多发性硬化疾病及模型小鼠中疾病相关细胞因子和转录因子研究进展

蔡盈盈 杜昌升*

(上海市信号转导与疾病研究重点实验室, 同济大学生命科学与技术学院, 上海 200092)

摘要 多发性硬化症(multiple sclerosis, MS)是一种原发于中枢神经系统的炎症性脱髓鞘疾病。实验性自身免疫性脑脊髓炎(experimental autoimmune encephalomyelitis, EAE)与MS有相似的临床症状和病理特征, 是被广泛应用于人类疾病研究的动物模型。MS确切的发病机制尚不清楚, 但普遍认为是在易感基因的基础上, 受环境因素触发, 由$CD4^+$ T细胞介导的中枢神经系统(central nervous system, CNS)自身免疫性疾病。初始$CD4^+$ T细胞在T细胞受体介导下活化, 继而可分化为至少4个主要亚型, 分别为T_H1、T_H2、T_H17和iT_{reg}细胞, 参与不同类型的免疫应答。细胞因子和转录因子网络对$CD4^+$ T细胞分化和效应细胞因子产物有重要意义。该文综述了各相关细胞因子和转录因子在$CD4^+$ T细胞向不同亚型分化及MS/EAE发病过程中的相互作用和调控, 揭示各因子在这些过程中的作用, 有助于进一步研究和治疗MS。

关键词 多发性硬化症; 实验性自身免疫性脑脊髓炎; 细胞因子; 转录因子

1 多发性硬化症与实验性自身免疫性脑脊髓炎模型

多发性硬化症(multiple sclerosis, MS)是一种原发于中枢神经系统的炎症性脱髓鞘疾病。80% MS病患初发时为复发–缓解型(relapsing-remitting multiple sclerosis, RRMS), 其中约65%病程趋缓, 进展为继发性进展型(secondary progressive multiple sclerosis, SPMS)。20%病患无法看到明确缓解的病程, 自病程开始便持续恶化, 逐渐丧失神经系统功能和上行性麻痹, 属于原发性进展型(primary progressive multiple sclerosis, PPMS)[1]。虽然不同类型的MS在临床过程、影像学、病理学和免疫机制上存在不同, 但病灶受损基本结构是一致的, 都发生于由淋巴细胞、巨噬细胞或小胶质细胞参与的炎症反应中, 表现为脱髓鞘但轴突被部分保留的神经系统受损的病理特征[2]。MS确切的发病机制虽尚不清楚, 但普遍认为是在易感基因的基础上, 受环境因素触发, 由$CD4^+$ T细胞介导的中枢神经系统(central nervous system, CNS)自身免疫性疾病。

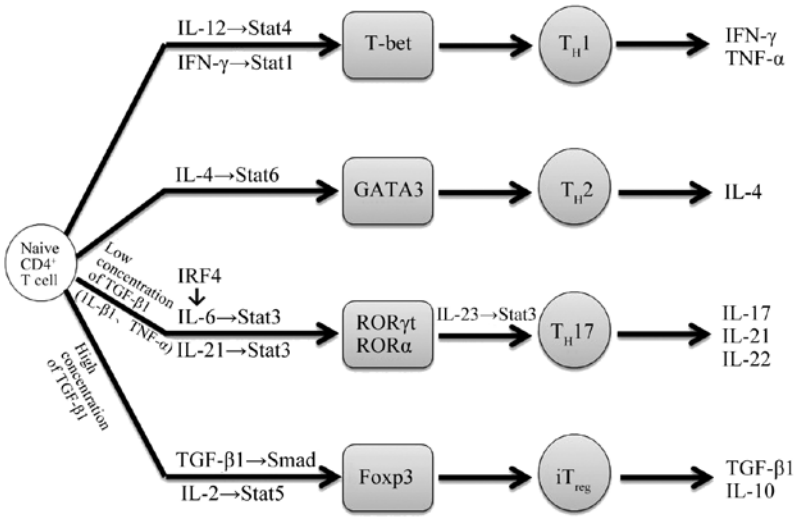
实验性自身免疫性脑脊髓炎(experimental autoimmune encephalomyelitis, EAE)是一种以特异性致敏的$CD4^+$ T细胞介导为主, 对实验动物进行髓鞘蛋白免疫构建的疾病模型。诱导发病过程中, 树突状细胞在淋巴结被活化并将髓鞘蛋白抗原提呈给初始T细胞, 特异性T细胞在外周被激活, 穿透血脑屏障迁移至CNS。进入CNS后, T细胞招募炎性细胞, 因为CNS中存在同源髓鞘蛋白抗原而被本地抗原递呈细胞(antigen-presenting cell, APC)再活化, 扩增和释放炎性介质, 并浸润递呈MHC II-抗原肽复合物的APC, 导致炎症发生, 进而脱髓鞘及轴突受损[3-4]。由于EAE与人类MS在临床、生化、免疫及病理等诸多方面具有相同的特征, 所以它是目前国际公认的MS理想动物模型。但人类MS与EAE动物模型仍存在差异, 例如IFN-γ在EAE中起保护作用, 但却会加重MS患者的病情。

近几年常用的缓解MS发病的药物如IFN-β、醋酸格拉默等, 其免疫调节作用机制的揭示佐证了MS的发生取决于促炎症反应和抗炎症反应的平衡, 表现为促炎症因子IFN-γ、IL-12、TNF-α和IL-17等上调及抗炎症因子IL-4、IL-10和TGF-β1等下调[4-5]。如图1所示, $CD4^+$ T细胞的定向分化需要亚型特异性转

