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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，IFNβ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名を超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[1]．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[2]．

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[3]．すなわち，IL-12の影響下ではIFNγ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IFNγ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，IFNγの投与が病気を軽減し，IFNγ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[4]．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TNFα，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[5]．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-βを加えると誘導され，IFNγ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RORγtとRORαt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

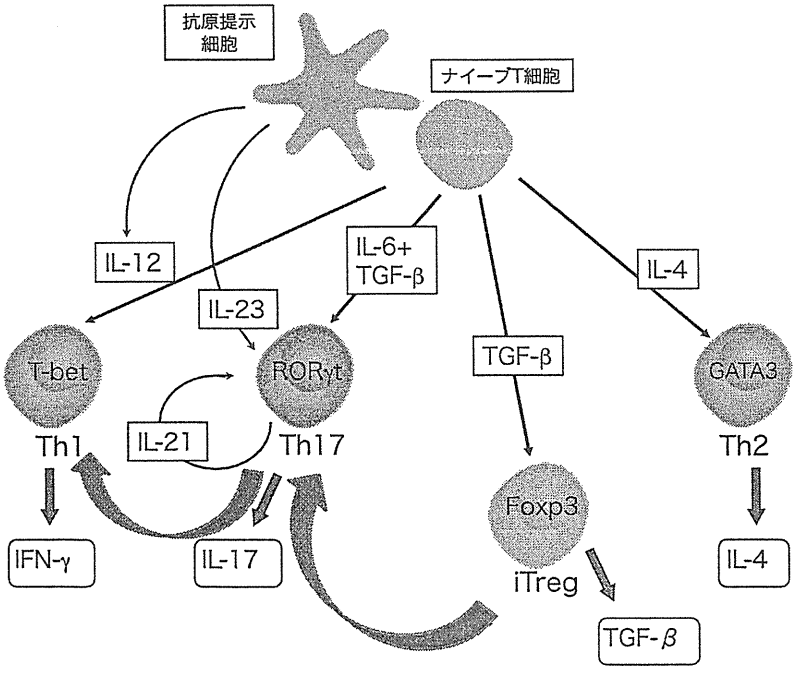

図1　ヘルパーT細胞の分化と機能

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[6]．

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[7]や，レチノイン酸誘導体による分化抑制[8]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・ハイドロカーボン受容体 aryl-hydrocarbon receptor（AhR）を発現することも報告され，注目を集めている[9]．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[10]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-α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[11]，ケモカイン受容体CCR6を発現すること[12]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[13]．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[14]，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[15]．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[16]や，CSF中に増加するマトリックスメタロプロテネース9（MMP9）によるBBB破壊などが強調されている[17]．

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[12,18]．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[19]．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[20]．

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[21]．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[22]．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，IFNγ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[23]．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IFNγ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[24]．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[25,26]，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[27]．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-β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-βは最初にTh17細胞・Treg両者の転写因子，RORγtとFOXP3を誘導する[28]．IL-6やIL-1β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RORγt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[29]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[30,31]，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が示されている．

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[15]，FTY720はTh17細胞のリンパ節外への移出を阻止し[32]，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[33]，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[34]，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[35]．

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[36]．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[37]．レチノイン酸（ATRA）はTh17細胞の分化に対して抑制的に働く[38]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[39]．

最後に，MSの治療薬として最も汎用されるIFNβ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（IFNα受容体1）の発現はTh1細胞よりも高く，IFNβはTh17細胞を抑制する効果をもつと報告された[40]．しかし，SteinmanらはTh1細胞で誘導したEAEはIFNβにより軽減するが，Th17細胞で誘導したEAEはIFNβにより悪化したと報告し[41]，IFNβ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IFNβ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IFNβ

有効群（responder）に比べ高く，Th17細胞の活動性とIFNβ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[42]，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[43,44]．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[45]，IFNβ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IFNβが病態の悪化につながることから[46,47]，NMOではTh17細胞の活動性が亢進している可能性がある[48]．

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[49]．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[50]，IFNβ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．

文献

1) McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol. 2007; 8: 913-9.
2) Weiner HL. The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? Ann Neurol. 2009; 65: 239-48.
3) Coffman RL. Origins of the T(H)1-T(H)2 model: a personal perspective. Nat Immunol. 2006; 7: 539-41.
4) Cua DJ, Sherlock J, Chen Y, et al: Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature. 2003; 421: 744-8.
5) Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. J Clin Invest. 2006; 116: 1218-22.
6) Korn T, Bettelli E, Oukka M, et al. IL-17 and Th17 Cells. Annu Rev Immunol. 2009; 27: 485-517.
7) Boniface K, Bak Jensen KS, Li Y, et al. Prostaglandin E2 regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. J Exp Med. 2009; 206: 535-48.
8) Mucida D, Park Y, Kim G, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science. 2007; 317: 256-60.
9) Veldhoen M, Hirota K, Westendorf AM, et al.

