

Review Article

T Lymphocytes and Anxiety: A Review of Clinical and Animal Studies

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While the role of the innate immune system's role in mood and anxiety disorders is well-studied, the role for the adaptive immune system in these disorders is less well defined. Animal studies link the adaptive immune system to stress-related behavior and stress circuitry, and in particular point to a role for T lymphocytes in immune-brain crosstalk that influences anxiety and brain function. Clinical studies also support the link between the adaptive immune system, in particular T lymphocytes, and anxiety disorders. Here we review this literature related to changes in adaptive immune markers in patient populations, considerations for treatment related to immune modulation, and the possible role that adaptive immune-brain crosstalk may play in gender differences in stress-related behaviors and brain function.

Keywords: T lymphocytes; Immune-brain crosstalk; Anxiety disorders; Flow cytometry analysis

Introduction

Behavioural neuroscience is ever expanding and incorporating interdisciplinary approaches to better understand the neurobiology of behaviour. Neuroimmunology, in particular, has yielded fresh insights into how immune functioning links to mood and psychiatric illnesses including anxiety and depression [1,2]. The field of neuroimmunology and the role for the immune system in behaviour developed from the seminal work by Hans Selye [3]. A key indicator that immune functioning could influence behaviour was the observation of sickness behaviours that include fatigue, loss of appetite, sleepiness, social withdrawal, weakness, and lethargy following immune activation [4,5]. These behaviours are consistently observed during infection irrespective of the infectious agent or the vertebrate species affected [4]. Additionally, these behaviours very closely resemble symptoms of anxiety disorders and depression suggesting that the immune system could contribute to behaviours associated with these disorders as well.

Clinical studies have indicated that administration of proinflammatory cytokines such as interferon- α results in sickness behaviours similar to those of infections and depression, namely, sadness, pessimism, suicide ideation, anorexia, loss of concentration, and sleep disturbance [6,7]. In animal studies, peripheral Interleukin (IL) -1 β cytokine injection activates the Hypothalamic Pituitary Adrenal (HPA) axis [8,9] and results in both sickness behaviours [9-12] and neuronal activation within the CNS [13,14]. Similar results, though with a longer induction period, were discovered using the gram negative bacterial endotoxin Lipopolysaccharide (LPS) as a peripheral immune challenge [15]. Importantly, innate immune challenge with peripheral IL-1 β or LPS results in an immune response centrally that mimics the immune response peripherally [6,16]. It is therefore evident that a peripheral immune response has a profound influence centrally on the brain and behaviour. However, emergent evidence indicates that adaptive immune functioning is also relevant to stress-related behaviours including anxiety.

In particular, animal studies have made a link between adaptive immunity and stress-related behaviours including anxiety-like behaviour. Several established behavioural tests are used to study anxiety-like and exploratory behaviour in rodents including the Elevated plus Maze (EPM), the open field test, and the light/dark test [17-19]. Recombines Activating Gene (RAG)-1 knockout mice, that lack B and T lymphocytes, showed increased exploratory behaviour and increased activity the Elevated Plus Maze (EPM) and open field [20]. Germ-free mice, that lack all gut microbiota and have an undeveloped adaptive immune system, have reduced trait anxiety, a behavioural phenotype that has been demonstrated in the EPM and light/dark test [21-23]. Interestingly, mice lacking functional class I MHC molecules and CD8+ lymphocytes [24] due to the genetic knock out of β 2-Microglobulin (β 2M) and Transporter Associated with antigen Processing (TAP) genes showed increased risk assessment behaviours including pokes into the open arms of the EPM, and head dips over the sides of the open arms of the EPM, however these mice did not show reduced open arm time in the EPM [25]. Another study using mice lacking all functional T cells (*TCR β -/- δ -/-*) showed reduced anxiety-like behaviours in the EPM, light/dark test, and the open field, however, sex differences in the reduced anxiety-like phenotype were evident [26]. In this recent study, no differences in anxiety-like behaviour were observed in B cell deficient mice, suggesting that T lymphocytes may mediate immune-brain crosstalk related to anxiety-like behaviour [26]. Together these animal studies in immunocompromised mice suggest a link between the adaptive immune system and anxiety.