收稿日期: 2012-03-23 接受日期: 2012-05-25

国家自然科学基金(No.31000399, No.31171348)和国家重点基础研究发展计划(973计划)(No.2012CB910404)资助项目

*通讯作者。Tel/Fax: 021-65986852, E-mail: duchangsheng@gmail.com



初始$CD4^+$ T细胞在IL-12作用下活化Stat4上调IFN-γ的表达量, IFN-γ活化Stat1上调转录因子T-bet, 促进T_H1细胞分化, 生成分泌IFN-γ和TNF-α的T_H1细胞。主要分泌IL-4的T_H2细胞分化需在IL-4作用下招募和磷酸化转录因子Stat6, 进一步诱导IL-4及重要转录因子GATA-3的表达。T_H17细胞以分泌IL-17、IL-21和IL-22为特点, IL-6和低浓度TGF-β1的共表达起始该亚型分化, 其中表达受转录因子IRF4影响的IL-6通过活化Stat3上调IL-21的表达, 并一起活化Stat3上调重要转录因子RORγt、RORα及细胞表面IL-23-R的表达, 继而IL-23发挥作用促进T_H17细胞彻底分化, IL-1β和TNF-α也促进其分化。T_{reg}细胞中nT_{reg}细胞是$CD4^+$ T细胞在胸腺自然选择过程中产生的, 而iT_{reg}细胞则需IL-2活化Stat5上调Foxp3, 高浓度TGF-β1通过Smad复合物信号通路上调Foxp3和IL-10表达量, 进而共同促进分泌TGF-β1和IL-10的iT_{reg}细胞分化。

The expressions of IFN-γ are up-regulated in naive $CD4^+$ T cells under the activation of Stat4 by IL-12. IFN-γ activates Stat1 to improve the expressions of transcription factors T-bet so as to promote the differentiation of T_H1 cells, which secrete IFN-γ and TNF-α. The differentiation of T_H2 cells mainly secreting IL-4 needs the recruitment and phosphorylation of transcription factors Stat6 under the action of IL-4 for further induced expression of IL-4 and key transcription factors GATA-3. T_H17 cells are characterized by secreting IL-17, IL-21 and IL-22. The co-expression of IL-6 and TGF-β1 at low concentration can induce the differentiation of T_H17 cells. IL-6, the expression of which may affected by transcription factor IRF4, can activate Stat3 to up-regulate the expression of IL-21, and then activate Stat3 together so as to improve the key transcription factors RORγt and RORα, as well as the expression of IL-23-R on cell surface, since when can IL-23 promote the complete differentiation of T_H17 cells. What's more, IL-1β and TNF-α can also promote the differentiation. T_{reg} cells include nT_{reg} cells which are derived from the thymus, and iT_{reg} cells differentiation of which needs the activation of Stat5 by IL-2 and TGF-β1 at high concentration to up-regulate Foxp3 and IL-10 through Smad complex signal pathway, and then promote the differentiation of iT_{reg} cells secreting TGF-β1 and IL-10 collaboratively.

图1　小鼠$CD4^+$ T细胞分化主要亚型及相关因子

Fig.1　The major subtypes and key factors in differentiation of $CD4^+$ T cells in mouse

录因子持续高表达, 在不同的细胞因子作用下主要分化为T_H1、T_H2、T_H17和iT_{reg}等效应T细胞和调节T细胞, 继而产生促炎症和抗炎症细胞因子, 两类细胞因子的失衡很可能就是导致MS/EAE发病的主要原因之一。所以以初始$CD4^+$ T细胞为源头, 对其向四个亚型分化过程中关键转录因子和细胞因子进行研究可以推断各因子在疾病发展中的作用, 并间接了解各亚型在发病与恢复中所处地位, 也为治疗提供新的靶点。

2　初始$CD4^+$ T细胞向各细胞亚型分化过程中相关细胞因子与转录因子

2.1　初始$CD4^+$ T细胞→T_H1细胞

临床研究发现, 在MS患者外周血和损伤处均发现激活状态的T_H1细胞, 可以识别髓鞘类蛋白抗原表位, 并分泌IFN-γ和TNF-α[6], 因此T_H1细胞被首先认为参与MS/EAE炎症反应。初始$CD4^+$ T细胞在淋巴结中由树突状细胞分泌的IL-12作用下活化Stat4上调IFN-γ的表达量, IFN-γ通过活化Stat1上调转录因子T-bet, 进一步提高IFN-γ的转录表达, 形成IFN-γ正反馈环路, 促进T_H1细胞分化, 最终生成分泌IFN-γ和TNF-α的T_H1细胞。进入CNS后T_H1细胞被小胶质细胞再活化, 进而激活巨噬细胞, 促进TNF-α、NO、蛋白酶和氧自由基的释放及Fcγ受体介导的吞噬作用, 最终引起脱髓鞘[5,7-9]。

IFN-γ是T_H1细胞的标记性细胞因子, 能上调内皮细胞上MHC II类分子、黏附分子和促炎症细胞因子IL-12等, 对T细胞迁移进入CNS及炎症发生有促

进作用, 所以一直以来IFN-γ被认为是起始MS/EAE的必要条件。然而进一步研究发现, IFN-γ并不是EAE发病的必要因素, 且缺失IFN-γ会加重病情[10-12]。*IFN-γ*$^{-/-}$小鼠不仅能正常构建EAE模型, 而且恢复受阻, IFN受体缺失型小鼠中CD4$^+$ T细胞浸润扩张且MHC II类分子和促炎症细胞因子上调。目前认为, IFN-γ的作用可能更倾向于通过目前还未知的受体介导调控促炎症因子, 降低巨噬细胞活性, 减少NO的产生, 下调CNS中炎症反应[13-15]。