The aryl hydrocarbon receptor links T(H)17-cell-mediated autoimmunity to environmental toxins. Nature. 2008; 453: 106-9.
10) Hofstetter HH, Ibrahim SM, Koczan D, et al. Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. Cell Immunol. 2005; 237: 123-30.
11) Cosmi L, De Palma R, Santarlasci V, et al. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. J Exp Med. 2008; 205: 1903-16.
12) Acosta Rodriguez EV, Rivino L, Geginat J, et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol. 2007; 8: 639-46.
13) Tzartos JS, Friese MA, Craner MJ, et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol. 2008; 172: 146-55.
14) Brucklacher-Waldert V, Stuerne K, Kolster M, et al. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. Brain. 2009; 132: 3329-41.
15) Polman CH, O'Connor PW, Havrdova E, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med. 2006; 354: 899-910.
16) Mahad D, Callahan MK, Williams KA, et al. Modulating CCR2 and CCL2 at the blood-brain barrier: relevance for multiple sclerosis pathogenesis. Brain. 2006; 129: 212-23.
17) Leppert D, Ford J, Stabler, G, et al. Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. Brain. 1998; 121: 2327-34.
18) Sato W, Aranami T, Yamamura T. Cutting edge: Human Th17 cells are identified as bearing CCR2+CCR5- phenotype. J Immunol. 2007; 178: 7525-9.
19) Singh SP, Zhang HH, Foley JF, et al. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. J Immunol. 2008; 180: 214-21.
20) Reboldi A, Coisne C, Baumjohann D, et al. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol. 2009; 10: 514-23.
21) Kebir H, Kreymborg K, Ifergan I, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med. 2007; 13: 1173-5.
22) Ifergan, I, Kebir, H, Bernard, et al. The blood brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. Brain. 2008; 131: 785-99.
23) Kebir H, Ifergan I, Alvarez JI, et al. Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. Ann Neurol. 2009; 66: 390-402.
24) Hirota K, Duarte JH, Veldhoen M, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. Nat Immunol. 2011; 12: 255-63.
25) Wei G, Wei L, Zhu J, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating $CD4^+$ T cells. Immunity. 2009; 30: 155-67.
26) Annunziato F, Romagnani S. The transient nature of the Th17 phenotype. Eur J Immunol. 2010; 40: 3312-6.
27) Domingues HS, Mues M, Lassmann H, et al. Functional and pathogenic differences of Th1 and Th17 cells in experimental autoimmune encephalomyelitis. PLoS One. 2010; 5: e15531.
28) Zhou L, Lopes JE, Chong MM, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008; 453: 236-40.
29) Zhou X, Bailey Bucktrout SL, Jeker LT, et al Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nat Immunol. 2009; 10: 1000-7.
30) Beriou G, Costantino CM, Ashley CW, et al. IL-17-producing human peripheral regulatory T cells retain suppressive function. Blood. 2009; 113: 4240-9.
31) Voo KS, Wang YH, Santori FR, et al. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A. 2009; 106: 4793-8.

32) Mehling M, Lindberg R, Raulf F, et al. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology. 2010; 75: 403-10.
33) Piccio L, Naismith RT, Trinkaus K, et al. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. Arch Neurol. 2010; 67: 707-14.
34) Leonardi CL, Kimball AB, Papp KA, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet. 2008; 371: 1665-74.
35) Segal BM, Constantinescu CS, Raychaudhuri A, et al. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol. 2008; 7: 796-804.
36) Serada S, Fujimoto M, Mihara M, et al. IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2008; 105: 9041-6.
37) Doi Y, Oki S, Ozawa T, et al. Orphan nuclear receptor NR4A2 expressed in T cells from multiple sclerosis mediates production of inflammatory cytokines. Proc Natl Acad Sci U S A. 2008; 105: 8381-6.
38) Mucida D, Park Y, Kim G, et al. Reciprocal Th-17 and regulatory T cell differentiation mediated by retinoic acid. Science. 2007; 317: 256-60.
39) Klemann C, Raveney BJ, Klemann AK, et al. Synthetic retinoid AM80 inhibits Th17 cells and ameliorates experimental autoimmune encephalomyelitis. Am J Pathol. 2009; 174: 2234-45.
40) Durelli L, Conti L, Clerico M, et al. T-helper 17 cells expand in multiple sclerosis and are inhibited by interferon-beta. Ann Neurol. 2009; 65: 499-509.
41) Axtell RC, de Jong BA, Boniface K, et al. T helper type 1 and 17 cells determine efficacy of interferon-beta in multiple sclerosis and experimental encephalomyelitis. Nat Med. 2011; 16: 406-12.
42) Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet. 2004; 364: 2106-12.
43) Misu T, Fujihara K, Kakita A, et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain. 2007; 130: 1224-34.
44) Wingerchuk DM, Lennon VA, Pittock SJ, et al. Revised diagnostic criteria for neuromyelitis optica. Neurology. 2006; 66: 1485-9.
45) Ishizu T, Osoegawa M, Mei FJ, et al. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. Brain. 2009; 128: 988-1002.
46) Warabi Y, Matsumoto Y, Hayashi H. Interferon beta-1b exacerbates multiple sclerosis with severe optic nerve and spinal cord demyelination. J Neurol Sci. 2007; 252: 57-61.
47) Shimizu J, Hatanaka Y, Hasegawa M, et al. IFNbeta-1b may severely exacerbate Japanese optic-spinal MS in neuromyelitis optica spectrum. Neurology. 2010; 75: 1423-7.
48) Axtell RC, Raman C, Steinman L. Interferon-beta exacerbates Th17-mediated inflammatory disease. Trends Immunol. 2011; 32: 272-7.
49) Disanto G, Berlanga AJ, Handel AE, et al. Heterogeneity in multiple sclerosis: scratching the surface of a complex disease. Autoimmune Dis. 2010; 2011: 932351.
50) Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000; 47: 707-17.