Adaptive immune role in anxiety

Immune dysregulation has been observed in psychiatric patient populations. In particular, the importance of cytokines in depression has been thoroughly reviewed [7,27-29]. Furthermore, axis I psychiatric illnesses have been correlated with immune dysfunction such as increased susceptibility to infection, bronchitis, and asthma [30]. However, the role of the adaptive immune system in psychiatric

illness has received considerably less attention. Nonetheless, clinical data related to T lymphocytes functioning in anxiety disorders has emerged and suggests that immune functioning plays an important role in the pathophysiology of these disorders.

The adaptive immune system, that can recognize a large diversity of antigens and mount targeted immune responses, consists of T and B lymphocytes [31]. B lymphocytes can produce antibodies against specific pathogens and form the humoral immune response to extracellular pathogens. This response may or may not involve effector CD4⁺ T helper cells, also a component of humoral immunity, depending on the antigen. Effector CD8⁺ T lymphocytes are responsible for the adaptive cellular immune response that targets intracellular pathogens. Both CD4⁺ and CD8⁺ T lymphocytes can also exist as Tregulatory cells (Tregs), which are involved in immune tolerance to self-antigens. In addition to CD4⁺ and CD8⁺ T lymphocytes, which contain α and β chain subunits for the T Cell Receptor (TCR), there are also $\gamma\delta$ T lymphocytes, though the function of these cells is less understood [31].

Flow cytometry analysis is one approach to characterize changes in T lymphocytes in patient populations compared to healthy volunteers. Flow cytometry analysis determines the composition of leukocytes in an individual's whole blood sample. Most studies using this approach have examined the presence of CD4⁺ (T helper) and CD8⁺ (cytotoxic) T lymphocytes. One study that examined a mixed group of patients with either generalized anxiety disorder or panic disorder showed a lower CD4⁺/CD8⁺ T cell ratio in blood leukocytes compared to healthy volunteers, which was due to an absolute increase in the number of CD8⁺ T cells in patients [32], however, a separate study of only patients with panic disorder showed decreased CD8⁺ T cells and a related increase in CD4⁺/CD8⁺ T cell ratio [33]. Flow cytometry analysis has also been used in studies of Posttraumatic Stress Disorder (PTSD). One such study showed that individuals who suffer from PTSD had significantly reduced overall lymphocyte counts, that reflected less absolute numbers of CD4⁺ and CD8⁺ T cells compared to individuals without PTSD [34]. In a separate study, women with PTSD symptoms had an altered T cell profile at baseline that included increased CD4⁺ T cells but decreased CD8⁺ T cells [35]. Across the entire sample in this study, there was a strong correlation between CD4⁺/CD8⁺ T cell ratio and presence of PTSD symptoms [35]. Most studies using flow cytometry analysis in anxiety disorders to date relied on relatively small number of subjects. Overall, these studies show alterations in adaptive immune profiles in anxiety disorders, and yet the composition of immune cells in blood samples may be influenced by gender, age, and other humoral factors. Therefore, these factors might contribute to the lack of consistency in results across studies. Moving forward it will be important to consider whether changes in lymphocyte blood profiles contribute to susceptibility to anxiety or if these observations are the result of an anxiety disorder.

Another approach to studying blood lymphocytes is to examine lymphocyte function by collecting cells and culturing them *ex vivo*; this approach provides outcome measures including cell proliferation and cytokine production following mitogen stimulation. Using this approach, investigators showed that patients with anxiety disorders, including generalized anxiety disorder and panic disorder, had

reduced T cell proliferation compared to healthy controls, and showed reduced Interleukin-2 (IL-2) production following mitogen stimulation with Phyto-Haemagglutinin (PHA) [36]. Similar findings were observed in male patients with a history of PTSD, where reduced T cell production of cytokines, Interferon- γ (IFN- γ) and IL-4 was observed in response to PHA stimulation [34]. Another study examined several leukocyte functions in women with anxiety disorders and showed reduced leukocyte chemotaxis, reduced proliferation to PHA stimulation and reduced IL-1 production [37]. Consistently these studies show reduced mitogen-stimulated T-cell proliferation in cell cultures from individuals with anxiety disorders. A series of papers examining T lymphocytes from patients with GAD compared to healthy controls confirmed reduced T-cell proliferation in GAD cells compared to control but has also demonstrated that the cytokine profile in GAD individuals showed Th1 (IL-1 and IFN- γ) and Th2 (IL-4, IL-5) deficiencies as well as increased production of Th17 cytokines including TNF- α and IL-17 [38-40]. Furthermore, these investigators have demonstrated that the Th17 phenotype was enhanced by the addition of dopamine [39] or substance P [38] suggesting that the adaptive immune deficiencies could increase susceptibility to inflammatory or autoimmune disorders in anxious individuals [38,39].