T-bet是IFN-γ强有力的转录因子, 在T细胞活化过程中能被IFN-γ诱导上调, IFN-γ通过一个自分泌反馈环控制T-bet的表达。尽管IFN-γ对T-bet的调节作用非常明显, 但调节并非专一, 如T-bet还受T_H2细胞相关因子IL-4、Stat6所抑制[16-18]。*T-bet*$^{-/-}$小鼠的CD4$^+$ T细胞不能分化成T_H1细胞, 在诱导EAE发病时, *T-bet*$^{-/-}$小鼠的CNS中出现少量炎性浸润, 但均呈现对EAE的抗性[19]。近年发现, T-bet可直接调控IL-23-R的表达, 促进T_H17细胞分化和稳定其表型[20]。此外, *T-bet*$^{-/-}$ CD4$^+$ T细胞中缺失关键黏附因子和趋化因子受体, 从而阻碍了CD4$^+$ T细胞向CNS迁移[21]。

IL-12的表达量在MS患者和EAE小鼠CNS中均高于正常个体, 与疾病严重程度呈正相关[22]。IL-12从多方面促进T_H1细胞分化, 一方面与IFN-γ一起活化转录因子T-bet, 另一方面激活Stat4蛋白直接增强*IFN-γ*基因的转录, 还能抑制T_H2特异性转录因子GATA3的转录, 使分化完全的T_H2细胞向T_H1转化[17,23]。IL-12处理后, EAE小鼠发病更快、更严重、发病期延长并较难恢复, 淋巴结细胞中IFN-γ和TNF-α明显上调[24]。

TNF-α和IFN-γ可协同上调IL-1和IL-6, 诱导巨噬细胞及其他APC表面MHC II分子的表达。TNF-α本身具有起始脱髓鞘、细胞凋亡和神经损伤的功能, 经抗TNF-α处理的小鼠可防止EAE发病, 且保护作用持续数月, 但处理过的小鼠发病后的进程和严重度与野生型小鼠发病无差别。所以, TNF-α对MS/EAE起始阶段有重要作用, 却并不为病情进一步发展或恢复所必要。此外, 一定浓度的TNF-α还会导致少突胶质细胞、星型细胞存活率下降, 从而影响发病。因此, 在发病过程中TNF-α可能为双向调控。目前推测, TNF-α的两个受体之一TNF-R1在促炎症和脱髓鞘中发挥作用, 而TNF-R2可抑制炎症, 甚至在髓鞘再生中起作用[25-28]。

2.2 初始CD4$^+$ T细胞→T_H2细胞

T_H2细胞因为分泌IL-4、IL-5和IL-13等抗炎性细胞因子而被认为在MS/EAE中起抑制作用。早期免疫反应中的产物IL-4通过上调细胞表面MHC II-抗原肽复合物和IL4受体的表达, 直接起始T_H2细胞的分化, *IL-4*$^{-/-}$小鼠CD4$^+$ T细胞不能分化产生T_H2细胞因子。IL-4可激活多重信号通路, 其中就包括招募和磷酸化转录因子Stat6, 从而调控IL-4基因表达活性, 形成IL-4的正反馈的环路, 并诱导重要转录因子GATA3的表达, 产生T_H2优势免疫应答。而*Stat6*$^{-/-}$小鼠在IL-4作用下仍不能分化成T_H2 细胞[29-31]。此外, IL-4和GATA3还可能通过抑制IL-12-Rβ2的表达阻止初始T细胞向T_H1分化[32-34]。

IL-4是重要的炎症调节因子, 抑制T_H1细胞的生成, 下调多种促炎症细胞因子。在IL-4作用下, CNS中不论静止状态还是活化状态的巨噬细胞都会成为替代性活化巨噬细胞, 这类巨噬细胞因金属蛋白酶的合成受抑制而缺乏NO, 有助于组织修复和病情恢复。EAE小鼠的自然恢复过程均伴有IL-4上调, CNS中缺失IL-4会导致小鼠EAE发病明显加重[35]。在诱导EAE发病早期使用IL-4可抑制CNS中IL-2和TNF-α的产生及小胶质细胞和巨噬细胞的增殖, 从而减轻脱髓鞘, 改善临床症状[26,36-37]。

转录因子GATA3是目前唯一确定的能调控IL-4、IL-5和IL-13等关键性T_H2细胞因子的特异性转录因子, 在MS/EAE发病起重要作用, 能上调抗炎性细胞因子的表达, 与蛋白Gfi-1作用, 特异性增殖T_H2细胞, 并抑制T_H1分化[38]。EAE小鼠在各个时期脑和脾中GATA3表达均低于对照组, 发病高峰期表达最低。T-bet通过抑制GATA3表达, 阻断GATA3与T_H2细胞因子间相互作用, 进而诱导已分化成熟的T_H2向T_H1逆转。GATA3在促进T_H2细胞发育的同时也抑制了T-bet的作用, T-bet和GATA3被认为共同决定着T_H细胞的分化方向, 所以, T-bet/GATA3的比值较单独的T-bet或GATA3更能灵敏反映EAE的病情变化[39-40]。此外, GATA3还具有在炎症环境下维持iT_{reg}细胞表型的作用[41]。

