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## 1) 多発性硬化症とTh17細胞

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### 要 旨

T細胞の介在する自己免疫応答は、多発性硬化症 (MS) の病態の理解に必須である。Th17細胞は新たに同定されたCD4陽性ヘルパー T細胞で、炎症促進作用をもつサイトカインIL-17産生能を有することを特徴とし、感染防御、アレルギー、および自己免疫疾患発症における役割について近年研究が進んでいる。Th17細胞の登場によりTh1細胞とTh2細胞のバランスでMSを理解するTh1/Th2パラダイムは崩れ、Th1細胞とTh17細胞の両者が炎症の促進に関与すると考えられている。一方、最近ではTh17細胞が制御性T細胞と共通の分化経路をもち、またTh1細胞への分化転換がみられるなど、Th17細胞の可塑性が話題になっている。

### 動 向

Th17細胞はIL-17を産生するCD4陽性ヘルパー T細胞で、近年Th1細胞やTh2細胞とは異なる分化経路を辿る細胞として同定された。MSにおける病原性については確立していないが、再発に伴って活動性が上がるという複数の報告がある。最近の話題として、Th17細胞が、環境によっては制御性T細胞やTh1細胞に形質転換する可塑性の問題がクローズアップされている。MSの

病態におけるTh17細胞の重要性が指摘される一方で、IFN $\beta$ の治療反応性とTh17細胞活動性の負の関連が報告されている。多様なMSの病態を説明する免疫細胞の一つとして、Th17細胞を正しく評価し、治療方針の決定につなげていくことが求められている。

### A. 多発性硬化症はTh1病か？

多発性硬化症 multiple sclerosis (MS) は、神経症状発現の時間的および空間的多発性を特徴とする中枢神経系の炎症性脱髄疾患である。発症から5年以内では大部分の症例が再発と寛解を繰り返す病型 (再発・寛解型MS) を取るが、発症から5~10年経過すると、一部の症例では明確な再発を示さずに神経障害が蓄積・進行する (二次進行型MS)。また再発・寛解型MSの経過を取らずに、最初から進行経過を取る症例もある (一次進行型MS)。若年世代に好発し、性差 (女性>男性)、人種差、地域差が認められ、欧米白人に多くアジア人には少ないことが知られている。しかし、我が国では、過去30年間に患者数の著増が認められ、現在14,000名を越す患者が特定疾患医療受給者として登録されている。

MSの病因はいまだ不明で、様々な病態を含む

疾患である可能性があるが、疾患感受性遺伝子の多くが免疫関連分子であることや、リンパ球を標的にした治療の有効性から、自己免疫機序は重要である<sup>1)</sup>。再発・寛解型MSの時期はT細胞やB細胞の関与する獲得免疫系の役割が大きく、進行期になるとマクロファージや樹状細胞などによる自然免疫系の役割が大きいと考えられている<sup>2)</sup>。

CD4陽性ヘルパーT細胞 T helper (Th) は獲得免疫系の「司令塔」であり、MSの再発において中心的な役割を担う。Th細胞の分化については、ナイーブCD4陽性T細胞が抗原提示を受けて活性化しメモリーT細胞に分化する際に、周囲のサイトカイン環境により、Th1とTh2の二つの異なる表現型が誘導されるというモデルが1980年代末に提示された<sup>3)</sup>。すなわち、IL-12の影響下ではIFN $\gamma$ を産生するTh1細胞が分化し、IL-4の影響下ではIL-4, IL-5, IL-13などを産生するTh2細胞が分化する。前者は主に細胞内感染病原体排除の役割を担い、後者は抗体産生やアレルギー反応に関わる。両者は互いに拮抗し、Th1-Th2のバランスが、様々な免疫応答や疾患発症を規定するという考えが、いわゆる「Th1-Th2パラダイム Th1-Th2 paradigm」である。このパラダイムでは、寄生虫・アレルギー性疾患ではTh2が優位になっているのに対し、MS、炎症性腸疾患、関節リウマチ、乾癬ではTh1優位な状態であると考えられた。MSは代表的なTh1病とされ、Th1とTh2のバランスをTh2に偏倚させることで軽快すると考えられた。MSがTh1病とされた根拠としては、MSの代表的な動物モデルEAE (experimental autoimmune encephalomyelitis; 実験的自己免疫性脳脊髄炎)において、Th1細胞を他の動物へ移入することによってEAEが誘導できることや、以前の臨床研究においてTh1応答を促進するIFN $\gamma$ の投与がMSの病態を悪化させたことなどがあげられる。しかし、動物モデルEAEでは、IFN $\gamma$ の投与が病気