Understanding how changes in immune-brain crosstalk contribute to anxiety disorders is central to work in this area. Notably, there is substantial overlap between the signaling molecules of the adaptive immune system and the nervous system. For example, it has long been known that T lymphocytes have benzodiazepine [41] and serotonin receptors [42]. The peripheral benzodiazepine receptors, found on T lymphocytes, are pharmacologically distinct from the receptors in the CNS [41]. However, they have similar binding affinity for benzodiazepines and are affected by this treatment [43]. For example, monocyte chemotaxis [44] and T-cell mediated humoral immune responses [45] are both enhanced by treatment with diazepam. In rats, diazepam treatment increased corticosterone and the percentage of CD8⁺ T cells but decreased B cells and the number of apoptotic cells [46]. In cell culture experiments, diazepam and tofizopam dose-dependently alter peripheral blood mononuclear cell proliferation and reduce plasma levels of Tumor Necrosis Factor- α (TNF- α). However, while tofizopam elevates T cell mitogen-induced production of IL-2, diazepam decreases it [47].

T cells have also been closely linked to the serotonergic system. T cells are known to express the serotonin transporter (5-HTT) [48], as well as serotonin receptors 5-HT_{2A} and 5-HT_{2C} and different splice variants of these receptors in leukocytes of patients with obsessive compulsive disorder and bipolar disorder [42] suggesting that serotonergic dysregulation in T cells could be related to anxiety behaviour and disorders. Furthermore, there are differences in serotonergic dysregulation specific to different anxiety disorders. In patients with generalized anxiety disorder, 5-HTT is expressed at normal levels but with altered functional efficiency [49]. 5-HTT's expressed by T cells are also susceptible to the effects of serotonin reuptake inhibitors and tricyclic antidepressants [48]. Also, it has been shown that mice deficient of T regulatory cells have reduced serotonin (5-HT) and serotonin metabolite (5-HIAA) in the hippocampus [50] indicating that the serotonergic link between the immune system and the brain is bidirectional.

Treatment of psychiatric illness through immune modulation

Providing further support for links between the adaptive immune system and anxiety behaviours is the evidence of alterations to the immune system by anxiolytic medication. Certain benzodiazepines, one of the most successful classes of anxiolytic medications, interfere with T lymphocyte proliferation in response to mitogen [51] while others boost cellular immunity [52]. Twelve weeks of treatment with a serotonin and norepinephrine reuptake inhibitor completely inhibited T lymphocyte proliferative capacity to phyto-haemagglutinin mitogen in a patient who had been immune typical prior to treatment [53] showing that this particular benzodiazepine potently modulates T cell functioning. This case study is in keeping with animal work going back as far as 1982, when diazepam administration to mice resulted in impaired delayed-type hypersensitivity and primary antibody response to red blood cells from sheep [54]. Additionally, the psychotropic medications imipramine, haloperidol, chlorpromazine, and meprobramate also reduce cellular immunity [55] indicating that the immunomodulatory effects of these medications are widespread. These studies indicate that some treatments for anxiety already have immune effects in addition to central targets, though this has received little attention in the following years. A decade after the first discovery that benzodiazepines have immunosuppressant effects, an investigation of treatment with alprazolam, a benzodiazepine receptor antagonist, observed increased natural killer cell activity and increased lymphocyte proliferation in mice treated with low doses [56], thus indicating that benzodiazepine receptors activation and inactivation influence T cell functioning.