转录因子Stat6具有稳步上调IL-4、IL-5、IL-13和GATA3的作用。*Stat6*$^{-/-}$小鼠免疫后CNS中T_H1细胞占主导地位, 发病更严重[42]。敲除*Stat6*基因的SH2结构域不同区域构建的模型中, 有T_H2分化被促进并且对EAE诱导具有抗性的模型, 也有T_H2分化受损病

情加重的模型。*Stat6*基因的修饰还会影响T_{reg}细胞的功能, 进而影响MS/EAE发病[43], 所以*Stat6*基因修饰会是具有前景的研究方向。

2.3 初始CD4$^+$ T细胞→T_H17细胞

T_H17细胞作为CD4$^+$ T细胞亚型, 以分泌IL-17、IL-21和IL-22为特点, 细胞因子IL-6和TGF-β1的共表达诱导T_H17细胞的分化, 其中TGF-β1可通过抑制T-bet和GATA3表达, 阻断T_H1和T_H2细胞分化[44-45]。IL-6则通过活化Stat3上调IL-21, 并一起进一步活化Stat3上调RORγt、RORα及细胞表面IL-23-R的表达。由巨噬细胞和树突状细胞分泌的IL-23也通过活化Stat3发挥作用, 可能是T_H17细胞彻底分化和增殖所必须的细胞因子, IL-1β和TNF-α则发挥促进分化的作用[46-48]。而T_H1和T_H2分泌的细胞因子IFN-γ和IL-4则抑制其分化[5]。此外, 3个重要转录因子Stat3、RORγt和IRF4的正向调控对T_H17的分化有重要作用, *Stat3*$^{-/-}$、*RORγt*$^{-/-}$和*IRF4*$^{-/-}$小鼠都会导致T_H17细胞分化出现障碍[49]。研究证实T_H17细胞能破坏小鼠血脑屏障, 并对人类神经元细胞有强烈的细胞毒性作用[50]。MS患者脑组织急性病灶内IL-17表达均高于静止病灶、慢性病灶及正常组织, 且分离出的髓鞘反应性T细胞被证实为T_H17细胞[51-52]。随着T_H17细胞日益受到关注, 现在有观点认为, T_H1细胞最先穿过血脑屏障进入CNS, 从而易化对T_H17细胞的招募, T_H17和T_H1细胞可能都参与了致病过程, 并且T_H17细胞比T_H1细胞具有更好的增殖能力和更难以被抑制的优势[4,53]。

IL-6、IL-2l和IL-23均可活化JAK激酶, 选择性激活Stat3, 再依靠Stat3通路上调IL-6、IL-2l和IL-23-R[54]。Stat3缺陷会严重阻断T_H17细胞分化, 而过表达Stat3, 即使不加入IL-23或IL-6/TGF-β1仍可高表达IL-17, 并且上调RORγt。IL-17A和IL-17F基因启动子区域均存在Stat结合位点, 可与Stat3直接结合, 参与IL-17的转录调节。目前还不能确定Stat3是否直接调节RORγt表达, 但Stat3缺陷确实影响RORγt的表达, 使T_H17向T_H1细胞分化, 并导致T-bet和Foxp3表达上调[55-58]。EAE小鼠在急性发病期CNS中Stat3表达显著上调, 并在恢复期下调, 且*Stat3*$^{-/-}$小鼠发病症状轻微[59]。RRMS患者复发期外周血也发现磷酸化Stat3明显上调[60]。

T_H17细胞分泌IL-17A和L-17F。其中IL-17A在免疫反应中起重要作用, 主要通过上调其它促炎症细胞因子和趋化因子、招募中性白细胞、提高抗体产量并激活T细胞促使组织发生炎症反应[61]。在自身免疫疾病患者和动物模型中都发现IL-17A表达水平上升。与RRMS缓解期病患和其他的非炎症性神经系统疾病患相比, RRMS复发期患者脑脊液(cerebrospinal fluid, CSF)中分泌IL-17A的T_H17细胞的数量显著升高[53]。*IL-17*$^{-/-}$小鼠可明显抑制EAE发病, 表现为发病迟、评分低、组织学转好且提早恢复, *IL-17*$^{-/-}$ CD4$^+$ T细胞诱导EAE发病效果差, 抗IL-17处理则可以抑制EAE小鼠发病[62]。值得注意的是, *IFN-γ*$^{-/-}$细胞中产IL-17细胞增多, 而*IL-17*$^{-/-}$细胞中产IFN-γ细胞增多[63], 即IFN-γ与IL-17间可能存在相互调节机制。

RORγt是T_H17分化过程中特异性转录因子, 其地位类似T_H1和 T_H2细胞中的T-bet和GATA3。RORγt可高效起始IL-17A的转录, IL-6和TGF-β1上调IL-17的作用必须在RORγt条件下才能发挥。RORγt还可以不需要其他因子的情况下有效提高IL-17A受体表达。在EAE发病期间, *RORγt*$^{-/-}$小鼠脊髓中T_H17细胞减少, T_H17细胞因子和粘附因子表达显著下降[64-65]。Foxp3可直接与 RORγt第二外显子区作用, 下调IL-17A的表达[54]。而IL-6、IL-21和IL-23可以减少Foxp3对RORγt的抑制作用, 促进T_H17细胞的分化[66]。所以, RORγt和Foxp3表达的动态变化可以反映出T_H17细胞与iT_{reg}细胞的分化情况。

