向精神薬の治験の進め方

——抗うつ薬の臨床試験を中心に——

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はじめに

世界保健機構（WHO: World Health Organization）による世界疾病負荷調査（GBD: The Global Burden of Disease Study）では、精神疾患は、主な機能障害の原因の1つであることが指摘（WHO (2009)；WHO（2004 update2008））され、疾病全体の中でも最も重要な疾患として位置づけられている。また海外における中枢領域の臨床試験の登録件数は悪性疾患領域についで2位であり、なかでもうつ病と統合失調症を対象とした試験の登録件数が多く（Karlberg (2008)）、当該領域の新薬開発は活発である。

臨床試験においては、科学的に有効性と安全性を示す必要があることは言うまでもない。本邦における向精神薬の治験は、これまでは実薬対照非劣性試験または同等性治験が中心であった。しかし、ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use、日米EU医薬品規制調和国際会議）のE10ガイドライン（厚生省医薬局審査課長（2001）でも指摘されているとおり、非劣性試験または同等性試験は、内部妥当性を示す指標が存在せず無効同等の可能性が排除できないことより、有効性に関する結果解釈が困難となる問題がある。近年は、塩酸セルトラリンやミルタザピンのようにプラセボ対照試験により承認された向精神薬が登場しており、明確なエビデンスが蓄積されるようになったと言える。

しかし、既承認の統合失調症治療薬の約4分の1が、そして抗うつ薬の約半数がプラセボ対照試験に失敗しており（Laughren (2001)）、プラセボに対する優越性を示すことも容易なことではない。また、初回の臨床試験から医薬品として承認に至るまで割合は10%未満と高

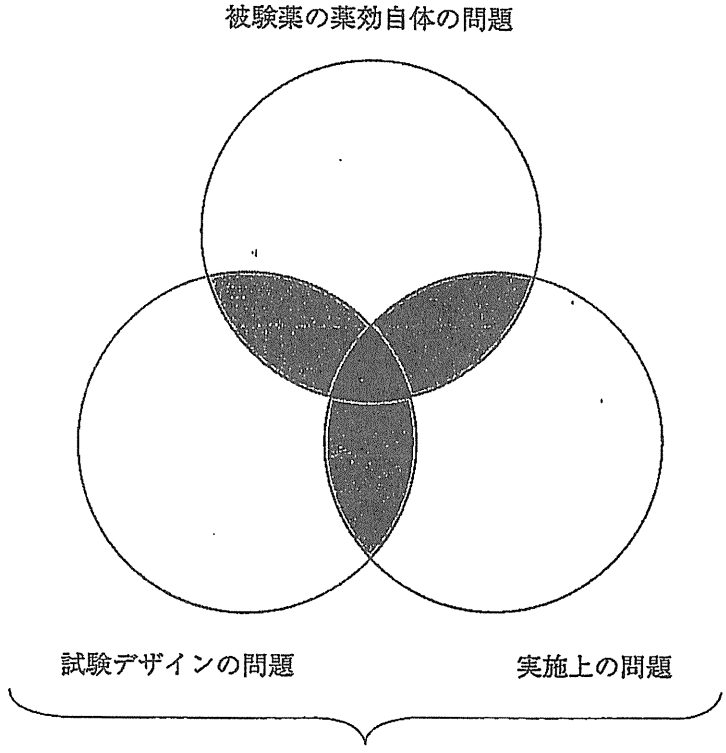
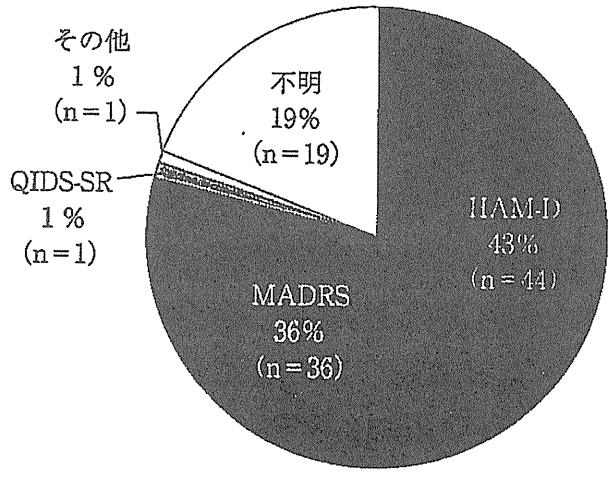


図１　臨床試験の成否に影響を及ぼす要因

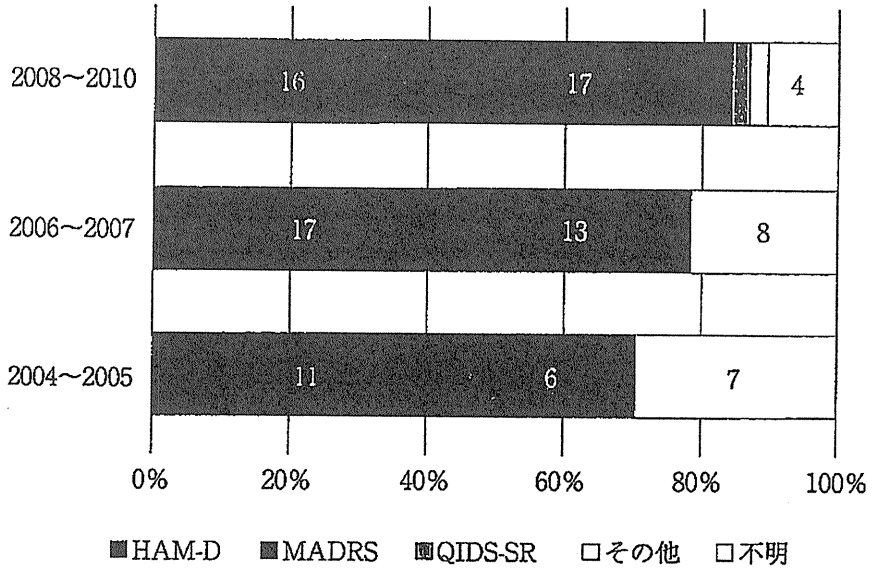
くはなく（Kola, et al（2004））、多くの場合は被験薬の有効性を示すことができない（Frank, et al（2003））ことが原因する。臨床試験の成否には、薬効自体の問題もあるが、試験デザインや試験実施上の問題等の方法論的問題も影響（図１）する。適切に臨床試験を実施していくためには、よく計画された試験計画が必要なだけではなく、治験を行うわれわれ臨床医が実施上の問題についても十分理解する必要がある。このため、本稿では、抗うつ薬の治験を例にその有効性評価に影響を及ぼす実施上の問題を取り上げ説明する。

