped the landscape of the journal for the past decade; as a consequence, in a single publication we have frequently observed the integration of several of the individual themes that permeated the first 10 years of *BBI*. Below we discuss five of these broader research topics that we find particularly noteworthy.

Theme 1. Neurotransmitters, neuropeptides, and T cell function

An interest in understanding the Th1/Th2 CD4⁺ T cell dichotomy, cytokine regulation, and β_2 -AR signaling led Ramer-Quinn et al. (Ramer-Quinn et al., 2000) to examine the effects of NE signaling on *in vitro* cultures of purified spleen derived CD4⁺ T cells from BALB/c and DO11.10 mice. The investigators observed a β_2 -AR dependent decrease in IL-2 production when resting CD4⁺ T cells were cultured in the presence of NE (or a β_2 -AR agonist) at the time of antigen presenting cell (APC)-independent T cell stimulation. Surprisingly, this modulatory effect on IL-2 production was restricted to recently activated naïve CD4⁺ T cells, as IL-2 concentrations in previously differentiated primary Th1 or Th2 effectors remained unchanged in the presence of NE. Furthermore, the production of the primary T-helper effector cell signature cytokines (IFN- γ , IL-4, and IL-5) was not affected by β_2 -AR stimulation. These data were among the first to suggest that the CD4⁺ T cell activation state (or history of activation) is related to the functional activity of the β_2 -AR and cytokine production. Thus, this paper supports the hypothesis that the SNS, through NE release into secondary lymphoid organs, provides an additional homeostatic mechanism that can control the magnitude of antigen-specific T cell expansion during the initiation of an immune response.

In 2002, we (Callahan et al., 2002) reported that spleen derived T cells from C57BL/6 mice that had been injected with the neurotoxin 6-hydroxydopamine to ablate peripheral sympathetic nerves had increased IL-2 and IFN- γ production following KLH stimulation. Interestingly, the response to the T cell mitogen Con A resulted in decreased production of these same cytokines. A series of mix and match experiments in which T cells from denervated mice were co-cultured with syngeneic APC from vehicle treated animals, and vice versa, demonstrated that APC

antigen presentation was not affected by the presence or absence of sympathetic nerves. We concluded that the nature of the immunological stimulus is critical for the outcome of the cytokine response in a NE deficient environment. A logical extension of this work might be to examine T cell responses following infection, a situation in which a diverse number of antigen derived peptides are produced and presented by APC. These peptides differ in their ability to stimulate T cells, establishing an immunodominance hierarchy, with only a few T cell receptor (TCR) clonal populations contributing to the bulk of the immune response. It would be interesting to see if the SNS can contribute to the antigen specific clonal selection process based on the quality of the stimulus provided by different peptides in an *in vivo* infection model.

The control over T cell survival, defined as the ratio between cell division and cell death, is key to homeostasis. Various mechanisms regulate T cell survival/proliferation to ensure proper responses to pathogens and prevent autoimmunity. In 2004, Abdouh et al. (Abdouh et al., 2004) reported a novel way in which lymphocyte survival could be enhanced by serotonin via signaling through the 5-HT_{1A} receptor. In this manuscript, spleen cells were stimulated with various T cell and B cell stimuli (Con A, PHA, and LPS) in the presence of physiological concentrations of 5-HT, a selective agonist to 5-HT_{1A}, or a 5-HT_{1A} receptor antagonist. A time dependent increase in lymphocyte viability was observed at 48 and 72 hours following PMA and ionomycin stimulation in cell cultures treated with 5-HT or the 5-HT_{1A} agonist. The receptor antagonist reversed this increase in viability. In further experiments, a decrease in apoptosis was observed to be correlated with an increase in the percentage of lymphocytes in the S-phase of the cell cycle. This phenotype was preceded by an increase in the transcription factor NF- κ B translocation into the nucleus of the lymphocytes. A caveat of the cell cycle and NF- κ B translocation observations is that they were obtained from unfractionated spleen cultures stimulated by PMA and ionomycin. Thus, the stimulation was not specific for T cells. In addition, various subpopulations of cells within the spleen may have different 5-HT_{1A} cell surface receptor densities. Nevertheless, it is interesting that signaling through the 5-HT_{1A} receptor may be another possible mechanism that contributes to the maintenance of homeostasis in the bulk population of lymphocytes.