RORγt的缺失不会完全阻断T_H17细胞分化, 原因在于T_H17细胞同时还可在IL-6和TGF-β1激活Stat3信号通路后表达类似受体RORα。RORα促进T_H17分化的机制可能与IL-17A和IL-17F非编码保守序列有关。RORα缺失会导致IL-17表达量降低, 而RORα和RORγt共表达能协同促进T_H17分化。同时敲除*RORα*和*RORγt*则使T_H17分化受阻, 对EAE发病起绝对抑制作用, 由此奠定了RORα和RORγt在T_H17分化中的关键地位[67]。同样, Foxp3能与RORα作用, 下调RORα的转录活性, 抑制T_H17分化[68]。

关于IL-12的研究大多针对p40亚单位, 但IL-23结构上与IL-12相似, 且与IL-12共用p40亚单位, 所以之前的研究可能夸大了IL-12在EAE发病中的作用。通过基因敲除获得IL-23缺陷、IL-23和IL-12同时缺陷及IL-12缺陷小鼠模型, 免疫后发现前二者不发病, 用抗IL-23处理也可抑制多重炎症发生[69-70], 所以IL-23被认为是EAE发病的必要条件。临床也发现,

MS患者CSF中IL-23表达量明显高于正常人[71]。单独IL-23或IL-6在诱导T_H17细胞分化中作用极弱, 而二者协同作用可以显著促进T_H17细胞分化, 其中IL-6通过活化Stat3可使IL-23-R表达量上调120倍[56]。初始$CD4^+$ T细胞表面并不表达IL-23-R, 随着分化过程中IL-23-R的上调, IL-23才开始与受体结合活化Stat3, 进而上调T_H17细胞特征因子的表达, 维持其表型和促进其增殖。排除IFN-γ和 IL-4影响, IL-23可通过磷酸化Stat3, 使IL-17表达量提高10%~20%[55,72-73]。IL-23的缺失会导致T_H17细胞分化停滞于早期活化状态, 最终使T_H17细胞数量减少[65,74]。IL-6和TGF-β1共处理虽可以诱导T_H17分化, 但分化的细胞缺乏致炎性, 而IL-23条件下活化的髓鞘特异性T细胞能100%诱导发病, 所以TGF-β1和 IL-6主要负责起始T_H17细胞分化, 但促炎症特质的发挥还需要IL-23的介导[75-76]。近期还发现, IL-23和IL-22共表达可以通过抑制T-bet和Foxp3促进T_H17细胞分化, 并且IL-23对MOG特异性T细胞向CNS迁移也有重要作用[4,77]。

急性复发期 MS患者血和CSF中IL-6显著高于缓解期及其他类神经疾病。在EAE中情况相同, 病情加重前IL-6表达增高, 病情稳定后又迅速下降[26]。被动移植髓鞘特异性T_H17细胞会提高小鼠血清中IL-6的水平, 所以T_H17细胞分化存在IL-6正反馈环路, 其中IL-17可能是主要刺激源[78-79]。初始$CD4^+$ T细胞中IL-6并不为RORγt表达所需, 单独TGF-β1即可上调RORγt。所以, *IL-6*$^{-/-}$小鼠发病受抑制的原因可能在于IL-6的缺乏导致对Foxp3的抑制被解除, 进而影响了T_H17细胞分化[48,64,80]。此外, IL-6还具有抑制IL-1β和TNF-α的抗炎作用, IL-6治疗可显著缓解EAE小鼠脱髓鞘和CNS中的炎症反应, 可能与IL-6作用于B细胞分化, 产生中和抗体有关[81]。

在正常CNS组织中几乎没有IL-1β的表达, 而在MS患者中IL-1β有较高表达, 可以上调内皮细胞黏附分子, 促进活化的白细胞穿过血脑屏障。并且IL-1β相关易感性呈家族聚集趋势, IL-1β与其天然拮抗剂IL-1-Ra相对表达量比值高的家族更容易出现RRMS患者[82-84]。实验证实, IL-1-Ra处理可以延缓EAE小鼠发病、减轻症状[85-86], 目前相对有效缓解MS/EAE病情的药物IFN-β和醋酸格拉默也是通过上调的IL-1-Ra控制IL-1β表达量[87]。所以, IL-1β可能是导致MS/EAE发病或加速发病的原因之一。在人类T_H17分化中, 无需RORC上调, 单独IL-1β即可诱导分化, 但其促分化作用需通过上调IL-23, 一旦阻断IL-23, IL-1β作用消失[88-89]。但也有研究发现IL-1β能上调Foxp3, 促进iT_{reg}细胞分化[90]。

IL-21为T_H17细胞特异性高表达, 能抑制IFN-γ表达和T_H1分化[91-92]。与TGF-β1共处理可以消除*IL-6*$^{-/-}$小鼠因为外周$Foxp3^+$ iT_{reg}细胞占主导地位对T_H17分化产生的抑制作用。在*IL-21-R*$^{-/-}$T细胞中T_H17细胞分化功能受损。*IL-21*$^{-/-}$小鼠中也出现T_H17分化和EAE发病受阻的现象。在诱导EAE小鼠发病前注射IL-21, 则加重CNS的炎症反应[80,93-94]。与IL-6类似, IL-21的表达必须依靠Stat3直接结合于*IL-21*启动子区进行调控, 通过自分泌途径扩增放大T_H17细胞分化[76]。在野生型小鼠中, 阻断IL-21并不会完全阻断T_H17细胞分化, 其原因可能在于IL-6的存在。在TGFβ-1缺失的情况下, 与IL-6一样, 单独IL-21处理不能诱导T_H17分化[95]。所以IL-21和IL-6在T_H17分化过程中可能具有类似作用且相互补偿, 并且IL-6对初始$CD4^+$ T细胞中IL-21的表达是必需的[96]。