抗うつ薬の臨床試験の特徴

米国国立衛生研究所（NIH: National Institutes of Health）の臨床試験登録データベース（http://www.clinicaltrials.gov/）には、企業主導によるうつ病を対象としたプラセボ対照の無作為化二重盲検比較試験（2004年から2010年1月までに開始された第Ⅱ相もしくは第Ⅲ相試験）のうち、安全性評価を主目的とした試験およびランダム化治療中止試験を除外すると、101試験が登録されている。これらの臨床試験の有効性の主要評価項目は、HAM-D（Hamilton Depression Rating Scale）またはMADRS（Montgomery-Åsberg Depression Rating Scale）が多く使用されている（図2a）。また最近では、HAM-Dと比較してMADRSの主要評価項目としての使用が増加する傾向もある（図2b）。対象となる被験者の重症度は、選択基準において症状評価尺度のカットオフポイントとして設定されるが、各カットオフポイントはHAM-D（17項目）では20点以上、MADRSでは22点以上と設定されることが多い（図3）。登録されている101試験のうち、選択基準および主要評価項目で設定されている症状評価尺度がいずれも公開されているのは37試験のみであるが、このうち12試験は選択基準および主要評価項目では同一の症状評価尺度が使用され、残り25試験は選択基準と主要評価項目とで異なる評価尺度（例えば、選択基準はHAM-D、主要評価項目はMADRS）が用いられている。以上の試験の投薬期間は、多くが6週週間もしくは8週間と設定されている（表1）。



(a) 2004年～2010年1月



(b) 開始年別（数値は試験数を表示）

図2　主要評価項目に使用された症状評価尺度

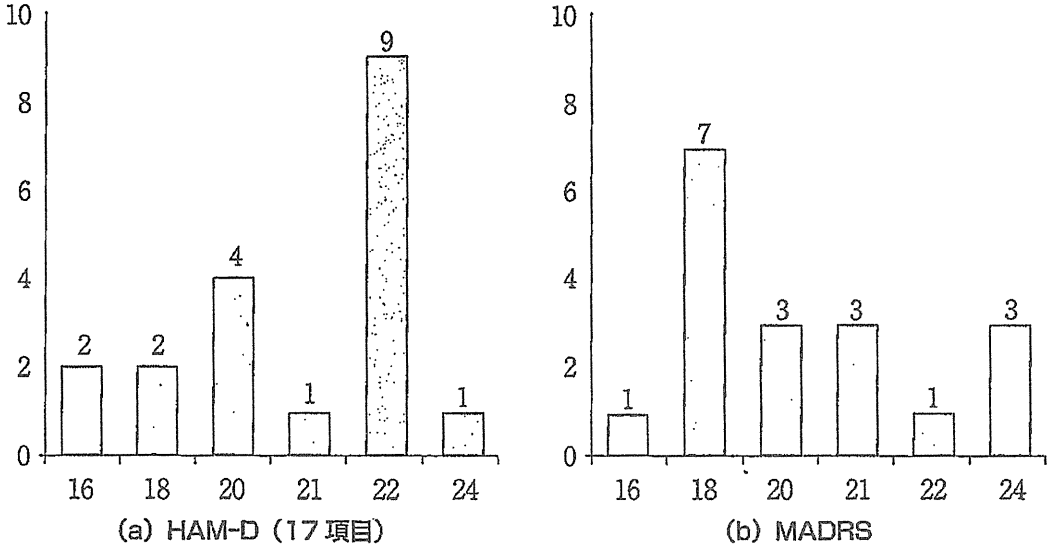


図３　選択基準における症状評価尺度のカットオフ・ポイント
（2004年-2010年1月）

投与期間	試験数
4週間	2
6週間	18
8週間	53
9～10週間	6
11～12試験	3
不明	19
合計	101

表１　各試験ごとの投与期間の分布
（2004年～2010年1月）

有効性評価に関わる問題

●プラセボに対する反応性

向精神薬の臨床試験においては、主要な有効性評価は、症状評価尺

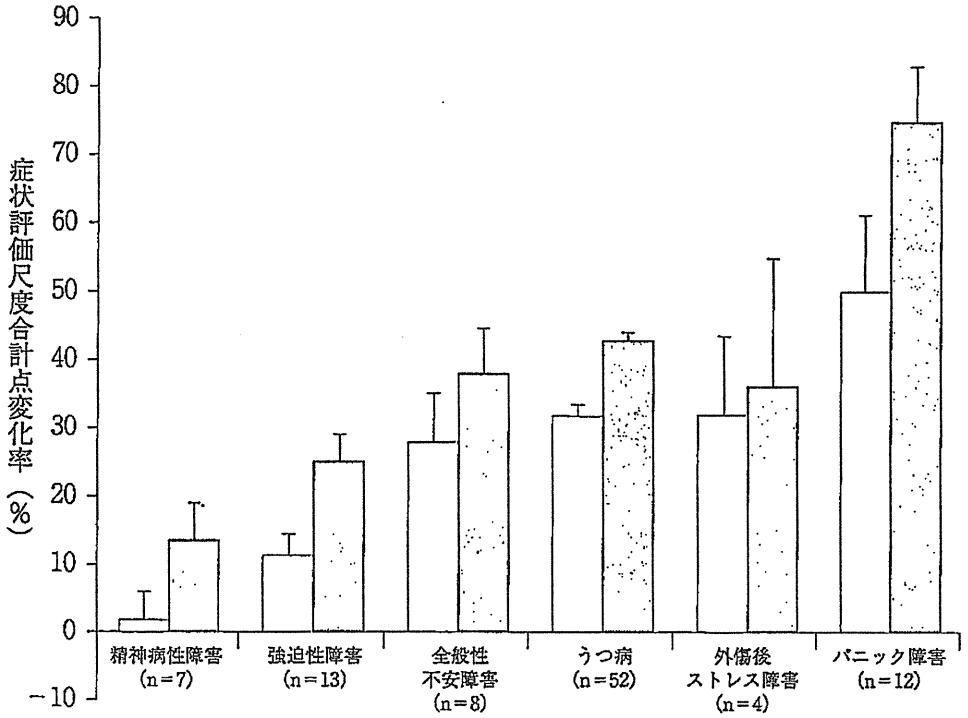


図４　疾患領域ごとのエフェクトサイズ

Knan, et al（2005）改編。

度の合計点のベースラインから最終観察時までの変化量が検討される。薬効はエフェクトサイズ（被験薬群とプラセボ群の差）が検討されるため、当然のことながらプラセボに対する反応性が影響する。精神疾患領域のなかでは、うつ病や全般性不安障害においてプラセボ反応性が高いことが知られている（図4）。プラセボ反応性に影響する要因についてもさまざまな検討（Walsh, et al（2002）；Fava, et al（2003））が行われているが、明確にはなっていない。治験薬に対しても、被験者のみならず担当医師も新たな治療としての効果を期待したバイアスが発生（Marks（2009））するため、このような事実を把握したうえで、より客観的に症状評価を行うことが重要である。症状評価は臨床試験の質に大きく影響するが、詳細は別章（樋口「日本の治験の現状と課題」）で解説する。