を軽減し、IFN $\gamma$ 遺伝子欠損マウスでEAEが増悪することから、Th1-Th2パラダイムには大きな矛盾のあることが指摘されるようになった。

## B. Th17細胞の登場

2003年にCuaらはMS/EAE = Th1病説の反証となる報告をした<sup>4)</sup>。Th1細胞を誘導するIL-12はp40とp35の二つのサブユニットから構成されるが、IL-12とp40を共有する別のサイトカインIL-23はもう一つのサブユニットとしてp19をもつ (p40-p19 heterodimer)。意外なことにIL-12を産生しないp35の遺伝子欠損マウスにおいてEAEは増悪し、IL-23を産生しないp19の遺伝子欠損マウスでEAEは消失した。すなわち、EAEを起こす脳炎惹起性の細胞はIL-12が誘導するTh1細胞ではなく、IL-23が誘導する細胞であることが示唆された。関節リウマチのモデルであるCIA (collagen induced arthritis) においても同様の結果が得られ、臓器特異的自己免疫疾患におけるIL-23の役割に注目が集まった。

IL-23が誘導するT細胞はIL-17AおよびIL-17Fを産生することが明らかにされていたが、IL-17A, IL-17Fは上皮細胞、血管内皮細胞、線維芽細胞にあるIL-17受容体に結合してIL-6やTNF $\alpha$ , GM-CSFなどのサイトカインや、ケモカインなどの産生を誘導し、好中球動員や炎症促進機能を有し、感染防御、アレルギー、自己免疫疾患発症に関わる<sup>5)</sup>。2006年には、IL-17産生T細胞が、ナイーブCD4陽性T細胞にIL-6とTGF- $\beta$ を加えると誘導され、IFN $\gamma$ やIL-4によって分化抑制がかかることが示された (図1)。さらにその後、固有に発現する転写因子としてTh1細胞がT-betを発現するのに対して、この細胞はROR $\gamma$ tとROR $\alpha$ tを発現することが見出された。ここにIL-23依存性に誘導されるIL-17産生T細胞は、Th1・Th2細胞とは異なる分化経路を

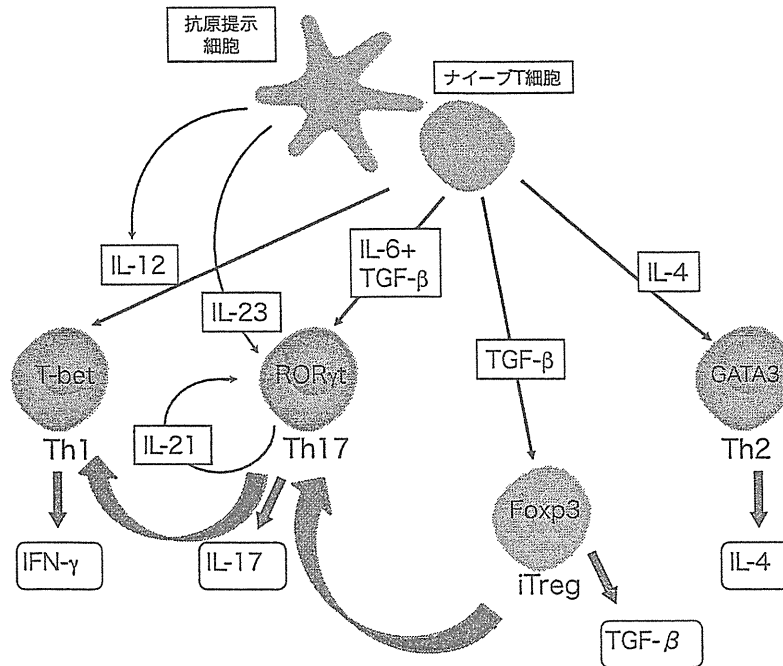


図1 ヘルパーT細胞の分化と機能

経た細胞として「Th17細胞」と名付けられた。なおIL-23はTh17細胞の分化誘導には必要ではなく、分化したTh17細胞の安定化と増殖に必要と考えられた<sup>6)</sup>。

その後、Th17細胞の生物学的な研究が発展し、プロスタグランジンE2 (PGE2) による分化促進<sup>7)</sup> や、レチノイン酸誘導体による分化抑制<sup>8)</sup> がTh17細胞の分化に影響することが明らかにされた。また、Th17細胞がダイオキシンなどの化学物質の受容体であるアリル・ hidrocarbon 受容体 aryl-hydrocarbon receptor (AhR) を発現することも報告され、注目を集めている<sup>9)</sup>。なおIL-17の遺伝子欠損マウスにおけるEAEの改善は軽度である<sup>10)</sup> が、その理由として、Th17細胞がIL-17AやIL-17F以外にもIL-21やIL-22, IL-26, IL-6, TNF- $\alpha$ を産生することが議論されている。