干扰素调节因子IRF4在T_H2细胞分化中可诱导转录因子GATA3的表达, 被认为是T_H2细胞分化中重要的转录因子。但研究发现, *IRF4*$^{-/-}$小鼠对EAE发病的抑制作用比*RORγt*$^{-/-}$小鼠更彻底, 存在显著的T_H17分化障碍, 并伴随IL-6的下调。由于缺乏IL-6的抑制作用, 在T_H17分化环境下Foxp3表达量剧增, 而RORγt和RORα表达量降低, 逆转了T_H17与iT_{reg}细胞比例, 抑制了发病。将正常$CD4^+$ T细胞移植到*IRF4*$^{-/-}$小鼠可恢复对EAE的敏感性, 无严重程度差别。但在*IRF4*$^{-/-}$ T细胞中过表达RORγt和RORα只能部分恢复T_H17的分化能力, 说明IRF4的缺失还通过其他途径对T_H17的分化造成影响[97]。另有研究认为, IRF4的缺失引起IL-6表达下调, 阻碍了IL-6对IL-21表达的诱导, 抑制了IL-21的自分泌环路, 降低了IL-17和IL-23-R的表达, 从而影响了T_H17细胞的表型稳定[98]。

T_H17细胞也特异性分泌IL-22[99], 在MS患者损伤处的BBB内皮细胞高表达IL-22-R和IL-17-R, IL-22和IL-17可能通过与受体结合破坏BBB内皮细胞间紧密连接, 易化T_H17细胞穿过BBB引发CNS中的炎症反应[50]。与IL-17类似, 在鼠源和人源初始$CD4^+$ T细胞中IL-22的表达都可以被IL-23或IL-6上调。经IL-23处理, IL-22的表达量提高较IL-17更明显。TGF-β1虽然可以上调IL-17, 却抑制IL-22的表

达。并且与IL-17不同, IL-22并不参与$CD4^+$ T细胞的分化[100]。早期IL-22因能导致银屑病而被认为有促炎症作用, 但*IL-22*$^{-/-}$小鼠对EAE的易感性并没有改变, 发病严重程度与野生型无差别, 不过还不能排除IL-22可能作为IL-17的补偿作用[101-102]。虽然IL-22的表达与RORγt和RORα呈正相关[93,103], 但IL-22表达是否直接受RORγt和RORα的调控还未知。所以IL-22在MS/EAE的发病中的作用还值得进一步研究。

2.4 初始$CD4^+$ T细胞→$CD4^+$ $CD25^+$ $Foxp3^+$ iT_{reg}细胞

调节型T细胞(T_{reg})具有免疫调节功能, 在维持外周免疫耐受、预防自身免疫性疾病的发生中起着重要作用。$CD4^+$ $CD25^+$ T_{reg}细胞占胸腺、外周血液和淋巴样组织总$CD4^+$ T细胞1%~10%[104], 缺失T_{reg}细胞会导致实验动物出现自发性自身免疫疾病[105]。$CD4^+$ $CD25^+$ T_{reg}细胞以细胞表面表达CD25和转录因子Foxp3为特点, 在外周通过与APC和效应T细胞直接接触或分泌抗炎性细胞因子IL-10和TGF-β1等控制炎症反应。可分为胸腺自然选择过程中产生的自然型$CD4^+$ $CD25^+$ T_{reg}细胞(nT_{reg})细胞和由外周初始$CD4^+$ T细胞分化而来的适应型$CD4^+$ $CD25^+$ T_{reg}细胞(iT_{reg})。iT_{reg}细胞的分化需要TGF-β1和IL-2, nT_{reg}细胞在二者作用下, 可诱导大量初始$CD4^+$ T细胞转变为$CD4^+$ $CD25^+$ $Foxp3^+$细胞。其中, IL-2活化Stat5上调Foxp3, TGF-β1通过Smad复合物信号通路上调Foxp3和IL-10, 共同促进iT_{reg}细胞分化, 形成TGF-β1正反馈环路。人源iT_{reg}细胞中活化Stat5可上调IL-10的表达[106-111]。虽然iT_{reg}自身会分泌IL-10, 但由于分化早期IL-10分泌量少, 所以通过旁分泌的形式维持iT_{reg}细胞Foxp3的表达, 发挥iT_{reg}的免疫调节作用[4,106,112]。用iT_{reg}细胞对小鼠治疗能明显减缓脑脊髓炎, 加速CNS的修复[113], 在恢复–复发型EAE小鼠中, 不同品系小鼠的易感性均与特异性iT_{reg}细胞分布频率呈负相关[114-115]。在MS病人的CSF中iT_{reg}细胞显著增多, 虽然外周血液中iT_{reg}细胞数量无明显变化, 但抑制功能下降[116]。