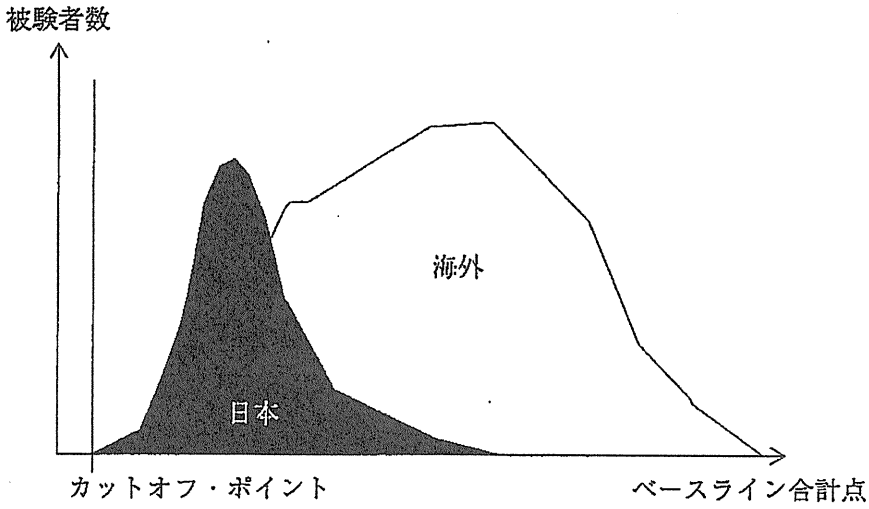


図５　国内外で同一の試験計画で実施した場合の被験者の重症度分布の差異

●被験者のベースライン重症度

前述のとおり、有効性評価は症状評価尺度合計点のベースラインから最終観察時までの変化量が検討されるため、ベースラインでの重症度は有効性評価に影響する（Kirsch, et al (2008)；Fournier, et al (2010)）。つまり、被験者集団のベースライン重症度が低い場合、得られたエフェクトサイズが小さくなり、統計学的な検出力が担保された試験計画でも検証が困難になることがある。現に、欧州医薬品委員会（CPMP: Committee for Proprietary Medical Products）も、抗うつ薬の臨床試験においては、軽度の患者では有効性を検討することが困難であることを指摘（European Medicines Agency (2002)）している。また、国内と海外で同一の計画により臨床試験が実施された場合に、被験者の重症度の分布が国内外で異なることが経験される（図5)。これにより、海外試験で有効性が示されても、国内試験でより軽度の患者の組み入れが多くなると、成功確率が低下し有効性が示されないことが起こりうる。前述の通り選択基準と主要評価項目とで異なる評価尺度を使用することは組み入れの際のバイアスを軽減するため

の方策であるが、安全確保策を十分に検討するとともに、薬効評価に適した重症度の被験者を選択していく必要がある。

●併用薬・併用療法の影響

うつ病の臨床においては、抗うつ薬の他にベンゾジアゼピン系薬剤等が併用されることもある。うつ病に対するベンゾジアゼピン系薬剤の長期投与の有効性は明確になっていないが、短期の使用ではその有効性が示されている（Furukawa（2000））。一般に、抗うつ薬の臨床試験の投与期間は6週間もしくは8週間と設定されることが多い（表1）ことから、ベンゾジアゼピン系薬剤をはじめとする併用薬や併用療法は、有効性評価に影響する可能性があることにも留意する必要がある。

●症例の集積性

臨床試験における症例の集積性については2つの問題があり、1つは施設当たりの被験者数であり、2つ目は被験者の組み入れ速度に関するものである。

これまでにも筆者は、本邦の臨床試験は海外と比較して、1施設当たりの被験者数が極端に少なく、参加施設数が多いことを報告（中林ほか（2010））している。これにより、被験者集団や症状評価の方法にもばらつきが大きくなり、有効性評価に影響する可能性がある。ICH E9ガイドライン（厚生省医薬安全局審査管理課長（1998））で指摘されている通り、参加施設の特性や施設ごとの被験者数に大きく差異がないように配慮するなど、実施体制につても検討していく必要がある。

本邦での治験における被験者の組み入れ速度については、初期の組み入れが少なく試験終了に近くなると急激に組み入れが多くなることが経験される（図6）。国際共同治験では、参加国ごとの目標症例数

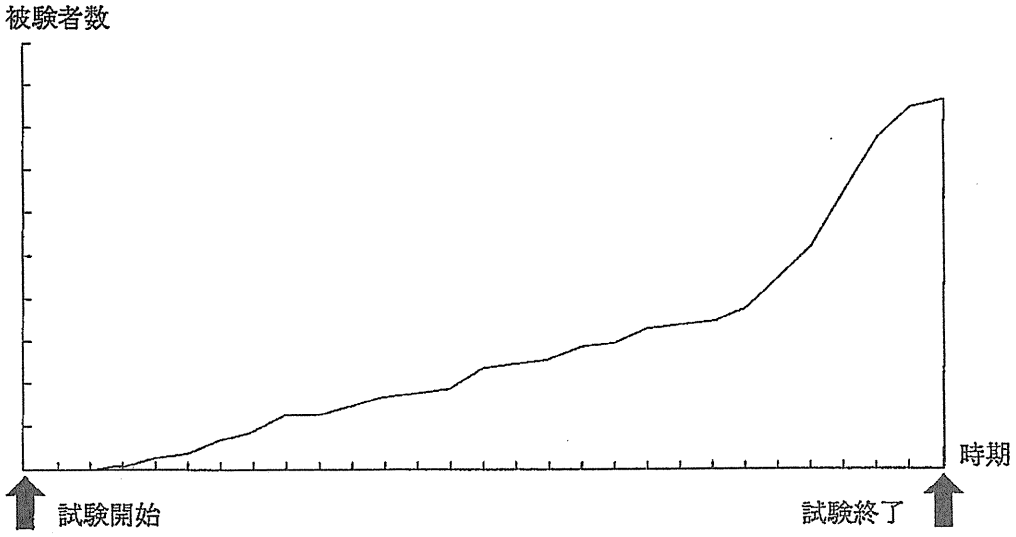


図６　試験開始から累積被験者数の一般的な推移

が設定（厚生労働省医薬食品局審査管理課長（2007））されていても、一般的に競争的に組み入れが行われるため、日本人症例の試験成績を検討するためには計画的な組み入れを行う必要がある。

おわりに

抗うつ薬の臨床試験の特徴を説明するとともに、有効性評価に影響を及ぼす可能性がある問題について説明した。これらは個々が独立した問題ではなく相互に関係することもあるため、試験の実施にあたっては種々の検討が必要となる。治験を行う医師や医療環境に関わる問題も多いため、より適切に試験を実施していくためには、今後も具体的な問題を理解し共有することが重要であると考える。また、欧米のみならずアジア諸国での臨床試験の実施も活発化（中林ほか（2010））しており、リーダーシップを保ち続けていくために、臨床試験のための基盤の整備についても継続的に行っていく必要があると考える。

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REVIEW ARTICLE

Mind over cytokines: Crosstalk and regulation between the neuroendocrine and immune systems

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Abstract

Crosstalk between the neuroendocrine and immune systems is essential for the maintenance of homeostasis in our bodies. Recent advances in neuroscience and immunology have elucidated the cellular and molecular basis for these bidirectional interactions. Neuronal and immune cells share a variety of neurotransmitters and cytokines as well as receptors, which enable these complex interactions. Individual hormones, neurotransmitters and neuropeptides have their own specific spatial and temporal niches, and these overlap to facilitate crosstalk with each other. The neuroendocrine system has multilevel modulatory properties that affect the functions of the immune system, contributing to both activation and suppression. Neural regulation of immune responses is accomplished systemically by hormones, regionally by innervation and locally by neurotransmitters. In turn, immune cells regulate neural function and integrity directly through cytokines or through the vagus nerve. In the present review, these complex, multifaceted interactions at the molecular level are explained based on current knowledge. (Clin. Exp. Neuroimmunol. doi: 10.1111/j.1759-1961.2011.00023.x, January 2012)