Theme 2. Depressive disorders may alter T cell sensitivity to SNS transmitters

In Volume 16 of *BBI*, Edgar et al. (Edgar et al., 2002) characterized a possible role for serotonin imbalance, such as has been observed in depressive disorders, in the modulation of T lymphocyte surface expression of the muscarinic cholinergic receptor (MR) and the β_2 -AR. The authors described an increase in surface expression of both receptors in mice subjected to chronic mild stress (CMS), a putative animal model for depression; binding affinity did not change as a function of stress. Whereas a parallel increase in cAMP expression was observed by stimulating cells from mice subjected to CMS via the β -AR, the opposite was observed for cGMP production upon MR stimulation. As a consequence of increased surface expression of β -AR and signaling, T cells from CMS animals (when compared to unstressed controls) were more sensitive to the β -agonist driven decrease in the proliferative response to Con A. The increase in MR expression and decrease in signaling in T cells from CMS mice did not affect their ability to proliferate in response to Con A stimulation. In contrast, an increase in T cell proliferation under the same conditions was observed with cells from unstressed mice. The altered phenotype of CMS T cells was reverted by daily administration of the antidepressant drug fluoxetine, a selective serotonin reuptake inhibitor (SSRI), during the CMS treatment.

This manuscript is one of the first to show a connection between depressive disorders and serotonin regulation directly affecting T cell sensitivity to the SNS. Currently, the mechanism(s) by which low levels of serotonin might induce β -AR and MR upregulation are yet to be determined. Furthermore, the paper reports a disconnect between MR upregulation and decreased receptor signaling. Whether serotonin affects MR coupling to small G-proteins or

induces factors that reduce small G-protein activity in T cells remains an open area for investigation. Additionally, we currently do not know how the modulation of β -AR and MR might play a role in immune responses to pathogens, or how SSRIs might affect the development of immune responses to these pathogens.

Theme 3. External pressures affect T cell effector function efficiency

The pages of *BBI* have been the host to a vast amount of research into the consequences of neuro-immune dysregulation in disease pathology and outcome. A noteworthy extension of prior work of Bonneau and colleagues was published in 2002. In this paper, Wonnacott and Bonneau (Wonnacott et al., 2002) showed that restraint stress administered during a secondary HSV infection could affect the ability of memory CD8⁺ T cells to clear the virus from clinically relevant mucosal sites. While these effects of stress on T cell memory function have been reported in the context of HSV infection specifically, little information is available about other viral and bacterial infection models or other memory T cells populations.

To address some of these issues, Olin et al. (Olin et al., 2007) injected swine with the *Mycobacterium bovis* strain of bacille Calmette Guerin (BCG) to study the effects of chronic morphine administration on T cell recall responses. Two major classes of T cells are identified on the basis of the type of antigen receptors they express; cells express either the $\alpha\beta$ T cell receptor (TCR) or the $\gamma\delta$ TCR. The $\gamma\delta$ TCR appears to have limited heterogeneity and may recognize a small number of natural ligands. Olin et al. observed that these $\gamma\delta$ T cells from vaccinated pigs that had undergone chronic morphine administration had reduced *ex vivo* proliferative capacity when incubated with *Mycobacterial* antigens. In addition, morphine treatment of vaccinated pigs diminished the $\gamma\delta$ T cell antigen specific cytotoxic properties against *M. bovis* infected targets. In this particular experiment, the cytolytic activity of the $\gamma\delta$ T cell from morphine treated animals was indistinguishable from that of unvaccinated pigs. While the authors propose that opiates modulate $\gamma\delta$ T cells' ability to respond to *Mycobacterium*, it would also be interesting to consider the repercussions of opiates, neurotransmitters, and stress hormones on memory T cell generation and effector activity modulation.

There remains fertile ground this type of research and, with the availability of receptor knockouts, T cell transgenic tools, and established adoptive transfer protocols, the field is poised to tackle a plethora of questions regarding the mechanism of the effects of neuroimmune interactions on memory T cells. It is known that the quality and timing of an initial encounter with pathogen is, among other things, critical for the generation of memory cells. It is not yet clear whether hormones might alter the early development, phenotype, function or ultimate location of memory cells, all important factors for the response to a second encounter with the pathogen.