Role of NK Cells and Invariant NKT Cells in Multiple Sclerosis

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Abstract Natural killer (NK) cells and invariant natural killer T (*i*NKT) cells are two distinctive lymphocyte populations, each possessing its own unique features. Although NK cells are innate lymphocytes with cytotoxic property, they play an immunoregulatory role in the pathogenesis of autoimmune diseases. NKT cells are T cells expressing invariant TCR α-chains, which are known to bridge innate and adaptive arms of the immune system. Accumulating data now support active involvement of these cells in multiple sclerosis (MS). However, unlike professionally committed regulatory cells such as $Foxp3^+$ regulatory T cells, NK, and *i*NKT cells have dual potential of acting as either protective or pathogenic lymphocytes depending on the disease setting, adding complexity to the interpretation of data obtained from human and rodent studies. They are potential therapeutic targets in MS, and further in-depth understanding of these cells will lead to designing new strategies to overcome the disabling disease MS.

1 Introduction

Over the past years, a growing number of evidence has indicated that multiple sclerosis (MS) is as an autoimmune disease mediated by T cell immunity (Sospedra and Martin 2005). As described in detail in other chapters, pathogenesis of MS would actually involve autoreactive T cells that recognize the central nervous system (CNS) antigens. The target antigens include myelin basic protein (MBP) (Bielekova et al. 2000; Martin et al. 1991; Ota et al. 1990; Pette et al. 1990; Richert et al. 1989), myelin proteolipid protein (PLP) (Correale et al. 1995; Illes et al. 1999; Kondo et al. 1996; Ohashi et al. 1995; Pelfrey et al. 1993), and myelin oligodendrocyte glycoprotein (MOG) (Iglesias et al. 2001; Koehler et al. 2002; Mendel et al. 1995).

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Although the dominant role of $CD4^+$ T cells in MS has long been emphasized (Hafler 2004), more recent works indicate that $CD8^+$ T cells (Huseby et al. 2001; Skulina et al. 2004) and B cells also play a critical role in the disease development, and actually comprise a proportion of the CNS infiltrating cells. $CD8^+$ cells are reported to be predominant in the CNS lesions of MS, although compositions of cellular infiltrates vary greatly, depending on types and stages of this disease (Sospedra and Martin 2005). Now, the key question in MS lies in what disrupts the T cell and B cell immunological tolerance against the CNS antigens that are usually kept well secluded from the systemic immune system (Goodnow et al. 2005; Kyewski and Derbinski 2004; Walker and Abbas 2002). The relevance of this question is obvious because better understanding of the mechanism for the disruption of self-tolerance will lead to development of various new approaches to prevent the onset of MS and to control its further progression.

One of the distinctive and intriguing aspects of MS is that individual patients show various patterns in the longitudinal changes of its disease activity. While a large majority of the patients exhibit a relapsing and remitting course, some patients develop into or even start out as a progressive chronic illness (Sospedra and Martin 2005; Steinman 2001). Despite the vigorous efforts to control the activity of MS, currently available therapeutics to do not halt the progression of disease in a majority of cases, although some patients do not exhibit any sign of worsening for a long period of time even without treatment.

To clarify the regulation of autoimmune responses, much efforts have been dedicated to investigate the role of specialized adaptive regulatory T cells, including $CD4^+$ T cells expressing transcription factor Foxp3 (Miyara and Sakaguchi 2007), IL-10 producing T regulatory 1 (Tr1) cells (Roncarolo et al. 2006), and TGF-β producing Th3 cells (Awasthi et al. 2007; Baecher-Allan and Hafler 2006). However, recent publications provide evidence that cells of the innate immune system also have an unexpected potential to inhibit autoreactive $CD4^+$ T cells from mediating autoimmune disease and to protect tissues from collateral damage by T cells reactive to exogenous pathogens (Carrol and Prodeus 1998; Fearon and Locksley 1996; Medzhitov and Janeway 1997; Shi et al. 2001). Natural killer (NK) cells and invariant natural killer T (*i*NKT) cells, the main focus of this review, are also now recognized as innate cells with immunoregulatory potentials. Although they sense external ligands with different receptors (TCR for *i*NKT cells and NK receptor for NK cells), they behave like innate cells when they need to rapidly respond to stimuli. Therefore, it was believed previously that both cell types would primarily function within the innate arms of immunity. However, recent works have provided evidence that they would actively regulate T cell responses, thereby influencing the adaptive immune system (Bendelac et al. 1997; Carrol and Prodeus 1998; Fearon and Locksley 1996; Medzhitov and Janeway 1997; Shi et al. 2001; Shi and Van Kaer 2006).

In summary, NK cells and *i*NKT cells are now considered as multipotent cells that work at the border of innate and adaptive immunity, to prevent the induction, propagation, and activation of autoimmune T cells. Here, we review the latest advances in the research of the regulatory NK and *i*NKT lymphocytes with regard to the pathogenesis of MS and discuss the possibilities that they may serve as an effective target for MS therapy.