Introduction

Interactions between the immune and neuroendocrine systems were discovered by Hans Selye et al. in the 1930s. Since then, we have come to appreciate that integration between these two systems is essential in order to maintain homeostasis and overall health; the immune and neuroendocrine systems work in harmony with all other physiological systems at the level of the whole organism. These two systems reciprocally regulate each other, and share common ligands and receptors. Neuroendocrine regulation of immune responses is important for survival during both physiological and mental stress, and is accomplished systemically through hormonal cascades, regionally through nerve pathways into lymphoid organs and locally through neurotransmitters. In turn, the immune system regulates the central nervous system (CNS) through cytokines (Fig. 1). Herein, an updated overview of these complex interactions will be discussed.

Neuroendocrine regulation of the immune system

Systemic regulation of the immune system through hormones

Neuroendocrine systems systemically control immunological functions at the level of the hypothalamic–pituitary–adrenal (HPA) axis through glucocorticoids (GC), the hypothalamic–pituitary–gonadal (HPG) axis through sex hormones and the hypothalamic–pituitary–thyroidal (HPT) axis through thyroid hormones.[1] In addition to these classical pathways, the renin–angiotensin–aldosterone system (RAAS) and feeding regulatory hormones are also involved in the regulation of immune functions.

The hypothalamic–pituitary–adrenal axis

On various physical and psychological stimuli, corticotrophin-releasing hormone (CRH) is secreted from the paraventricular nucleus of the hypothalamus into the hypophyseal portal blood supply and stimulates the release of adrenocorticotropin hormone

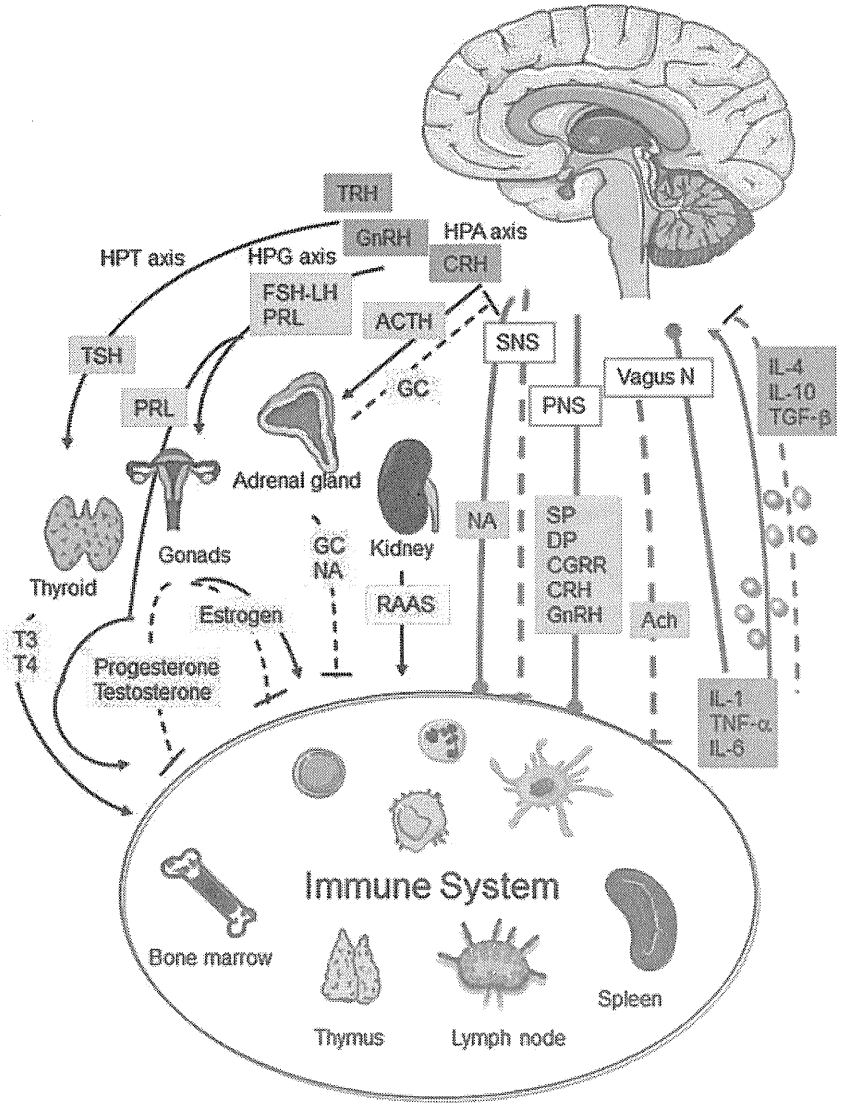

Figure 1 Crosstalk and regulation between the neuroendocrine and immune systems. The interactions between the immune system and the neuroendocrine system are regulated on the level of systemic routes by hormones and cytokines, regional routes by innervation of the sympathetic, parasympathetic and peripheral nervous systems, and local routes by neurotransmitters and cytokines. The hypothalamic–pituitary axis (black lines) controls the release of glucocorticoids, sex hormones and thyroid hormones. The autonomic and peripheral nervous systems participate in regional and local control of the immune system (green lines). In turn, immune cells produce cytokines on activation and affect neural functions (pink lines). Dashed lines indicate inhibitory signals. Red boxes indicate cytokines, green boxes indicate neurotransmitters, yellow boxes indicate hormones from peripheral endocrine organs, gray boxes indicate hormones from pituitary gland, purple boxes indicate hormones from the hypothalamus. Ach, acetylcholine; ACTH, adrenocorticotropin hormone; CGRR, calcitonin gene-regulated peptide; CRH, corticotrophin-releasing hormone; DP, dopamine; FSH, follicle stimulating hormone; GC, glucocorticoids; GnRH, gonadotropin releasing hormone; HPG, hypothalamic–pituitary–gonadal; HPT, hypothalamic–pituitary–thyroidal; IL, interleukin; LH, luteinizing hormone; NA, noradrenaline; PNS, peripheral nerve system; PRL, prolactin; RAAS, renin–angiotensin–aldosterone system; SNS, sympathetic nervous system; SP, substance P; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone.