ヒトのTh17細胞については、施設間で分化・誘導の条件が異なり、見解の一致しない点がある

が、炎症性サイトカインの存在下に分化する点についてはマウスの場合と同様である。ヒトTh17細胞の特徴として、NK細胞、NKT細胞のマーカーでもあるCD161を発現すること<sup>11)</sup>、ケモカイン受容体CCR6を発現すること<sup>12)</sup> が海外の研究で明らかにされた。

### C. MSとTh17細胞

MSの病理組織を用いた検討では、MS病変にCD4陽性(およびCD8陽性の)IL-17陽性細胞が存在することが報告されている<sup>13)</sup>。MS患者におけるTh17細胞の活動性について、CD4陽性T細胞中におけるIL-17産生細胞の割合を末梢血と脳脊髄液 cerebrospinal fluid (CSF) で比較したところ<sup>14)</sup>、MSの再発期ではTh1細胞、Th17細胞ともにCSF中で割合が増加していた(ただし、絶対数においてTh17細胞はTh1細胞より一桁少ない)。他の神経疾患ではTh1細胞は増加してい

たが、Th17細胞の増加は有意ではなく、Th17細胞の増加は再発期MSに特異的であると報告されている。また再発期MS患者のCSF由来のTh17クローンの解析では、接着因子MCAM/CD146の発現がTh1クローンよりも高く、ヒト脳の上皮細胞に接着しやすく、また活性化能・増殖能ともに高い傾向にあった。以上からMSの再発にTh17細胞が関わっていると結論している。

MSの再発において、脳炎惹起性のT細胞が血液脳関門 blood brain barrier (BBB) を通って脳実質内へ浸潤するステップが重要であり、現在国内で臨床治験中の抗VLA4抗体 (Natalizumab) はこの過程で重要なT細胞の脳血管内皮への接着を阻害してMSの再発を抑制する<sup>15)</sup>。なお、脳炎惹起性T細胞の脳内浸潤を促進する分子機序としては、その他に、ケモカインCCL2によるCCR2陽性T細胞の動員<sup>16)</sup> や、CSF中に増加するマトリックスメタロプロテナーゼ9 (MMP9) によるBBB破壊などが強調されている<sup>17)</sup>。

ケモカイン受容体の発現とThのサブタイプの間にはある程度の関連性が認められ、Th1細胞はCXCR3やCCR5を特徴的に発現することが知られている。ヒトのTh17細胞のそれは、CCR4+CCR6+あるいはCCR2+CCR5-と報告されている<sup>12,18)</sup>。両者は大部分オーバーラップするが、Th17細胞を*in vitro*で誘導した実験系ではCCR6がTh17細胞に強く関連すると言われている<sup>19)</sup>。将来MSになる可能性のあるCIS (clinically isolated syndrome) 患者のCSFを調べたところ、CSF中のCCR6陽性T細胞の頻度が末梢血に比べて増加しており、CCR6と結合するCCL20が脈絡叢上皮細胞に発現していることから、Th17細胞は脈絡叢から脳内に浸潤するという仮説が提出されている<sup>20)</sup>。

BBBの*in vitro*モデルを用いた解析では、Th17細胞の中でIL-22陽性細胞が、Th1細胞やIL-22陰性Th17細胞よりもBBBモデルを通過す

る能力が有意に高いことが示された。このIL-22産生性Th17細胞は、CD8細胞の代表的な細胞障害性因子であるGranzyme Bを有し、上皮細胞を障害し浸潤する能力がある<sup>21)</sup>。Th17細胞を分化・誘導する抗原提示細胞の関与については、BBBモデルを通過した単球がTh17細胞の分化・誘導能をもつことが示されている<sup>22)</sup>。これらの観察はTh17細胞がMSの再発に関わっていることを示唆するが、免疫学的な異常の一つの結果をみているにすぎない可能性も残されている。

#### D. Th1/Th17細胞とTh17細胞の可塑性

マウスに比べてヒトでは、IFN $\gamma$ とIL-17の両方を同時に産生する細胞 (Th1/Th17細胞) の存在が比較的多く認められるが、その意義は不明であった。最近、この細胞が再発期MS患者の末梢血で増加しており、BBBモデルを効果的に通過し、組織学的にもMS病変に存在することが報告された<sup>23)</sup>。これに関連してIL-17Aのリポーターマウスを用いた検討で、EAEが発症する過程でIL-17A産生性の細胞がIFN $\gamma$ 産生性の細胞に分化転換 conversion することが報告された。この結果に基づき、Th17細胞はTh1細胞あるいはTh1/Th17細胞に変化してEAEを発症するというモデルが提唱された<sup>24)</sup>。*in vitro*の実験下でもTh17細胞からTh1細胞への分化転換は容易に起こる。分化したTh細胞であっても、周囲のサイトカイン環境の変化によって比較的容易に他のThに分化転換することがエピジェネティクスによる解析でも明らかとなっているが<sup>25,26)</sup>、Th17細胞はTh1細胞に比べると不安定であり、高い可塑性 plasticity を有することが明らかになってきた。