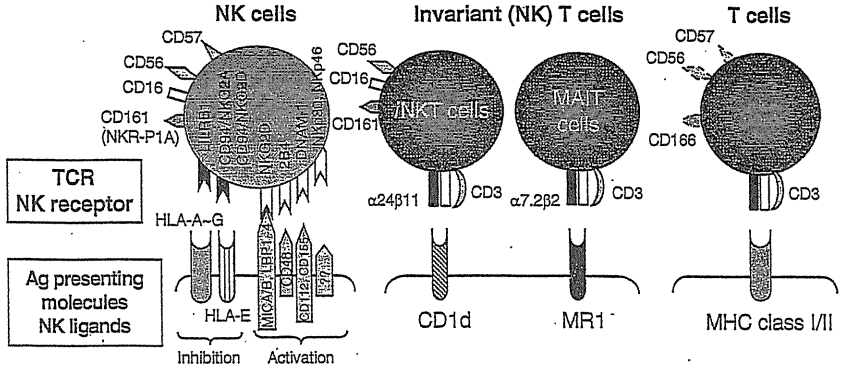
2 NK Cells and MS

2.1 General Properties of NK Cells

Natural killer cells are evolutionary primitive lymphocytes that lack antigen-specific receptors. They were originally identified as lymphoid cells capable of lysing tumor cell lines in the absence of prior stimulation *in vivo* or *in vitro*, which was the basis of their denomination (Trinchieri 1989). Constituting about 10% of the lymphocyte in human peripheral blood mononuclear cells (PBMC), NK cells possess cytotoxic properties, directed against virus-infected cells, thus considered as an important part of the innate immune system. Their cytotoxic reaction is determined by collective signaling of an array of inhibitory and stimulatory receptors expressed on their surface (Kirwan and Burshtyn 2007) (Fig. 1). Inhibitory receptors, commonly referred to as killer inhibitory Ig-like receptors (KIRs), interact with shared allelic determinants of classical and non classical MHC class I. Hence, NK cells are kept in an inactivated state through contact with self MHC class I molecule expressed on healthy cells. For example, CD94/NKG2A heterodimer expressed on NK cell surface recognize HLA-class Ib molecule, HLA-E (Borrego et al. 2006; Lopez-Botet et al. 1997). On the contrary, stimulatory receptors on NK cell surface bind to NK stimulatory receptor ligand up-regulated on other cells upon undergoing cellular stress. The main activating receptors constitutively found on all NK cells in peripheral blood are NKG2D, 2B4, and the two of the three natural cytotoxicity receptors (NCRs), NKp30, and NKp46. One example of NK stimulatory receptor ligand is the protein encoded by retinoic acid early inducible gene (RAE-I), which was isolated from tumor lines. RAE-1 is also expressed on virus-infected cells (Backstrom et al. 2007), and binds to the stimulatory receptor expressed on NK cells, NKG2D (Diefenbach et al. 2000; Smyth et al. 2005). As an overall effect, NK cells would lyse target cells that have lost or express low amounts of MHC class I molecules, including tumor cells or cells infected by viruses such as certain Herpes viruses or Adenoviruses.

Once activated, NK cells display cytotoxic functions which is mediated by direct cell-to-cell contact as well as secretion of cytokines and chemokines. The cell contact pathways include perforin/granzyme (Warren and Smyth 1999), Fas/Fas-ligand (Screpanti et al. 2005), and TRAIL/TRAIL ligand interaction (Takeda et al. 2001). They also produce inflammatory cytokines such as IFN-γ, TGF-β, and GM-CSF. Despite these cytotoxic actions against tumor cells and virus infected cells, it is now well conceived that some NK cells could act as modulator of adaptive immunity and have the potential to eliminate self-reactive T cells.

Although the diversity of NK cells remained to be ambiguous some time ago, recent works have greatly contributed to clarifying their heterogeneity in phenotypes and functions. The majority of human NK cells in PBMC belong to $CD56^{dim}CD16^{+}$ cytolytic NK subset. These cells express homing markers for inflamed peripheral sites and carry perforin to rapidly mediate cytotoxicity. $CD56^{bright}$ $CD16^{-}$ cells constitute a minor NK subset that lacks perforin but secrete large amounts of IFN-γ and



	Natural Killer cells	Invariant T cells		Conventional T cells
		iNKT cells	Vα7.2 iT cells	
TCR-Ag presenting molecules	None	α24β11- CD1d	α7.2β2/13- MR1	αβ- CD8:MHC class I CD4:MHC class II
NK marker	CD161 (NKR-P1) CD16 CD56 CD57 CD122	CD161 (NKR-P1) CD16 CD56	CD161 (NKR-P1)? CD16 ? CD56 ?	CD161, CD56, CD57 + in some subsets
NK receptor-ligands	Inhibition (KIR): CD94/NKG2A-HLA-E CD94/NKG2C-HLA-E LILRB1-HLA-A-G Activation: NKG2D-MICA/B ULBP1-4 NKp30- ??	CD94/NKG2A -HLA-E NKG2D-MICA/B ULBP1-4	??	Some cells pos. by induction
Memory phenotype		Majority CD69+	Majority CD69+	+ (Memory T cells)
Cytokine production	NK1: IFN-γ, TNF-α NK2: IL-5	DN: IFN-γ,TNF-α CD4: IL-4, IL-5, IL-13 (IL-17, IL-21)	? ? IFN-γ Th2 cytokine	CD8: IFN-γ CD4: Th1cell: IFN-γ Th2cell: IL-4, IL-5 Th17cell: IL-17
Perforine activation	+ (mainly NK1)	+ (mainly DN cells)	??	+ (mainly CD8 cells)
Frequency in PBMC	10 %	0.1 - 0.5 %	??	30-40 %

Fig. 1 Comparative features of human NK cells, invariant *i*NKT cells, and conventional T cells

TNF-α upon activation. They are superior to $CD56^{dim}$ cells in the regulatory functions that are mediated by these cytokines (Moretta et al. 2001). Moreover, they express surface markers such as CCR7 and CD62L that allow their homing to the lymph nodes, which results in the predominance of this NK cell subset in the secondary lymphoid organs.

Recent studies have shown that human NK cells are able to polarize *in vitro* into two functionally distinct subsets NK type 1 (NK1) or NK2 cells, analogous to T cell subsets Th1 or Th2. NK cells cultured in a condition favoring Th1 deviation (cultured with IL-12) would differentiate into NK1 cells producing IFN-γ and IL-10, whereas NK cells grown in a Th2 condition (cultured with IL-4) differentiate into NK2 cells producing IL-5 and IL-13 (Peritt et al. 1998). Although it was ambiguous whether the polarization actually occurs *in vivo*, an expansion of NK2 like cells producing IL-5 and IL-13 was observed in IFN-γ knockout mice (Hoshino et al. 1999), indicating that NK cells could functionally polarize into NK2-like cells in vivo.