(ACTH) from the anterior pituitary gland. ACTH then stimulates the adrenal cortex to produce GC. The secretion of CRH is upregulated by dopamine, serotonin, noradrenalin and histamine, and downregulated by opiates and γ-aminobutyric acid (GABA) as well as hormones downstream of CRH, such as GC and ACTH, through negative feedback.[2] GC bind to cytoplasmic glucocorticoid receptors (GCR), and modulate the transcription and protein synthesis of genes, such as activating protein-1 (AP-1) and nuclear factor κB (NFκB).[3–5] Although GC play various roles in modulating immune responses, their overall effect is suppressive for both innate and acquired immunity through the inhibition of differentiation, maturation, proliferation and functions of immune cells.[5] GC inhibit the production of pro-inflammatory (interleukin [IL]-1, IL-6, tumor necrosis factor [TNF]-α) and Th1-related cytokines (IL-2, IL-12, γ-interferon [IFN-γ], granulocyte macrophage colony-stimulating factor [GM-CSF]), as well as inflammatory mediators, such as prostaglandin and nitric oxide, and enhance the production of anti-inflammatory cytokines (IL-4, IL-10). GC also suppress the proliferation and function of cytotoxic T cells. GC inhibit antigen presentation by suppressing the maturation of dendritic cells and reducing the expression of major histocompatibility complex (MHC) class II molecules.[1] GC suppress cell trafficking by inhibiting the production of chemoattractants (IL-5, IL-8, regulated on activation, normal T cell expressed and secreted [RANTES], eotaxin, monocyte chemotactic protein-1 [MCP-1]) and the expression of cell adhesion molecules (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion

molecule-1 [VCAM-1], E-selectin). Furthermore, GC induce the apoptosis of T cells and thymocytes through increasing the expression of Bad, Bcl-XS and Bcl-XL genes.

CRH is released from nerve endings at sites of inflammation, as well as from immune cells, and CRH receptors are expressed on immune cells including macrophages, T cells, B cells, mast cells and eosinophiles.[6–9] The local effect of CRH is inflammatory rather than anti-inflammatory. CRH receptor antagonists suppress the production of IL-6, IL-1 and TNF-α from macrophages resulting in disease inhibition in a model of endotoxin shock.[10]

Hypothalamic–pituitary–gonadal axis

Females show greater humoral and cellular immune responses than males. The importance of sex hormones on immune reactions has been inferred from the higher frequency of many autoimmune diseases in females. Gonadotropin releasing hormone (GnRH) released from the hypothalamus stimulates gonadotropins including follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the anterior pituitary gland, and subsequently stimulates the release of estrogen and progesterone from ovary glands. Immune cells express GnRH and GnRH receptors.[11,12] GnRH enhances T cell development, proliferation, cytokine production (including IFN-γ) as well as IgG production,[12] and is therefore immunostimulatory. In agreement with this, administration of GnRH antagonist in a murine lupus model ameliorates disease by reducing autoantibody production, whereas administration of GnRH agonists exacerbates disease severity.[13]

Estrogen binds to two forms of cytoplasmic estrogen receptors (ER), ERα and ERβ. ERα is expressed on the endometrium, ovarian stromal cells, breast and hypothalamus, whereas ERβ is widely expressed in tissues including brain, kidney, bone, heart, lungs, intestine and endothelial cells[14]. Estrogen has dual roles in the modulation of immune responses depending on the plasma levels. High levels of estrogen suppress macrophages to produce TNF-α and IL-12, and promote them to produce IL-10.[15] In addition, estrogen promotes the HPA axis and noradrenaline (NA) production, resulting in further inhibition of inflammation, and favors a Th2 pattern of cytokines.[16] Estrogen has a potent modulatory effect on B cell development and survival, interfering with B cell tolerance and enhancing autoantibody production.[17–19] Consistent with these findings, hyperestrogenic states, such as an ingestion of oral contraceptives and pregnancy, are associated with disease flare-up of systemic lupus erythematosus (SLE), in which a humoral immune response is an important pathogenic factor.[11] In contrast, pregnancy has been reported with decreased disease activities in rheumatoid arthritis or multiple sclerosis, which are Th1/Th17-mediated responses that dominate in the pathogenesis.[11] Several studies in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) that is mainly mediated by myelin reactive T cells, have shown the inhibitory effects of estrogens on disease acitivities.[20–25] The mechanisms that underlie the inhibition of EAE include suppression of myelin-specific Th1 and Th17 immune suppression, increased Th2 responses, induction of $CD4^+CD25^+$ regulatory T cells (T regs) and downregulation of inflammation. The studies using knockout mice and a specific ligand for ERα or ERβ suggest that the suppressive effects of estrogen in EAE are mediated by ERα,[26–28] although ERβ seems to be involved in neuroprotection.[29,30] More recently, G-protein coupled estrogen receptor (GPR30), a membrane estrogen receptor, has been shown to be important in the inhibition of EAE by estrogen.[31–33] Based on these findings and a promising pilot trial of oral estriol, there are several clinical trials of estrogens in MS underway.[34–36] Progesterone shows anti-inflammatory effects through the inhibition of NFκB.[15,37] Testosterone inhibits both innate and acquired immunity.[11] Dihydrotestosterone decreases immunoglobulin and cytokine production and lymphocyte proliferation.[38]

Prolactin (PRL) is released from the anterior pituitary gland and stimulates mammary growth and differentiation. PRL and PRL receptor, members of a cytokine receptor superfamily, are expressed on immune cells. PRL is stimulated by suckling and stress, and inhibited by dopamine. The production of PRL in T cells is inhibited by IL-2 and IL-4.[39] The effects of PRL on immune responses are immunostimulatory and PRL enhances production of cytokines, such as IFN-γ, IL-12 and IL-10, as well as T cell proliferation. PRL also alters the functions and selection of B cells, resulting in the breaking of tolerance of autoreactive B cells.[40–42] Consistent with this, bromocriptine administration abrogates the estradiol-induced breakdown of B cell tolerance.[43]

Hypothalamic–pituitary–thyroidal axis

Thyrotropin-releasing hormone (TRH) secreted from the paraventricular nucleus of the hypothalamus stimulates the release of thyroid stimulating hormone (TSH) in the anterior pituitary gland and subsequently stimulates the release of thyroid hormones

from the thyroid gland. The existence of receptors for thyrotropic and thyroid hormones in immune cells, and the production of TSH by immune cells established the presence of interactions between pituitary–thyroid hormones and the immune system. Studies using mice deficient in thyroid hormone receptors suggested that B cells, macrophages and granulocytes were decreased in the spleens of these mice.[44] Experimentally-induced hypothyroidism resulting from propylthiouracil (PTU) treatment in rodents, as well as hypothyroidism in humans, reduces thymic activity and humoral and cell-mediated immune responses, and this suppression was relieved by the administration of thyroid hormones.[45–47] Consistent with these results, lymphocytes from hyperthyroid mice treated with thyroxin showed higher T and B cell mitogen-induced proliferation. Recall responses to sheep red blood cell immunization showed increased or decreased IL-2 and IFN-γ production in hyper- or hypothyroid mice, respectively. In addition, the production of IL-6 and IFN-γ on stimulation with lipopolysaccharide (LPS) was upregulated in hyperthyroid mice, suggesting enhancement of innate immune responses.[47] Furthermore, proliferative responses and cytotoxic activity were reduced in chronically stressed mice in which the levels of thyroid hormones, but not GC and NA, were reduced, and thyroxin replacement reversed the reduction in T cell responses.[48] These findings suggest that stress induces an alteration of the HPT axis leading to modulation of immune responses.