このテーマに関連して興味ある動物実験の結果が発表されている<sup>27)</sup>。Dominguesらは、髄鞘抗

原反応性のT細胞をin vitroでTh1およびTh17細胞に誘導したのち、T細胞やB細胞を欠損するRAG2欠損マウスに移入してEAEを発症させたところ、Th1細胞を移入した場合には、脊髄を病変の主座とし下肢から上行する麻痺症状を示す古典的タイプのEAEが発症した。一方Th17細胞を移入すると、失調症状を有する非典型的なEAEが観察された。浸潤細胞は脳内に分布し、Th17細胞以外に、Th1細胞やTh1/Th17細胞が確認された。Th1/Th17細胞は末梢組織にはみられないことから、Th17細胞が脳へ浸潤する過程でTh1/Th17細胞へ分化転換したと考察している。Th17細胞がMSの病変部位と関連する可能性を示唆する興味深い報告である。

Th17細胞の分化に必要なTGF- $\beta$ は制御性T細胞(Treg)の分化を誘導するサイトカインでもある。TGF- $\beta$ は最初にTh17細胞・Treg両者の転写因子、ROR $\gamma$ tとFOXP3を誘導する<sup>28)</sup>。IL-6やIL-1 $\beta$ などの炎症性サイトカインの存在下では、FOXP3の発現低下とROR $\gamma$ tの発現上昇が生じTh17細胞に分化すると考えられている。FOXP3陽性細胞がTh17細胞に分化転換し病原性細胞となった例<sup>29)</sup>やIL-17を産生するFOXP3陽性Treg(抑制機能も有する)の存在が報告されており<sup>30,31)</sup>、正反対の作用をもつ両者が分化経路を共有し、中間的な表現型をもちうることを示されている。

## E. 治療標的としてのTh17細胞

従来の治療(ステロイドや免疫抑制剤)や現在開発中の薬剤(VLA4抗体やFTY720など)は、特にTh17細胞を標的として開発されたものではないが、Th17細胞の機能を抑制して治療効果を発揮する。抗VLA4抗体はTh17細胞の中枢神経内への侵入を抑制し<sup>15)</sup>、FTY720はTh17細胞のリンパ節外への移出を阻止し<sup>32)</sup>、抗CD20抗体

はB細胞除去を通じてT細胞を減少させることにより<sup>33)</sup>、それぞれ治療効果を示すと考えられる。

現在様々な領域で臨床応用が検討されている抗p40抗体は、IL-12とIL-23の両者を阻害し、Th1・Th17細胞両者の分化を抑制する効果が期待できる。この抗体は乾癬に対しては顕著な効果を認めたが<sup>34)</sup>、MSの再発を抑制する効果は証明できなかった。理由は明らかでないが、抗体が中枢神経内に十分到達しなかった可能性が指摘されている<sup>35)</sup>。

Th17細胞の分化に関わる転写因子やサイトカインを阻害する治療の可能性についても、現在活発に研究が進められている。Th17細胞の分化に関わるIL-6を阻害する抗IL-6抗体は、抗リウマチ薬としてすでに臨床の場で使用されているが、Th17細胞を抑制しEAEを抑制することが報告されている<sup>36)</sup>。オーファン核内受容体NR4A2は寛解期MS患者のT細胞で発現の高いことが遺伝子発現の網羅的解析で見出されたが、siRNAiで抑制するとTh17細胞機能が抑制される<sup>37)</sup>。レチノイン酸(ATRA)はTh17細胞の分化に対して抑制的に働く<sup>38)</sup>ことが知られている。すでにATLの治療薬として臨床で使用されているレチノイン酸の誘導体Am80はTh17細胞抑制作用をもつと報告されている<sup>39)</sup>。

最後に、MSの治療薬として最も汎用されるIFN $\beta$ とTh17細胞の関係について、大変興味深い報告を紹介する。Th17細胞上のIFN受容体(IFN $\alpha$ 受容体1)の発現はTh1細胞よりも高く、IFN $\beta$ はTh17細胞を抑制する効果をもつと報告された<sup>40)</sup>。しかし、SteinmanらはTh1細胞で誘導したEAEはIFN $\beta$ により軽減するが、Th17細胞で誘導したEAEはIFN $\beta$ により悪化したと報告し<sup>41)</sup>、IFN $\beta$ はTh17細胞の介在する自己免疫病態には無効である可能性を示唆した。実際、MS患者のうちIFN $\beta$ の無効群の血清では、IL-17Aとよく似た性質をもつIL-17Fの発現がIFN $\beta$