Theme 4. Neuroprotective effect of T cells

In an elegant study published in 2003, Serpe et al. (Serpe et al., 2003) discovered a direct link for CD4⁺ T cell involvement in neuron survival. The authors used a facial motor neuron (FMN) injury model in mice deficient in functional T cells (CD4KO, CD8KO mice), B cells (M μ MT mice), or both cells (RAGKO mice). By reconstituting these mice with lymphocytes from a particular lineage (CD4⁺, CD8⁺, B220⁺) or by examining FMN survival in the lineage knockouts, the authors observed that CD4⁺ T cells were sufficient to increase survival of FMN by up to 30% four weeks after injury.

More recent data from these investigators suggest that regulatory T cells (CD4⁺CD25⁺ T cells) do not play a role in FMN neuroprotection (Deboy et al., 2006a), but that the IL-4/STAT-6 pathway (Th2 cells) is required for motor neuron survival (Deboy et al., 2006b). Is there a

particular population of phenotypically distinct CD4⁺ T cells that mediate neuroprotection? Is this effect mediated via cell to cell contact and, if so, are these T cells autoreactive or are they antigen experienced cells with some degree of crossreactivity? Do the products of neuron injury instruct CD4⁺ T cells to release neuroprotective factors? These and other interesting questions will help us understand the role of CD4⁺ T cell subsets in neuron survival and nervous system integrity.

Theme 5. Brain lateralization can have an impact in T cell function

Goldstein et al. (Goldstein et al., 2002), using a kindled temporal lobe seizure model in rats, observed that T lymphocytes would proliferate normally in response to Con A and PHA stimulation independent of the side of temporal lobe excitation. However, changes in proliferation were observed when the anatomical site of the stimulus was taken into account. Here, T lymphocytes derived from rats in which seizure foci were in the piriform cortex (of the right temporal lobe) failed to proliferate when stimulated with PHA. Interestingly, with the exception of IL-4, both Th1 and Th2 cytokine (IFN- γ and IL-10) production to mitogen stimulation was significantly increased in T cells acquired 30 minutes post-seizure induction on the left temporal lobe.

The mechanisms by which the stimulation of certain anatomical regions in the right or left temporal lobe controls proliferation and cytokine production are currently not known. While this research focused on seizures and epilepsy, it is possible that these findings could extend to immunity in humans that have acquired brain injuries, have had abnormal brain development, or suffer from neurodegenerative disease. It would be interesting to see whether lateralization of brain injuries might alter susceptibility to infection, or pose different challenges for treatments of other diseases, such as immunotherapy.

Concluding remarks: twenty years of *BBI*: T cells and the brain

This short review highlights only a fraction of the papers devoted to studies of nervous system-T cell interactions that have appeared on the pages of *BBI*. They highlight the evolution of our understanding of the mechanisms underlying the relationships between T cells and the nervous system. From the first studies that relied on non-specific mitogen stimulation, to newer studies performed with knockout animals that define subsets of T cells involved in neuron protection, this review clearly documents that we have come a long way since 1987. In the next decade, we should expect to see great strides in understanding how the nervous system affects development of T cell memory responses, and the role of T cells in the nervous system, particularly in neuroinflammatory processes.

The basic science research we have discussed in these pages—findings that stress can modulate T cell subsets, for example—has important implications for translational research. Many researchers are conducting intervention studies which strongly suggest that relieving stress in both non-diseased and diseased individuals can improve a variety of immune responses. As well, the early conditioning studies discussed in this review are now leading investigators, including Robert Ader and new colleagues, to explore the role of conditioning in the pharmacotherapy of disease in human subjects. To be able to decrease amounts of drugs with harmful side effects for treatment of a variety of diseases, such as cyclosporin for psoriasis patients, is an important advancement.

Here is to the next twenty years of *BBI*, and to the continued remarkable journey!

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Figure 1.

On the path from immature, double positive (CD4⁺CD8⁺) thymocyte to memory cell, each arrow in this figure represents a maturational stage at which T cells are subject to regulatory processes. These processes are dictated by complex mechanisms, including the actions of other cells in the immune cascade, chemokines, and by the quality of an encounter with antigen. As well, the brain exerts regulatory control over T cell development, and many of the important papers that have documented the relationship between the brain and T cells have been published in *Brain, Behavior and Immunity*.