Renin–angiotensin–aldosterone systems

In addition to the aforementioned pathways, other systems, such as RAAS, are important modulators of immune functions. RAAS regulates blood pressure and body fluid homeostasis. Renin converts angiotensin (Ang) to AngI, and Ang-converting enzyme (ACE) catalyzes the conversion of AngI to AngII. Although there are two subclasses of receptors for AngII, AngII type 1 receptor (AT1R) and AngII type 2 receptor, AT1R mediates the major effects of AngII. RAAS is also expressed in immune cells like the other hormones described earlier. Monocytes and dendritic cells produce AngII and AT1R.[49–51] AngII stimulation promotes the production of inflammatory mediators, including cytokines, chemokines and adhesion molecules, through the activation of NFκB.[52,53] These mediators also promote the differentiation of dendritic cells and accumulation of neutrophils, which then drives diseases, such as atherosclerosis and inflammation.[51,54,55] Inhibition of ACE or AT1R suppresses the production of inflammatory cytokines, including TNF-α, IL-1, IL-6, IL-12 and IL-18,[56–58] leading to disease suppression in arthritis models.[59,60] Inhibition of RAAS regulates not only innate immunity, but also adaptive immunity, as antigen-specific Th1 responses are suppressed in collagen-induced arthritis and experimental autoimmune uveitis.[60,61] More recently, suppression of both Th1 and Th17 responses, and induction of Tregs and transforming growth factor-β (TGF-β) by the blockade of ACE or AT1R were shown in EAE.[62,63]

Leptin, ghrelin, neuropeptide Y

Recent studies have shed light on the immunomodulatory potency of feeding regulatory hormones, such as leptin, neuropeptide Y (NPY) and ghrelin. Leptin is predominantly produced by adipocytes and is actively transported through the blood–brain barrier (BBB) and acts on the hypothalamic satiety center to decrease food intake. The receptors for leptin (OB-R) belong to the class I cytokine receptor family, which includes the IL-2 receptors, and have at least six isoforms. The short leptin receptor isoform (OB-Ra) and the long leptin receptor isoform (OB-Rb) are the main leptin signaling receptors, and are expressed in the hypothalamus and other cells including immune cells.[64] Leptin induces the release of inflammatory cytokines, such as TNF-α and IL-6, as well as CC-chemokine ligand 2 (CCL2) and vascular endothelial growth factor (VEGF).[65] Leptin also stimulates the production of chemokines by eosinophils[66] and neutrophils.[67,68] Serum leptin is decreased after acute starvation in parallel with immunosuppression or Th2 bias, whereas exogenous leptin enhances proliferation of T cells and skews cytokine balance towards Th1, leading to the suppression of EAE.[69,70] Serum levels of leptin correlate with body fat mass. In contrast, serum levels of adiponectin, another hormone secreted from adipocytes, are markedly decreased in individuals with visceral obesity and insulin resistance. Interestingly, adiponectin inhibits the ability of macrophages to produce inflammatory cytokines and chemokines, and carry out phagocytosis.[71,72]

NPY is increased after starvation. NPY regulates a variety of physiological activities, including energy balance and feeding, anxiety, neuroendocrine secretion, neuronal excitability and vasoconstriction. NPY is synthesized and released with NA from sympathetic nerves, the adrenal medulla and immune cells.[73] NPY receptors are G-protein-coupled receptors and consist of five subsets (Y1–5), which are differentially expressed in tissues. Y1 receptors are

rather ubiquitous and are also expressed in immune cells. Exposure of macrophages to NPY suppresses the production of IL-6 *in vitro*.[74] Exogenous NPY shifts the Th1/Th2 balance towards Th2 through NPY receptor 1 and ameliorates the severity of EAE.[75] In contrast, studies using NPY1 receptor-deficient mice have shown that NPY promotes APC activation in addition to its role in downregulating Th1-responses.[76] Leptin and NPY are linked to ghrelin, as ghrelin is increased after starvation, it potently stimulates the release of NPY in the CNS[77] and antagonizes the effects of leptin.[78]

Ghrelin is predominantly secreted from the mucosal endocrine cells of the stomach and the ghrelin receptor, a G protein-coupled receptor called GH secretagogue receptor (GHS-R), is widely distributed throughout various organs. Ghrelin stimulates GH release, increases food intake, regulates energy homeostasis and decreases energy expenditure by lowering the catabolism of fat.[79,80] Ghrelin and the GHS-R have been detected in immune cells and lymphoid tissues. Ghrelin induced increases in peripheral blood lymphocytes, as well as thymic cellularity and differentiation; the resulting increases in cytotoxic lymphocytes reduce tumor initiation and subsequent metastases.[81] More recent studies have highlighted the anti-inflammatory functions of ghrelin. Ghrelin inhibits the nuclear translocation of NFκB and suppresses the production of inflammatory cytokines from macrophages and T cells.[82,83] As a consequence, ghrelin inhibits bowel disease,[84] arthritis,[85,86] sepsis and endotoxemia.[82,86,87] Furthermore, ghrelin inhibits the production of inflammatory cytokines from microglia and subsequently suppresses EAE.[88,89]

Regional regulation of the immune system through the autonomic nervous system

Regional control of immune responses is mediated by innervation of primary and secondary lymphoid organs. Nerve terminals lie adjacent to T cells, B cells and dendritic cells, with the neuroimmune junction measuring approximately 6-nm wide, in contrast to a typical CNS synapse, which is 20-nm wide. Innervation of lymphoid organs changes depending on the pathological conditions. Innervation to the lymph node increases under psychosocial stress in primates,[90,91] whereas it decreases under conditions of viral infection or inflammation, such as arthritis.[92,93] The predominant nerve fibers are sympathetic, but in addition, acetylcholine (Ach), calcitonin gene-regulated peptide (CGRP), vasoactive intestinal polypeptide (VIP), dopamine, substance P and somatostatin can be found at these sites. In this section, sympathetic and parasympathetic effects are discussed. Other neurotransmitters will be discussed in the next section.

Sympathetic nervous system control of immune responses

The sympathetic nervous system (SNS) contains regions of the brain, as well as sympathetic nerves, that innervate primary and secondary immune organs and release noradrenaline (NA) from their nerve terminals on stimulation. In addition, adrenaline is systemically released from chromaffin cells in the adrenal medulla. Most studies show that activation of the SNS inhibits the immune system, although some studies show the opposite effects including induction of chemokines, such as CXCL8.[1,74] Catecholamines bind to α- and β- adrenergic receptors, seven-transmembrane domain G protein-coupled receptors composed of heterodimers of two different subunits that form multiple subtypes. Immune cells predominantly express β2-adrenergic receptors (β2AR). The signals through β2AR on dendritic cells and macrophages upregulate cyclic AMP (cAMP), activate protein kinase A and inhibit NFκB. These intracellular events attenuate the production of inflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-12, and upregulate IL-10 production,[94–96] resulting in the suppression of Th1 responses. Interestingly, β2AR are expressed on naïve $CD4^+$ T cells and Th1 cells, but not Th2 cells. Suppression of the Th1 response seems to be influenced by the time-point, as IFN-γ production decreases if NA is added before activation, but increases when NA is added after activation.[97,98] In addition to the suppression of Th1 responses, chemical sympathectomy increased splenic and lymph node $CD4^+$FoxP3 Treg cells through a TGF-β-dependent mechanism to further suppress excess immune responses.[99]