有効群 (responder) に比べ高く, Th17細胞の活動性とIFN $\beta$ の治療効果の間に負の関連があると指摘している。

抗アクアポリン4抗体 anti-aquaporin 4 (AQP4抗体) の発見に伴い<sup>42)</sup>, 視神経脊髄型のMS (optico-spinal MS: OSMS) の多くが, 視神経脊髄炎 neuromyelitis optica (NMO) としてMSとは区別して扱われるようになってきた<sup>43,44)</sup>。日本人の視神経脊髄型MSでは脳脊髄液中のIL-17が通常型のMSに比べ高値であること<sup>45)</sup>, IFN $\beta$ によって病態が悪化した症例がNMOで報告されていること, Th17細胞の活動性の高いSLE, 乾癬, 関節リウマチなどではIFN $\beta$ が病態の悪化につながることから<sup>46,47)</sup>, NMOではTh17細胞の活動性が亢進している可能性がある<sup>48)</sup>。

#### むすび

Th17細胞が発見された意義を3つあげてみたい。一つ目は免疫学における学術的な意義である。Th17細胞はTh1-Th2パラダイムという従来のドグマを崩し, その後の多様なTh細胞の発見のさきがけとなった。二つ目は「MSを予防する」可能性を開いたことである。MSは一卵性双生児における発症率が30%前後であり, その発症には環境因子の影響が大きいが, これまで議論されてきた危険因子—喫煙, ビタミンD摂取量やストレス—だけでは, ここ30年の日本人MS患者の顕著な増加を説明することはできない。ライフスタイルの変化・食生活の変化が腸内細菌を変え, TregやTh17細胞を介して免疫系が動き, MSが発症しやすくなったという可能性が考えられる。発症の誘因が分かればそこから逆にMSの予防戦略が立てられるかもしれない。三つ目として, MSの多様な病態を説明する一つの指標を提供したことがあげられる。MSの病態は多様であり, 個々の患者ごと, また病期ごとに, Th17細胞の

活動性が異なる可能性がある。近年MSは, 単一疾患ではなく, 多様な病態を含むとの認識が広まりつつある<sup>49)</sup>。経過の多様性, 再発寛解型と進行型のタイプの多様性, 病理学的分類の多様性<sup>50)</sup>, IFN $\beta$ などの治療効果の多様性などである。しかしバイオマーカーが存在しないために多様なMSの病態を適切に分類することができず, 病態に応じた治療を選択することができていない。もしTh1やTh17, さらにTh1/Th17細胞の活動性を評価するアッセイが確立すれば, MSの病態を説明する一つの指標となり, 治療法の選択も可能になり, MS診療が進歩することが期待される。

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## REVIEW ARTICLE

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

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**Keywords**

autonomic nervous system; cytokine; hypothalamic–pituitary–adrenal axis; neuropeptide; neurotransmitter

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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 multi-level 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.

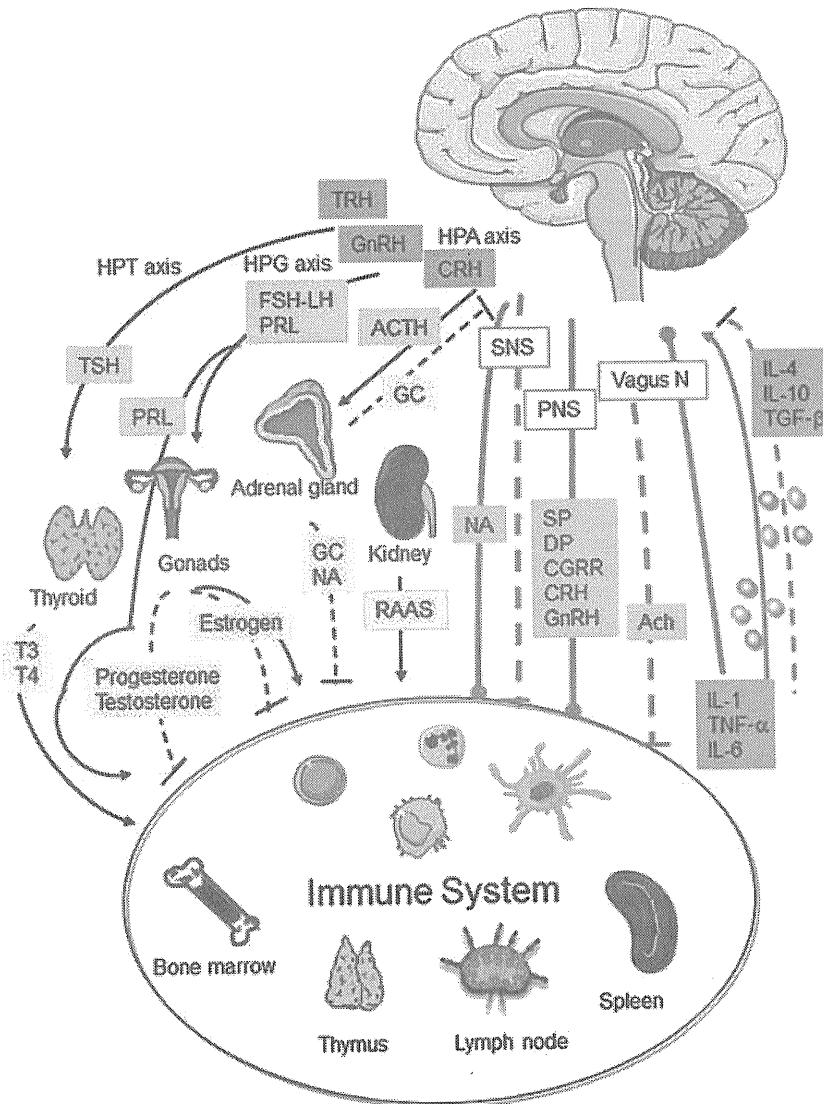
**Neuroendocrine regulation of the immune system**