转录因子Foxp3是iT_{reg}细胞的特异性标志, 能上调iT_{reg}相关表面分子如CD25、CTLA-4和GITR等的表达, 抑制IL-2、IL-4和IFN-γ的产生, 促进iT_{reg}细胞分化并发挥免疫调节作用。*Foxp3*基因的突变或缺失都会阻断iT_{reg}细胞分化并导致致命性自身免疫和炎症性疾病发生, 移植iT_{reg}细胞可以弥补新生*Foxp3*$^{-/-}$小鼠免疫缺陷[117]。Foxp3不仅促进iT_{reg}细胞的分化, 对稳定其表型和调节作用的持续发挥有重要意义, 将Foxp3在初始$CD4^+$ T细胞或效应T细胞中过表达, 可以使其获得iT_{reg}的表型并降低其它T细胞亚型的增殖。对成熟iT_{reg}细胞诱导敲除Foxp3, 会导致其抑制功能的丧失, 并转向分泌T_H1细胞因子[118-119]。所以, MS患者外周的Foxp3表达水平降低, 可能是外周iT_{reg}细胞抑制功能下降的直接原因[120]。但也有研究表明, 随着iT_{reg}细胞Foxp3表达量下降, 细胞会倾向于转化为T_H2细胞, 但T_H2调节作用不明显[121]。

IL-10最主要的功能是限制和终止炎症反应, 可激活Stat3和Stat5信号通路, Stat3的活化进一步上调IL-10的表达, 放大其抑制作用, 下调IL-1β、IL-2、IL-6、IL-12、IFN-γ和TNF-α等一些主要的促炎症因子表达。激活Stat5则有利于$Foxp3^+$ T淋巴细胞的表型稳定和增殖, 降低T_H17细胞RORγt和IL-17的表达, 从而抑制T_H17细胞分化[122-125]。在EAE小鼠模型中, IL-10的两个表达峰值分别出现于发病高峰和恢复期, *IL-10*$^{-/-}$小鼠EAE易感性提高且发病更重, 对应的$CD4^+$ T细胞具有强致病性[113,126-128]。将IL-10直接作用于CNS可以完全阻断EAE小鼠发病, 但有时间、空间及剂量限制[129-130]。所以IL-10会是非常具有前景的治疗靶点。

TGF-β1具有免疫调节功能, 如抑制IFN-γ、TNF-α、IL-6表达, 拮抗IFN-γ、TNF-α诱导的MHC II表达, 抑制巨噬细胞活化, 直接下调T_H1细胞的活性并抑制其增殖等[131]。T_H17细胞分化中, 低浓度TGF-β1与IL-6或IL-21共同提高IL-23-R表达量。而高浓度TGF-β1则抑制IL23-R的表达, 转向促进$Foxp3^+$ iT_{reg}细胞分化[64]。MS患者CSF中TGF-β1表达水平较其他CNS免疫炎性疾病及正常组高, 血及CSF中表达TGF-β1的细胞增多程度与MS严重度负相关。缺乏TGF-β1会导致EAE病情加重且不能恢复[26,132]。*TGF-β1*$^{-/-}$小鼠外周iT_{reg}细胞显著减少, 并伴随Foxp3表达量的下调和抑制作用的减小[133]。所以, 虽然还不能确定TGF-β1是否起始iT_{reg}细胞分化, 但在稳定Foxp3表达量中TGF-β1具有重要作用。此外, TGF-β1还可以诱导小胶质细胞分泌肝细胞生长因子, 从而促进脱髓鞘损伤恢复[134]。

3 小结

综上所述, 从初始$CD4^+$ T细胞分化为四大主要

亚型涉及到许多细胞因子和转录因子。在MS/EAE中这些细胞因子和转录因子在不同组织器官和发病阶段中不仅会呈现各自特征表达, 而且存在相互作用, 构成免疫网络体系。深入研究相关细胞因子和转录因子, 一方面有助于疾病机制的揭示, 另一方面为临床治疗提供药物靶点, 发展新的免疫治疗方案。

参考文献 (References)

1 Compston A, Coles A. Multiple sclerosis. Lancet 2008; 372 (9648): 1502-17.

2 Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: An overview. Brain Pathol 2007; 17(2): 210-8.

3 Steinman L. Assessment of animal models for MS and demyelinating disease in the design of rational therapy. Neuron 1999; 24(3): 511-4.

4 Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. Clin Exp Immunol 2010; 162(1): 1-11.

5 Boppana S, Huang H, Ito K, Dhib-Jalbut S. Immunologic aspects of multiple sclerosis. Mt Sinai J Med 2011; 78(2): 207-20.

6 Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culpepper JA, *et al*. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. J Immunol 1995; 154(10): 5071-9.

7 Billiau A. Interferons in multiple sclerosis: Warnings from experiences. Neurology 1995; 45(6 Suppl 6): S50-3.

8 Elenkov IJ, Chrousos GP. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. Ann N Y Acad Sci 2002; 966: 290-303.

9 Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: Are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta 2011; 1812(2): 246-51.

10 Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, *et al*. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J Immunol 1996; 156(1): 5-7.

11 Krakowski M, Owens T. Interferon-gamma confers resistance to experimental allergic encephalomyelitis. Eur J Immunol 1996; 26(7): 1641-6.

12 Willenborg DO, Fordham S, Bernard CC, Cowden WB, Ramshaw IA. IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. J Immunol 1996; 157(8): 3223-7.

13 Willenborg DO, Fordham SA, Staykova MA, Ramshaw IA, Cowden WB. IFN-gamma is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: A possible role for nitric oxide. J Immunol 1999; 163(10): 5278-86.