Phenotypical analysis of NK cells in rodents has also identified a distinct population of NK cells that express CD11c, a prototypical dendritic cell (DC) marker. As the CD11c NK cells were shown to exhibit both NK and DC function, they are often referred to as "bitypic NK/DC cells" (Homann et al. 2002; Pillarisetty et al. 2005). CD11c molecule is known to be associated with integrin CD18 and form CD11c/CD18 complex. Although the precise function is not clear, CD11c is reportedly involved in binding of iC3b (Bilisland et al. 1994), adhesion to stimulated endothelium (Stacker and Springer 1991), and phagocytosis of apoptotic cells (Morelli et al. 2003). Bearing in mind that we have only very little knowledge of how these NK cell subsets are correlated to each other, we will next discuss on the recent progress which correlates the regulatory aspects of NK cells with the pathogenesis of MS.

2.2 NK Cell in MS

Despite the extensive studies in the past, there has been no simple uniform consensus regarding the role of NK cells in MS. Some of the earlier studies have found an inverse relationship between the number or the functional activity of circulating NK cells and the clinical or radiological activity of the patients with MS. NK cells isolated from MS patients were reported to be inefficient at cytotoxic killing and IFN-γ production (Benczur et al. 1980; Kastrukoff et al. 1998; Munschauer et al. 1995; Vranes et al. 1989). Furthermore, a longitudinal study showed that the functional activities of NK cells would decline during the relapse and then normalized during remission (Kastrukoff et al. 2003). On the contrary, several earlier studies failed to reveal any quantitative or qualitative difference between NK populations in MS patients versus controls (Hauser et al. 1981; Rauch et al. 1985; Rice et al. 1983; Santoli et al. 1981). The reason for these controversial findings remains to be unclear. However, it is of note that the criteria used to classify NK cells have been variable among the researchers and as a result the assays and protocols used to measure their functions and frequencies differ widely among the studies above mentioned. Moreover, because of difficulties in enrollment of patients, each of the studies might have examined the group of patients in different conditions. We also assume that they did not unify various confounding factors, some of which were not recognized when the study was conducted. Even duration of time between blood sampling and examination may affect the condition of NK cells (Takahashi et al. 2001).

In spite of the setbacks, the notion that NK cells have a significant role in reducing neuroinflammation and CNS injury stems from indirect evidences that were extracted from studies of an animal model experimental autoimmune encephalomyelitis (EAE) and from human clinical trials.

2.2.1 Protective Role of NK Cells in EAE

Monophasic EAE can be induced in C57BL/6 strain of mice (B6 mice) by immunizing the mice with an encephalitogenic myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}). When NK cells were depleted in vivo by antibody specific for NK1.1 molecule (CD161), mice developed an aggravated form of EAE in terms of onset and clinical severity (Zhang et al. 1997). Furthermore, NK cell depletion was found to increase proliferation and production of Th1 cytokines by memory $CD4^+$ T cells in the recall response to MOG. Similarly, NK cell depletion augmented the severity of EAE induced in β_2-microglobulin –/– mice. As the mice are lacking expression of CD1d molecule necessary for $NK1.1^+$ T cell development, it was assumed that NK cells would play a regulatory role in a manner independent of $NK1.1^+$ T cells. Furthermore, co-transfer of whole splenocytes, but not of NK cell-depleted splenocytes, ameliorated EAE that was induced by adoptive transfer of MOG-specific T cells into $Rag2^{-/-}$ hosts. Taken together, it was concluded that NK cells play a regulatory role in EAE. Involvement of NK cells was also demonstrated in Lewis rat EAE model which can be induced by sensitization to MBP (Matsumoto et al. 1998). When NK cells were depleted by antibody specific for either NKR-P1 (analogous to NK1.1) or asialo GM1, the rats developed an aggravated form of EAE, characterized by higher maximal clinical scores and increased mortality rates. Subsequently, Swanborg et al. have shown that rat bone marrow-derived NK cells would exhibit potent inhibitory effects on proliferation of auto-reactive T cells (Smeltz et al. 1999), further strengthening the postulate that NK cells play a regulatory role in the CNS autoimmunity.

More recently, Huang et al. have reported that mice deficient in CX3CR1 (the fractalkine receptor) develop a more severe form of EAE (Huang et al. 2006). Compared with their littermates, $CX3CR1^{-/-}$ mice immunized with MOG_{35-55} would exhibit a higher incidence of CNS hemorrhage, leading to a higher mortality rate. Moreover, the survived mice failed to recover neurological functions after they reached the peak of EAE. Although the $CX3CR1^{-/-}$ mice developed more serious manifestations of EAE, recall responses to MOG_{35-55} and generation of encephalogenic T cells in the peripheral lymphoid organs were not augmented in the mice. Notable differences were found in the CNS infiltrating cells. Namely, $NK1.1^+CD3^-$ cells were selectively depleted from mononuclear cells isolated from the spinal cord of the $CX3CR1^{-/-}$ mice, whereas they comprised 10–20% of the CNS infiltrates in wild-type mice and heterozygous $CX3CR1^{+/-}$ littermates. These findings led the authors to speculate that the exacerbated disease in $CX3CR1^{-/-}$ mice was due to a failure of regulatory NK cells to enter the target organ. In support of this, the majority of CNS-infiltrating NK cells in the littermate mice suffering from EAE expressed CX3CR1.