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.<sup>1</sup> 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, adrenocorticotropic 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- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; 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 down-regulated by opiates and  $\gamma$ -aminobutyric acid (GABA) as well as hormones downstream of CRH, such as GC and ACTH, through negative feedback.<sup>2</sup> 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  $\kappa$ B (NF $\kappa$ B).<sup>3-5</sup> 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.<sup>5</sup> GC inhibit the production of pro-inflammatory (interleukin [IL]-1, IL-6, tumor

necrosis factor [TNF]- $\alpha$ ) and Th1-related cytokines (IL-2, IL-12,  $\gamma$ -interferon [IFN- $\gamma$ ], 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.<sup>1</sup> 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 chemoattractant 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.<sup>6-9</sup> The local effect of CRH is inflammatory rather than anti-inflammatory. CRH receptor antagonists suppress the production of IL-6, IL-1 and TNF- $\alpha$  from macrophages resulting in disease inhibition in a model of endotoxin shock.<sup>10</sup>

#### *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.<sup>11,12</sup> GnRH enhances T cell development, proliferation, cytokine production (including IFN- $\gamma$ ) as well as IgG production,<sup>12</sup> 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.<sup>13</sup>

Estrogen binds to two forms of cytoplasmic estrogen receptors (ER), ER $\alpha$  and ER $\beta$ . ER $\alpha$  is expressed on the endometrium, ovarian stromal cells, breast and hypothalamus, whereas ER $\beta$  is widely expressed in tissues including brain, kidney, bone, heart, lungs, intestine and endothelial cells<sup>14</sup>. Estrogen has dual roles in the modulation of immune responses depending on the plasma levels. High levels of estrogen suppress macrophages to produce TNF- $\alpha$  and IL-12, and promote them to produce IL-10.<sup>15</sup> In addition, estrogen promotes the HPA axis and noradrenaline (NA) production, resulting in further inhibition of inflammation, and favors a Th2 pattern of cytokines.<sup>16</sup> Estrogen has a potent modulatory effect on B cell development and survival, interfering with B cell tolerance and enhancing autoantibody production.<sup>17-19</sup> 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.<sup>11</sup> 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.<sup>11</sup> 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 activities.<sup>20-25</sup> The mechanisms that underlie the inhibition of EAE include suppression of myelin-specific Th1 and Th17 immune suppression, increased Th2 responses, induction of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells (T regs) and downregulation of inflammation. The studies using knockout mice and a specific ligand for ER $\alpha$  or ER $\beta$  suggest that the suppressive effects of estrogen in EAE are mediated by ER $\alpha$ ,<sup>26-28</sup> although ER $\beta$  seems to be involved in neuroprotection.<sup>29,30</sup> 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.<sup>31-33</sup> Based on these findings and a promising pilot trial of oral estriol, there are several clinical trials of estrogens in MS underway.<sup>34-36</sup> Progesterone shows anti-inflammatory effects through the inhibition of NF $\kappa$ B.<sup>15,37</sup> Testosterone inhibits both innate and acquired immunity.<sup>11</sup> Dihydrotestosterone decreases immunoglobulin and cytokine production and lymphocyte proliferation.<sup>38</sup>

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.<sup>39</sup> The effects of PRL on immune responses are immunostimulatory and PRL enhances production of cytokines, such as IFN- $\gamma$ , 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.<sup>40-42</sup> Consistent with this, bromocriptine administration abrogates the estradiol-induced breakdown of B cell tolerance.<sup>43</sup>