Although several pieces of conflicting data exist concerning the effects of sympathectomy on cytotoxic T cells, it has been reported recently that chemical sympathectomy by 6-hydroxydopamine or treatment with β2-blockers (but not β1- or α-blockers) enhanced $CD8^+$ T cell responses to viral and cellular antigens in mice, suggesting that the sympathetic nervous system plays an inhibitory role in $CD8^+$ T cell responses.[100] In humoral responses, β2-adrenergic stimulation or cAMP accumulation enhances B cell proliferation, B7-2 expression, differentiation to antibody-secreting cells and antibody production.[101] Inhibition of Th1 responses might also

contribute to enhanced humoral responses. However, antibody production seems to depend on the duration of cAMP accumulation. Short-term elevation enhances, whereas long-term elevation suppresses antibody production. Consistent with these results, high level spinal cord injury (T3) caused sustained increases in splenic NA and GC along with impaired antibody production, and these immunosuppressive effects were reversed by β2AR blockers.[102] Catecholamines affect other innate immune cells, and induce acute mobilization of NK cells and chronic inhibition of NK-cell activity directly and indirectly through the inhibition of IL-12 and IFN-γ. In addition, catecholamines suppress the migration, phagocytosis and degranulation of neutrophils.[103,104]

Parasympathetic control of immune responses

The parasympathetic nervous system uses Ach as a primary neurotransmitter and modulates immune responses through the efferent and afferent fibers of the vagus nerve. Inflammatory cytokines, such as IL-1, stimulate paraganglia cells resulting in signals through afferent fibers, which activate the parasympathetic brainstem regions to release Ach from efferent vagus nerves to control inflammation through negative feedback. Vagotomy shuts down the signals to the brain and the subsequent negative feedback resulting in enhanced inflammatory conditions, such as toxic shock[105] and CIA,[106] whereas electrical vagus nerve stimulation acts to ameliorate disease using a model of sepsis.[107] ACh binds to two types of receptors – nicotinic and muscarinic cholinergic receptors. Both types of receptors consist of many different subunits, thus comprising a variety of receptors. Among them, α7-nicotinic AChR (nAChR), expressed on macrophages, lymphocytes and neutrophils, is essential for the anti-inflammatory effects of vagal nerve signaling.[108] Activation of nicotinic AChR inhibits NFκB transcriptional activity and the production of inflammatory cytokines and high mobility group box 1 (HMGB1).[108,109] In agreement, stimulation of α7-nAChR by nicotine or Ach leads to the attenuation of inflammation in conditions such as sepsis or CIA through the suppression of inflammatory cytokines.[106–108] In addition, nicotine administration inhibited aspects of acquired immunity, including antigen-specific Th1 and Th17 responses and the subsequent development of EAE.[110,111] Furthermore, in α7-nAChR-deficient mice, the production of TNF-α, IFN-γ and IL-6, as well as antigen-specific IgG1 antibodies by spleen cells, was significantly facilitated.[112] Besides the effect on cytokine secretion, nAChR activation also modulates endocytosis and phagocytosis by macrophages. This effect, however, is mediated through α4β2-nAChR.[113] Interestingly, miR-132 has recently been shown to target acetylcholineesterase (AChE), a functional regulator of the cholinergic system.[114] Inflammatory stimuli induced overexpression of miR-132 in lymphocytes, and miR-132 attenuates inflammation by reducing AChE levels.

Local regulation of the immune system through neurotransmitters

Neurotransmitters are synthesized in neurons and reside in presynaptic terminals. They act on the postsynaptic neurons and other organs. Amino acids, such as glutamate and GABA, amines such as dopamine, NA and serotonin, and peptides termed neuropeptides, such as somatostatin, substance P, NPY, opioid, GnRH, CRH, CGRP and VIP, are all neurotransmitters. These molecules are released from the peripheral nervous system, as well as from immune cells including T cells, B cells, macrophages, dendritic cells, granulocytes,[6–9,11,12,73,115–130] and, therefore, contribute to the modulation of immune responses. Due to space limitations, only some of these neurotransmitters will be discussed here.

Glutamate

Glutamate is a primary excitatory neurotransmitter in the CNS and regulates motor, sensory and affective functions, as well as cognition, memory and learning. Glutamate binds to two families of multiple receptors, ionotropic glutamate receptors (iGluR) and G protein-coupled metabotropic glutamate receptors (mGluR). iGluR are subdivided into three groups based on their amino acid sequence and selective activation by the agonists N-methyl-D-aspartate (NMDA) and kainate or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). mGluR include eight subtypes and are classified into three subgroups according to their sequence homology and G protein coupling. Group I consists of mGluR1 and mGluR5, which are coupled to the Gq protein. Group II consists of mGluR2 and mGluR3, which are coupled with Gi and Go proteins, and L-2-amino-4-phosphonobutyric acid is their most potent agonist. Group III consists of mGluR4, mGluR6, mGluR7 and mGluR8, which are coupled with Gi and Go proteins, and for which L-2-(carboxycyclopropyl)-glycine is the most potent agonist. Even though iGlu3R signals have been reported to impair IL-10 production, but enhance the chemotactic migration and integrin-mediated adhesion of resting T cells,[131] administration of AMPA/kainate antagonist to mice

suffering EAE increased oligodendrocyte survival with no reduction of inflammation, suggesting a minor effect of iGluR on immune cells.[132] In contrast, some mGluR have recently been reported to be involved in immune responses. Regarding group I GluR, the expression of mGlu1R is induced after T cell activation in contrast to mGlu5R, which is constitutively expressed on T cells. Signals through mGlu5R inhibit T-cell proliferation through suppression of IL-6 production,[133] whereas signals through mGlu1R enhance the secretion of IL-2, IL-6, IL-10, TNF-α and IFN-γ, and counteract the mGlu5R-mediated inhibitory effect on T-cell proliferation.[133,134] Recent studies using mGluR4, a member of group III GluR, showed that mGluR4-deficient mice were vulnerable to EAE and that this was associated with enhanced Th1 and Th17 responses. These mice showed increased production of inflammatory cytokines, such as IL-6, IL-12 and IL-23, as well as anti-inflammatory cytokines including IL-10 and TGF-β.[135] In accordance with these findings, administration of N-phenyl-7-(hydroxyimino) cyclopropa[b] chromen-1a-carboxamide (PHCCC), an mGluR4 selective enhancer, increased EAE resistance by inducing Tregs, showing the immunosuppressive effect of mGluR4-mediated signaling.[135]