When NK cells were depleted in vivo by injecting anti-NK1.1 antibody, difference between CX3CR1$^{-/-}$ and the littermate CX3CR1$^{+/-}$ mice in the severity of EAE was no more evident. Of interest, soluble CX3CL1 was increased in the CNS of the EAE mice, and protein extracts from the CNS tissues showed a chemotactic activity for NK cells. It is of particular interest that a reduced number of circulating CX3CR1^{+} NK cells has recently been reported in patients with MS (Infante-Duarte et al. 2005), which would prompt further investigation to examine a possible correlate between EAE and MS with regard to NK cell-mediated immunoregulation.

2.2.2 Ex Vivo Analysis Revealed an Alteration of NK cells in MS

Given putative roles of NK cells in MS, one may ask if there is a significant correlation of NK cell functions and the disease activity of MS. By analyzing surface phenotypes and cytokine secretion profile of peripheral blood NK cells, we demonstrated in 2001 that NK cells from MS patients during clinical remission are characterized by a higher frequency of CD95^{+} cells as well as a higher expression level of IL-5, which represents a feature highly reminiscent of NK2 cells (Takahashi et al. 2001). The patients were selected from those who were not given any disease-modifying drugs, including corticosteroids. Remarkably, the NK2 cell-like feature, that is, a strong bias toward producing IL-5, was lost during the relapse of MS and regained after recovery. It was also found that NK2 cells induced *in vitro* from the peripheral blood of healthy subjects would inhibit the induction of Th1 cells, suggesting that the NK2 cells in vivo may also prohibit autoimmune effector T cells. Subsequently, we showed that when MS patients in remission are divided into two groups, according to the CD95^{+} NK cell frequency, memory T cells reactive to MBP are increased in patients who possess a higher number of CD95^{+} NK cells (Takahashi et al. 2004). Interestingly, NK cells from the "CD95 high patients" exhibited an ability to actively suppress the autoimmune T cells. These results allowed us to propose a model that CD95 low patients are enjoying very stable remission wherein an actual frequency of pathogenic autoimmune T cells is low, whereas CD95 high patients are in a more active state (which we call "smoldering state") wherein a higher number of autoreactive T cells are counter-regulated by NK cells (Fig. 2).

In a separate study, we found that CD11c expression on peripheral NK cells tends to correlate with temporal disease activity of MS (Aranami et al. 2006). Our study has revealed that surface CD11c expression on NK cells is significantly up-regulated in a proportion of patients with MS in remission, compared with healthy subjects or the rest of the patients. In the group of patients whose NK cells express higher levels of CD11c ("CD11c high patients"), IL-5 production from NK cells was significantly down-regulated and conversely, HLA-DR class II molecule was up-regulated. Accordingly, NK cells from "CD11c low patients" are NK2-biased, whereas those from "CD11c high patients" are not. NK cells from human PBMC would up-regulate expression of both CD11c and HLA-DR molecules after culture with IL-15 or a combination of IL-12 and IL-18 inflammatory cytokines commonly found in MS. Remarkably, the "CD11c high patients" tended to relapse significantly

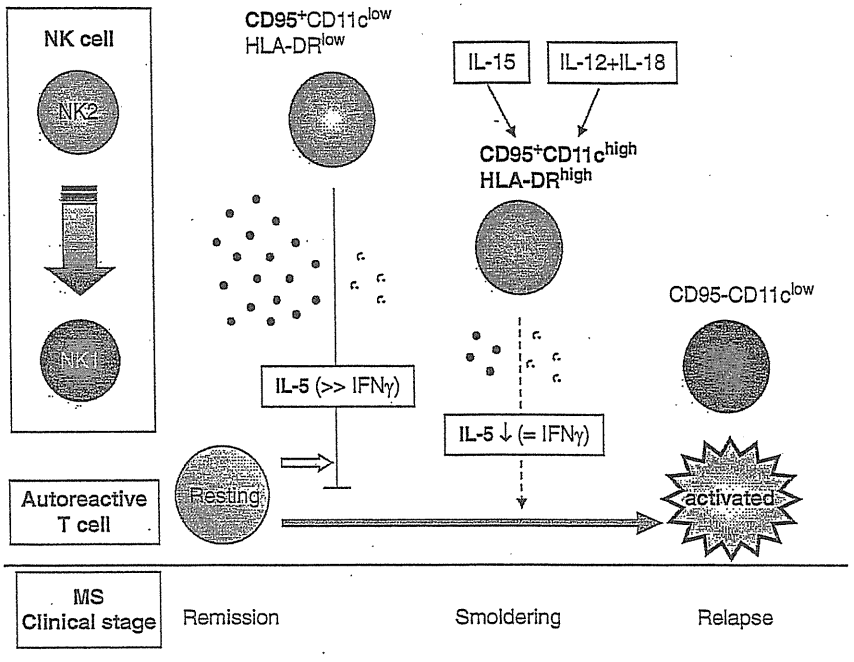


Fig. 2 Regulatory role of CD95+ NK 2 cell in MS remission

earlier than "CD11c low patients," indicating that "CD11c high patients" are clinically more active. We, therefore, propose that expression levels of CD11c on NK cells may serve as a good indicator of the disease activity (Fig. 2).