#### *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.<sup>44</sup> 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.<sup>45-47</sup> 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- $\gamma$  production in hyper- or hypothyroid mice, respectively. In addition, the production of IL-6 and IFN- $\gamma$  on stimulation with lipopolysaccharide (LPS) was upregulated in hyperthyroid mice, suggesting enhancement of innate immune responses.<sup>47</sup> 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.<sup>48</sup> 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.<sup>49-51</sup> AngII stimulation promotes the production of inflammatory mediators, including cytokines, chemokines and adhesion molecules, through the activation of NF $\kappa$ B.<sup>52,53</sup> These mediators also promote the differentiation of dendritic cells and accumulation of neutrophils, which then drives diseases, such as atherosclerosis and inflammation.<sup>51,54,55</sup> Inhibition of ACE or AT1R suppresses the production of

inflammatory cytokines, including TNF- $\alpha$ , IL-1, IL-6, IL-12 and IL-18,<sup>56-58</sup> leading to disease suppression in arthritis models.<sup>59,60</sup> 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.<sup>60,61</sup> More recently, suppression of both Th1 and Th17 responses, and induction of Tregs and transforming growth factor- $\beta$  (TGF- $\beta$ ) by the blockade of ACE or AT1R were shown in EAE.<sup>62,63</sup>

#### *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.<sup>64</sup> Leptin induces the release of inflammatory cytokines, such as TNF- $\alpha$  and IL-6, as well as CC-chemokine ligand 2 (CCL2) and vascular endothelial growth factor (VEGF).<sup>65</sup> Leptin also stimulates the production of chemokines by eosinophils<sup>66</sup> and neutrophils.<sup>67,68</sup> 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.<sup>69,70</sup> 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.<sup>71,72</sup>

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.<sup>73</sup> 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*.<sup>74</sup> Exogenous NPY shifts the Th1/Th2 balance towards Th2 through NPY receptor 1 and ameliorates the severity of EAE.<sup>75</sup> In contrast, studies using NPY1 receptor-deficient mice have shown that NPY promotes APC activation in addition to its role in downregulating Th1-responses.<sup>76</sup> Leptin and NPY are linked to ghrelin, as ghrelin is increased after starvation, it potently stimulates the release of NPY in the CNS<sup>77</sup> and antagonizes the effects of leptin.<sup>78</sup>

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.<sup>79,80</sup> 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.<sup>81</sup> More recent studies have highlighted the anti-inflammatory functions of ghrelin. Ghrelin inhibits the nuclear translocation of NF $\kappa$ B and suppresses the production of inflammatory cytokines from macrophages and T cells.<sup>82,83</sup> As a consequence, ghrelin inhibits bowel disease,<sup>84</sup> arthritis,<sup>85,86</sup> sepsis and endotoxemia.<sup>82,86,87</sup> Furthermore, ghrelin inhibits the production of inflammatory cytokines from microglia and subsequently suppresses EAE.<sup>88,89</sup>

#### 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,<sup>90,91</sup> whereas it decreases under conditions of viral infection or inflammation, such as arthritis.<sup>92,93</sup> 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.<sup>1,74</sup> Catecholamines bind to  $\alpha$ - and  $\beta$ -adrenergic receptors, seven-transmembrane domain G protein-coupled receptors composed of heterodimers of two different subunits that form multiple subtypes. Immune cells predominantly express  $\beta$ 2-adrenergic receptors ( $\beta$ 2AR). The signals through  $\beta$ 2AR on dendritic cells and macrophages upregulate cyclic AMP (cAMP), activate protein kinase A and inhibit NF $\kappa$ B. These intracellular events attenuate the production of inflammatory cytokines, such as TNF- $\alpha$ , IL-1, IL-6 and IL-12, and upregulate IL-10 production,<sup>94-96</sup> resulting in the suppression of Th1 responses. Interestingly,  $\beta$ 2AR are expressed on naive CD4<sup>+</sup> T cells and Th1 cells, but not Th2 cells. Suppression of the Th1 response seems to be influenced by the time-point, as IFN- $\gamma$  production decreases if NA is added before activation, but increases when NA is added after activation.<sup>97,98</sup> In addition to the suppression of Th1 responses, chemical sympathectomy increased splenic and lymph node CD4<sup>+</sup>FoxP3 Treg cells through a TGF- $\beta$ -dependent mechanism to further suppress excess immune responses.<sup>99</sup>

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  $\beta$ 2-blockers (but not  $\beta$ 1- or  $\alpha$ -blockers) enhanced CD8<sup>+</sup> T cell responses to viral and cellular antigens in mice, suggesting that the sympathetic nervous system plays an inhibitory role in CD8<sup>+</sup> T cell responses.<sup>100</sup> In humoral responses,  $\beta$ 2-adrenergic stimulation or cAMP accumulation enhances B cell proliferation, B7-2 expression, differentiation to antibody-secreting cells and antibody production.<sup>101</sup> 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  $\beta$ 2AR blockers.<sup>102</sup> 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- $\gamma$ . In addition, catecholamines suppress the migration, phagocytosis and degranulation of neutrophils.<sup>103,104</sup>