Dopamine

Dopamine is an important neurotransmitter in the CNS and plays a key role in the control of movement, endocrine regulation and cardiovascular function. Dopamine also plays an important function outside of the CNS in peripheral nerve systems, as dopamine is released from peripheral nerve terminals that innervate lymphoid organs, as well as from immune cells. Dopamine has been shown to inhibit proliferation of human lymphocytes, and even to induce apoptosis in peripheral mononuclear cells.[136,137] Dopamine receptors are seven-transmembrane G protein-coupled receptors with five subtypes (D1R-D5R) classified into two subgroups – D1-like receptors and D2-like receptors.[138] Murine and human lymphocytes express all subtypes of these receptors. D1-like receptors, including D1R and D5R, are coupled to Gαs, whose increases in cAMP are often linked to inhibitory effects. In contrast, D2-like receptors, including D2R, D3R and D4R, are coupled to Gαi, which decreases cAMP and is often linked to immunostimulation. Signals through D1-like receptors inhibit the function of cytotoxic T cells and Tregs.[119,139,140] In contrast, signals through D2R trigger integrin activation and IL-10 production.[141] Furthermore, signals through D3R induce the secretion of TNF-α from T cells[142] and induce the migration of naïve $CD8^+$ T cells.[143] However, recent *in vivo* studies showed that administration of D1-like receptor antagonists ameliorated EAE in association with a reduction of IL-17 and an increase in IFN-γ, whereas administration of D2-like receptor antagonists worsened EAE.[144] Furthermore, in an arthritis model, the D2-like receptor antagonist, haloperidol, significantly induced accumulation of IL-6+ and IL-17+ T cells, and exacerbated cartilage destruction, whereas D1-like receptor antagonists suppressed these responses,[145] suggesting that dopamine signals through D1-like receptors enhance Th17-mediated diseases by promoting the IL-6/Th17 axis in conjunction with the suppression of Tregs.

Substance P

Substance P (SP) is produced by the primary afferent neuronal terminals of the CNS and peripheral nerve endings, as well as by immune cells including monocytes, dendritic cells and lymphocytes. The diverse functions of SP include a role as a neuronal sensory transmitter associated with pain, stress, anxiety, secretion stimulation, smooth muscle contraction and immune stimulation. SP binds to both the neurokinin-1 (NK1R) and neurokinin-2 receptor, but the effects of SP are mainly mediated by NK1R, a G protein-coupled receptor. In the immune system, SP enhances the production of inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, by activating NFκB from monocytes. SP also increases NK cell activity and induces the release of CXCL8 and CCL2 from leukocytes and vasoactive mediators, such as serotonin and histamine, from mast cells.[146] In terms of T cells, SP potentiates acquired immune cell responses by enhancing T cell proliferation. Furthermore SP augments the generation of Th1 and Th1/Th17 cells from memory T cells by inducing IL-1β, TNF-α and IL-23 production by monocytes leading to the control of infections.[147–149] Consistent with these findings, NK1R antagonists are effective for the treatment of sepsis.[150–152]

Immune regulation of neuroendocrine systems

The neural regulation of immune responses has been extensively studied as aforementioned. However, the interactions between the neuroendocrine and immune systems are bidirectional and, recently, increased attention has been given to the immunological regulation of the neural system through cytokines. Activation of innate immune responses, not just by pathogens but also by

damage-associated molecules, such as HMGB1, heat shock protein and ATP, leads to the release of inflammatory cytokines. IL-1, TNF-α and, to a lesser extent, IL-6 stimulate the HPA axis at the level of vagus afferent nerves, the hypothalamus, the pituitary and the adrenal glands to release GC as well as the SNS to release NA, providing a negative feedback loop to stop inflammation. Cytokines produced in the periphery activate primary afferent nerves, such as the vagus nerves, enter the brain through the areas with a poorly developed blood–brain barrier, such as the circumventricular region or are actively transported. In addition, neurons, glial cells and endothelial cells produce these cytokines in the CNS.[153] In contrast to HPA axis, inflammatory cytokines have negative effects on the HPG axis, leading to the reduction of gonadal functions.[154]

The effects of cytokines on the CNS are not limited to the HPA axis and SNS, but are also involved in behavior induced by sickness including changes in behaviour that occur in ill patients, and even in depression. Systemic or intrathecal administration of IL-1β or TNF-α induces signs of behavior resulting from sickness, such as decreased motor activity, social withdrawal, altered cognition and fatigue. Although administration of IL-6 does not induce these behavioral changes, LPS-induced sickness behaviors are reduced in IL-$6^{-/-}$ mice, suggesting its involvement in these behavioral changes, although the degree is less compared with IL-1β or TNF-α. In contrast, anti-inflammatory cytokines, such as IL-10, attenuate LPS-induced sickness behaviors.[155–158]

Type I interferon, IFN-α and IFN-β, are used for the treatment of hepatitis C and MS, respectively. These cytokines show neuropsychiatric complications, including sleep disorders and depression, which serves as evidence of the cytokine-mediated modulation of neural activities. The potential link between inflammatory cytokines and depression is tryptophan metabolism. Tryptophan is an essential amino acid and is a source of serotonin, as it is metabolized to serotonin and kynurenine. In the latter pathway, tryptophan is metabolized by tryptophan 2,3 dioxygenase (TDO) and indoleamine 2,3 dioxygenase (IDO) to kynurenine, and then metabolized either to 3-hydroxykynurenine or kynurenic acid, an antagonist of NMDA receptors. 3-Hydroxykynurenine is further metabolized to 3-hydroxyanthranilic acid and quinolinic acid, an agonist of NMDA receptors. TDO is primarily located in the liver and is activated by GC, whereas IDO is widely expressed and is activated by inflammatory cytokines and downregulated by IL-4.[153,159] In patients treated with type I IFN, plasma levels of kyurenic acid as well as serotonin are decreased, suggesting that the behavior and depression caused by inflammatory cytokines including IFN might be a result of an alteration in glutamatergic neurotransmission.

Although much of the focus on T cells in a variety of pathogenic conditions, has been on classical immune-mediated inflammation, including infection and autoimmune disorders, in diseases newly related to inflammation, such as ischemia, neurogenerative and psychiatric disorders, a neuroprotective role has emerged as an important task for T cells. The production of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) from T cells,[160] reduced learning capacity in T cell-deficient mice and its restoration by passive T cell transfer[161] and enhanced hippocampal neurogenesis by T cells,[162,163] suggest a fundamental function of T cells in the maintenance of cognitive functions. Anti-inflammatory cytokines, such as IL-4 and TGF-β, are detectable in the CNS, and IL-4 is downregulated in a mouse model of Alzheimer's disease.[164] These cytokines, in addition to BDNF, might contribute to the maintenance of homeostasis of the CNS. Although the precise mechanisms remain elusive, T cells might serve as important players in the maintenance of neuronal integrity.

Future directions

Acute stress responses in the autonomic and peripheral nervous systems amplify local immune responses to eliminate pathogens and other dangerous occurrences. Subsequent to these initial responses, the neuroendocrine and autonomic systems act to inhibit immune responses and terminate inflammation. In contrast, chronically sustained stress induces unusual conditions, such as inadequate secretion of GC, as well as resistance to GC, increased sympathetic tone propelling the RAAS, functional loss of sympathetic nerve fibers at the inflammation site and a local β to α adrenergic shift.[165,166] Further studies to clarify the consequences of stress on chronic inflammatory conditions will provide novel strategies for the control of complex pathogenic conditions including autoimmune diseases, and neurogenerative and psychiatric disorders.

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