Another evidence for the role of NK cells in MS was obtained in the clinical trial of a new humanized monoclonal antibody against IL-2 receptor α-chain. In a recent phase II trial with the antibody (daclizumab), Bielekova et al. have noticed that an expansion of $CD56^{bright}$ immunoregulatory NK cells and their increased perforin expression would highly correlate with the reduction of the disease activity (Bielekova et al. 2006). In fact, contrast enhanced lesion on brain MRI was significantly suppressed along with an expansion of circulating $CD56^{bright}$ NK cells. NK cells isolated from patients being given daclizumab were found to exhibit cytotoxity towards autologous activated T cells, even without prestimulating NK cells with IL-2. These results raise a possibility that induced regulatory NK cells may at least partly mediate daclizumab effects on MS. In another study, an increase of $CD56^{bright}$ NK cells was demonstrated in the blood of newly diagnosed patients with relapsing-remitting MS who were started on interferon-β treatment a few months ago (Saraste et al. 2007). This work also supports a role for induced regulatory NK cells in patients who respond to immunomodulatory therapy. Taking the available data together, we assume that NK cells harbor functional subpopulations that play a protective role in CNS autoimmunity. Regulatory NK cells could be $CD56^{high}$, $CD95^{+}$, or $CX3CR1^{+}$, although mutual relationship of the populations still remains unclear. Further attempts to find a way to selectively activate regulatory NK cells are warranted, because it

will lead to developing a new treatment strategy for MS. It is known that NK cells show cytotoxic insults against CNS components in some *in vitro* conditions (Morse et al. 2001). To develop safe and effective drugs targeting NK cells, it is also important to know if regulatory NK cells could be selectively induced without augmenting cytotoxic NK cells that are potentially harmful for MS.

3 *i*NKT Cells in MS

3.1 What Is iNKT Cell?

3.1.1 General Properties of Invariant NKT (*i*NKT) Cells

Invariant NKT (*i*NKT) cells are a unique subset of lymphocytes that recognize a glycolipid antigen such as α-galactosylceramide (α-GC) (Kawano et al. 1997), that is bound to a monomorphic MHC class I-like molecule CD1d (Bendelac et al. 2007; Kronenberg 2005; Taniguchi et al. 2003). The term "NKT cells" was first introduced in mice to define a broader range of T cells that express the NK cell-associated marker NK1.1 (CD161) (Ballas and Rasmussen 1990; Fowlkes et al. 1987). The term "*i*NKT cells" defines a more limited population among $NK1.1^+$ T cells that express a single invariant α-chain (Vα14-Jα18 in mice and Vα24-Jα18 in humans) and respond to α-GC bound to CD1d (Dellabona et al. 1994; Exley et al. 1997; Koseki et al. 1991) (Fig. 1). The invariant α-chain is coupled with a noninvariant β-chain which selectively uses Vβ8.2, Vβ7, and Vβ2 gene segments in mice and Vβ11 (a molecule homologous to mice Vβ 8.2) in humans. It is currently known that mouse $NK1.1^+$ T cells (or NKT cells in the classic definition) are composed of *i*NKT cells, CD1d-restricted noninvariant T cells, conventional T cells that are not restricted by CD1d, and MAIT cells (see Sect. 4). On the other hand, there are a significant number of NK1.1-negative T cells that express the invariant Vα14-Jα18 TCR and react to α-GC/CD1d. In most of the current literatures, such T cells are also called *i*NKT cells.

*i*NKT cells constitutively express memory/activated T cell phenotype and are capable of robustly producing pro and antiinflammatory cytokines within hours after TCR engagement. The cytokine burst following *i*NKT cell activation then triggers a maturation process of downstream cells, such as NK cells, DCs, B cells, and T cells, which leads to subsequent alteration of a broad range of adaptive immune responses. Although *i*NKT cells utilize TCR for sensing a specific antigen, the behavior of the cells in response to external stimuli resembles that of innate lymphocytes (Mempel et al. 2002). Owing to the swift responsiveness to external stimuli, it is thought that *i*NKT cells play an important role in bridging innate and adaptive arms of immune response.

Another striking property of *i*NKT cells is to produce diverse combinations of cytokines, depending on how they are stimulated. Mouse *i*NKT cells can produce IFN-γ, IL-2–5, -13, -17, -21, GM-CSF, TNF-α, and osteopontin after an optimal engagement of TCR (Yamamura et al. 2007). In fact, they can produce a broad range

of pro- and anti-inflammatory cytokines upon stimulation with α-GC, a highly potent ligand for *i*NKT cells (Kawano et al. 1997). In contrast, cytokine production by *i*NKT cells is much more finely regulated under physiological environment, which could result in production of a set of Th2 cytokines (Sakuishi et al. 2007).

*i*NKT cells are segregated into $CD4^+CD8^-$ and $CD4^-CD8^-$ double negative (DN) subsets. It has been shown that each subset differs remarkably in their functional properties. In humans, about 40–60% of *i*NKT cells are $CD4^+$, and a large majority of the remaining cells are DN cells. Some *i*NKT cells express CD8α, but only very few cells co-express CD8β. The $CD4^+$ subset potently produces both Th1 and Th2 cytokines, whereas the DN population selectively produces the Th1 cytokines (IFN-γ and TNF-α) and preferentially up-regulates perforin in response to IL-2 or IL-12 (Gumperz et al. 2002; Lee et al. 2002). It is also known that the $CD4^+$ and DN *i*NKT cells differentially express chemokine receptors: CCR4 on $CD4^+$ cells and CCR1, CCR6, and CXCR6 on DN cells (Kim et al. 2002). These results suggest the presence of a functional dichotomy in *i*NKT cells.