Interestingly, immunomodulating psychotropic medications are currently used to treat psychiatric [1,57] and even immune illnesses [58]. Treatment of patients with anxiolytics and anti-depressants can modulate immune factors such as cytokines, cell profiles, and inflammatory markers. Men who suffer from depression have elevated C Reactive Protein (CRP), an indicator of inflammation, and IL-6 than healthy controls, an observation that was absent in women. CRP was most highly elevated in men taking SNRIs, tri- or tetracyclic antidepressant medication while IL-6 levels were lowest in those taking SSRIs [1]. In rats, the SSRI fluoxetine increased the percentage of 5-HTT positive T cells and the production of IL-2. This drug also increases the percentage of CD8+ T cells while lowering the percentage of CD4+ cells thus changing the lymphocyte profile [59]. In fact, SSRIs are so potent as immunosuppressant that they have shown some success treating graft-versus-host disease in patients who have received organ transplants [58]. TNF- α level, which are also elevated in some depressed patients prior to treatment, are normalized following treatment with amitriptyline and lorazepam in both patients who respond and who do not respond clinically to treatment [57]. These findings are striking as one study has shown that depressed patients who enter remission tend to have lower pretreatment levels of IL-6 and there is a strong negative correlation between pretreatment IL-6 and clinical improvement scores using the HAMD and MADRS assessments [57].

Currently, it remains unclear whether the effects of psychotropic medications are coincidental or complementary to the treatment effects of psychotropic medications. There is also the possibility, as was suggested early in the reporting of suppressed immunity [54],

that excessive immune suppression could compromise patient safety through increased susceptibility to infection. Ideally, there is a level of immune activation that is optimal for anxiety, it may be possible to inform clinicians which medications a patient is likely to respond to given their immune status. At minimum, the close interconnectedness of adaptive immunity and anxiety warrants careful consideration of immune competency in patients receiving any treatments that act through the immune system.

Sex differences in adaptive immunity - is there a role in anxiety?

An important context for adaptive immune influence on anxiety-like behaviour is evidence that this branch of immunity may contribute to sex differences in the prevalence and presentation of anxiety behaviours. Sex differences in immune function are well established [60-62] and contribute to gender differences in immune-related disorders such as infections and autoimmunity [63,64]. Clinically this is an important consideration as sexual dimorphism influences the incidence, age of onset, and clinical presentation of mental illness [65-73], however determining the precise roles for various sex-specific factors has received limited attention. Immune-brain crosstalk may be an important factor to the biological basis of sex differences in clinical psychiatry. In fact, there are known differences in immunity between the sexes and it is possible that these differences combine with endocrine effects to account for the sexually dimorphic behaviour observed. For example, endocrine-immune interactions contribute to gender differences in the immune response to pathogens such as bacteria, viruses, and parasites leading to increased susceptibility to infectious disease in males [63]. In contrast, females show increased risk of autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis [64]. It is possible that sex differences in immunity are influenced by endocrine function as estrogens enhance the immune response in females and evidence suggests that estrogens may also modulate T cell trafficking, contributing to increased susceptibility to autoimmune processes in females [74]. Understanding how immunological differences influence behaviour is needed to provide new insights into sex differences in the prevalence of anxiety disorders and differences in susceptibility to specific anxiety disorders between men and women.

Conclusion

Understanding how immunological differences influence behaviour is needed to provide new insight into sex differences in the prevalence of anxiety disorders in women and the differences in susceptibility to specific anxiety disorders between the two sexes. Furthermore, the discovery that adaptive immunity potently influences anxiety behaviours provides interesting possibilities within psychiatric treatment. Despite decades long knowledge of the interconnectedness and shared biology of the immune system and the brain; immune targets for psychotherapy remain largely unexplored. Presumably, this is due to immunological risk; however, if immune dysfunction is contributing the pathology of anxiety, avoiding the immune system may be treating the symptoms rather than the disease. Additionally, it appears as though the immune system is already being modulated by current anxiolytic therapies, though this is inadvertent and considered a side effect. It is worth investigating

immune parameters prior to and during treatment for anxiety to determine if immune profile can predict responsiveness to specific classes of anxiolytics or, inform safety considerations in patients who are already immune compromised. Finally, the differences between the sexes immunologically should be taken into account with neuroimmune contributions to anxiety. There are known differences between men and women for immune functioning, susceptibility to anxiety diseases and differences and the presentation of anxiety. Differing immune profiles between the sexes and the known relationship between immune functioning and anxiety suggests that men and women may benefit from different therapeutic approaches. Further neuroimmune investigations of anxiety disorders the treatment thereof should focus more attention on differences between men and women.

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