#### *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<sup>105</sup> and CIA,<sup>106</sup> whereas electrical vagus nerve stimulation acts to ameliorate disease using a model of sepsis.<sup>107</sup> 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,  $\alpha$ 7-nicotinic AChR (nAChR), expressed on macrophages, lymphocytes and neutrophils, is essential for the anti-inflammatory effects of vagal nerve signaling.<sup>108</sup> Activation of nicotinic AChR inhibits NF $\kappa$ B transcriptional activity and the production of inflammatory cytokines and high mobility group box 1 (HMGB1).<sup>108,109</sup> In agreement, stimulation of  $\alpha$ 7-nAChR by nicotine or Ach leads to the attenuation of inflammation in conditions such as sepsis or CIA through the suppression of inflammatory cytokines.<sup>106–108</sup> In addition, nicotine administration inhibited aspects of acquired immunity, including antigen-specific Th1 and Th17 responses and the subsequent development of EAE.<sup>110,111</sup> Furthermore, in  $\alpha$ 7-nAChR-deficient mice, the production of TNF- $\alpha$ , IFN- $\gamma$  and IL-6, as well as antigen-specific IgG1 antibodies by spleen cells, was significantly facilitated.<sup>112</sup> Besides the effect on cytokine secretion, nAChR activation also modulates endocytosis and phagocytosis

by macrophages. This effect, however, is mediated through  $\alpha$ 4 $\beta$ 2-nAChR.<sup>113</sup> Interestingly, miR-132 has recently been shown to target acetylcholinesterase (AChE), a functional regulator of the cholinergic system.<sup>114</sup> 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,<sup>6–9,11,12,73,115–130</sup> 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  $\alpha$ -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 iGluR3R signals have been reported to impair IL-10 production, but enhance the chemotactic migration and integrin-mediated adhesion of resting T cells,<sup>131</sup> 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.<sup>132</sup> 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,<sup>133</sup> whereas signals through mGlu1R enhance the secretion of IL-2, IL-6, IL-10, TNF- $\alpha$  and IFN- $\gamma$ , and counteract the mGlu5R-mediated inhibitory effect on T-cell proliferation.<sup>133,134</sup> 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- $\beta$ .<sup>135</sup> 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.<sup>135</sup>

#### 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.<sup>136,137</sup> 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.<sup>138</sup> Murine and human lymphocytes express all subtypes of these receptors. D1-like receptors, including D1R and D5R, are coupled to G $\alpha$ s, whose increases in cAMP are often linked to inhibitory effects. In contrast, D2-like receptors, including D2R, D3R and D4R, are coupled to G $\alpha$ i, which decreases cAMP and is often linked to immunostimulation. Signals through D1-like receptors inhibit the function of cytotoxic T cells and Tregs.<sup>119,139,140</sup> In contrast, signals through D2R trigger integrin activation and IL-10 production.<sup>141</sup> Furthermore, signals through D3R induce the secretion

of TNF- $\alpha$  from T cells<sup>142</sup> and induce the migration of naïve CD8<sup>+</sup> T cells.<sup>143</sup> 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- $\gamma$ , whereas administration of D2-like receptor antagonists worsened EAE.<sup>144</sup> 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,<sup>145</sup> 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-1 $\beta$ , IL-6 and TNF- $\alpha$ , by activating NF $\kappa$ B 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.<sup>146</sup> 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-1 $\beta$ , TNF- $\alpha$  and IL-23 production by monocytes leading to the control of infections.<sup>147–149</sup> Consistent with these findings, NK1R antagonists are effective for the treatment of sepsis.<sup>150–152</sup>

#### 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- $\alpha$  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.<sup>153</sup> In contrast to HPA axis, inflammatory cytokines have negative effects on the HPG axis, leading to the reduction of gonadal functions.<sup>154</sup>

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-1 $\beta$  or TNF- $\alpha$  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<sup>-/-</sup> mice, suggesting its involvement in these behavioral changes, although the degree is less compared with IL-1 $\beta$  or TNF- $\alpha$ . In contrast, anti-inflammatory cytokines, such as IL-10, attenuate LPS-induced sickness behaviors.<sup>155-158</sup>

Type I interferon, IFN- $\alpha$  and IFN- $\beta$ , 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.<sup>153,159</sup> 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, neurodegenerative 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,<sup>160</sup> reduced learning capacity in T cell-deficient mice and its restoration by passive T cell transfer<sup>161</sup> and enhanced hippocampal neurogenesis by T cells,<sup>162,163</sup> suggest a fundamental function of T cells in the maintenance of cognitive functions. Anti-inflammatory cytokines, such as IL-4 and TGF- $\beta$ , are detectable in the CNS, and IL-4 is downregulated in a mouse model of Alzheimer's disease.<sup>164</sup> 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  $\beta$  to  $\alpha$  adrenergic shift.<sup>165,166</sup> 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 neurodegenerative and psychiatric disorders.

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