3.1.2 iNKT Cells and Their Ligands

To evaluate the potential of *i*NKT cells to regulate autoimmune diseases, it is particularly important to understand how they recognize a glycolipid antigen bound to CD1d. The CD1d molecule, highly conserved among mammalian species (Exley et al. 2000), is primarily expressed on the cells of hematopoietic origin, including thymocytes, B cells, macrophages, and DCs, and could also be induced on T cells upon activation. The binding cleft of the CD1d molecule consists of two nonpolar lined grooves, which makes it ideal for the presentation of hydrophobic antigens such as glycolipids. In 1997, a marine sponge-derived glycosphingolipid, α-GC, was identified as a potent ligand for mouse *i*NKT cells (Kawano et al. 1997). It was subsequently found that α-GC is stimulatory for human *i*NKT cells as well (Brossay et al. 1998). Thereafter, a synthetic α-GC has been used extensively for research (Fig. 3). A widely supported view on the topology of TCR/ligand/CD1d is that the two lipid chains of α-GC would be inserted into the CD1d hydrophobic grooves and α-linked sugar moiety becomes accessible for the TCR of *i*NKT cells (McCarthy et al. 2007). More recently, crystal structure analysis has demonstrated that the invariant α-chain of the *i*NKT cells would selectively recognize the α-linked sugar of α-GC (Borg et al. 2007). It is of note that glycolipids with a-linked sugars such as α-GC could not be found in mammalian tissues, but are rather ubiquitously present in the environment. After LPS-negative α-proteobacteria extracts were found to contain glycosphingolipids stimulatory for *i*NKT cells, a growing number of bacterial lipid antigens has been shown to stimulate *i*NKT cells (Bendelac et al. 2007), including diacylglycerol glycolipid extracted from Borrelia burgdorferi (Kinjo et al. 2005). Given that the TCRs of *i*NKT cells recognize such pathogen-derived antigens, the lipid antigens may be an important initiator for triggering the immune response in bacterial and parasite infection. However, it has recently been demonstrated that *i*NKT cells are activated during infection without recognizing a bacteria component

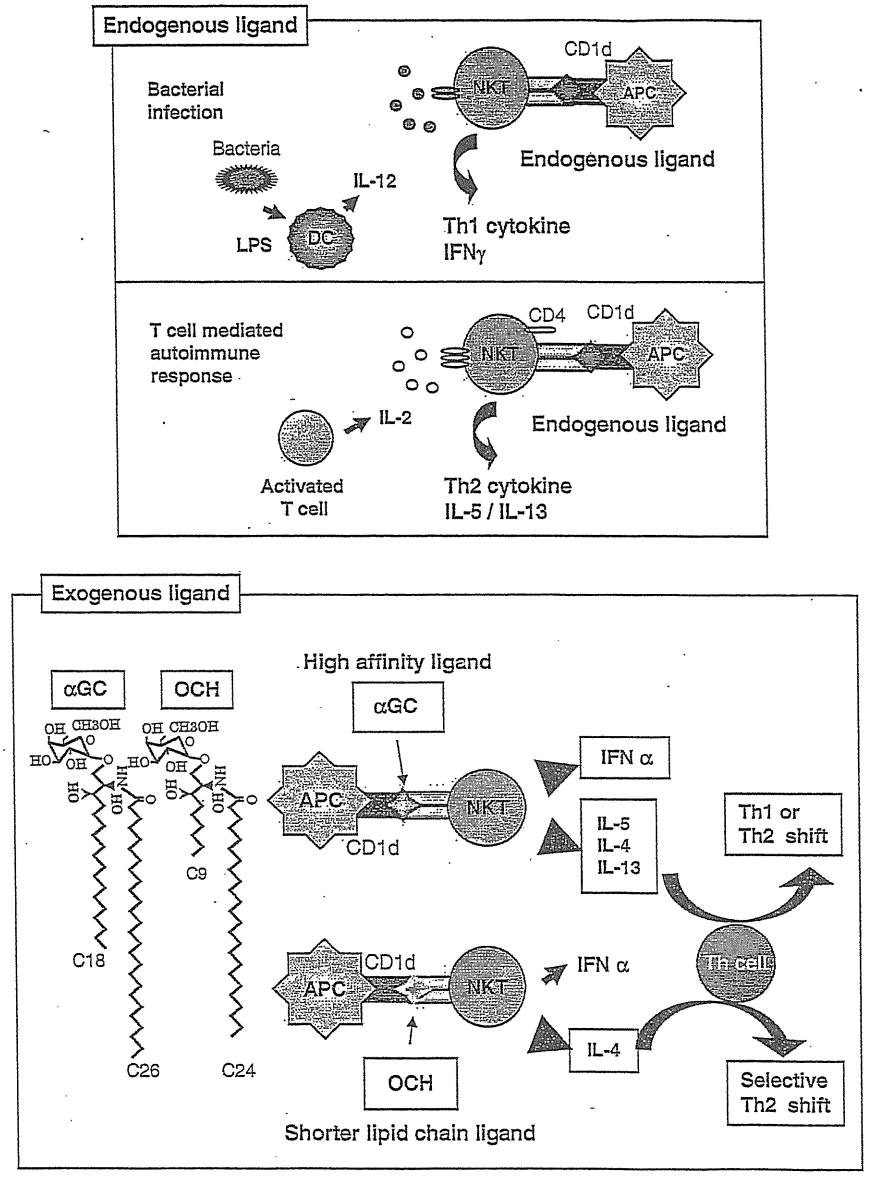


Fig. 3 Effects of lipid chain lengths in alpha-galactosylceramides on cyotokine release by natural killer T cells

via TCR (Brigl and Brenner 2004; Mattner et al. 2005). The antigen recognized by the TCR of *i*NKT cells is thought to be an endogenous ligand bound with CD1d, but not an exogenous microbial ligand. These studies also showed that the role for the bacterial LPS is to trigger production of IL-12 from DCs. Although *i*NKT cells