14 Tran EH, Prince EN, Owens T. IFN-gamma shapes immune invasion of the central nervous system via regulation of chemokines. J Immunol 2000; 164(5): 2759-68.

15 Espejo C, Penkowa M, Saez-Torres I, Xaus J, Celada A, Montalban X, *et al*. Treatment with anti-interferon-gamma monoclonal antibodies modifies experimental autoimmune encephalomyelitis in interferon-gamma receptor knockout mice. Exp Neurol 2001; 172(2): 460-8.

16 Yeh WI, McWilliams IL, Harrington LE. Autoreactive Tbet-positive CD4 T cells develop independent of classic Th1 cytokine signaling during experimental autoimmune encephalomyelitis. J Immunol 2011; 187(10): 4998-5006.

17 Smits HH, van Rietschoten JG, Hilkens CM, Sayilir R, Stiekema F, Kapsenberg ML, *et al*. IL-12-induced reversal of human Th2 cells is accompanied by full restoration of IL-12 responsiveness and loss of GATA-3 expression. Eur J Immunol 2001; 31(4): 1055-65.

18 Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, *et al*. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. Nat Immunol 2002; 3(6): 549-57.

19 Nath N, Prasad R, Giri S, Singh AK, Singh I. T-bet is essential for the progression of experimental autoimmune encephalomyelitis. Immunology 2006; 118(3): 384-91.

20 Gocke AR, Cravens PD, Ben LH, Hussain RZ, Northrop SC, Racke MK, *et al*. T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. J Immunol 2007; 178(3): 1341-8.

21 Mathur AN, Chang HC, Zisoulis DG, Kapur R, Belladonna ML, Kansas GS, *et al*. T-bet is a critical determinant in the instability of the IL-17-secreting T-helper phenotype. Blood 2006; 108(5): 1595-601.

22 梁军利, 唐玉兰. IL-12家族与多发性硬化的研究进展. 国际免疫学杂志(Liang Junli, Tang Yulan. Research progress of IL-2 families and multiple sclerosis. Int J Immunol) 2010; 33(1): 4.

23 李　峻, 周永明, 胡明辉. T-bet、GATA-3在Th1、Th2细胞分化中的作用及意义. 国际免疫学杂志(Li Jun, Zhou Yongming, Hu Minghui. The effects of T-bet/GATA-3 on the differentiation of Th1/Th2 cell and their clinical significances. Int J Immunol) 2009; 32(2): 6.

24 Leonard JP, Waldburger KE, Goldman SJ. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J Exp Med 1995; 181(1): 381-6.

25 Selmaj K, Raine CS, Cross AH. Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann Neurol 1991; 30(5): 694-700.

26 靳雁斌, 颜光涛. 细胞因子与多发性硬化病的关系. 标记免疫分析与临床(Jin Yanbin, Yan Guangtao. Labeled Immunoassays Clin Med) 2002; 9(3): 4.

27 Kassiotis G, Pasparakis M, Kollias G, Probert L. TNF accelerates the onset but does not alter the incidence and severity of myelin basic protein-induced experimental autoimmune encephalomyelitis. Eur J Immunol 1999; 29(3): 774-80.

28 Kassiotis G, Kollias G. Uncoupling the proinflammatory from the immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor level: Implications for pathogenesis and therapy of autoimmune demyelination. J Exp Med 2001; 193(4): 427-34.

29 Kotanides H, Reich NC. Interleukin-4-induced STAT6 recognizes and activates a target site in the promoter of the interleukin-4 receptor gene. J Biol Chem 1996; 271(41): 25555-61.

30 Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. J Immunol 1990; 145(11): 3796-806.

31 Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature 1993; 362(6417): 245-8.

32 Zhou M, Ouyang W. The function role of GATA-3 in Th1 and Th2 differentiation. Immunol Res 2003; 28(1): 25-37.

33 Kurata H, Lee HJ, O'Garra A, Arai N. Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity 1999; 11(6): 677-88.

34 O'Garra A, Arai N. The molecular basis of T helper 1 and T helper 2 cell differentiation. Trends Cell Biol 2000; 10(12): 542-50.

35 Ponomarev ED, Maresz K, Tan Y, Dittel BN. CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. J Neurosci 2007; 27(40): 10714-21.

36 Furlan R, Poliani PL, Marconi PC, Bergami A, Ruffini F, Adorini L, *et al*. Central nervous system gene therapy with interleukin-4 inhibits progression of ongoing relapsing-remitting autoimmune encephalomyelitis in Biozzi AB/H mice. Gene Ther 2001; 8(1): 13-9.

37 郭正良, 陈生弟. 细胞因子与多发性硬化的关系. 神经病学与神经康复学杂志(Guo Zhengliang, Chen Shengdi. Journal of Neurology and Neurorehabilitation) 2005; 2(2): 113-5.

38 Zhu J, Yamane H, Cote-Sierra J, Guo L, Paul WE. GATA-3 promotes Th2 responses through three different mechanisms: Induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. Cell Res 2006; 16(1): 3-10.

39 Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science 2005; 307(5708): 430-3.

40 吴 敏. 慢性EAE模型T-bet/GATA-3的平衡失调及TGF-β1对其调节的相关研究. 中南大学(Wu Min. The change of T-bet/GATA-3 ratio in chronic sustained model of experimental autoimmune encephalomyelitis and the regulation of exogenous TGF-β1. Central South University) 2007.

41 Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, Ribeiro CH, *et al*. GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. J Clin Invest 2011; 121(11): 4503-15.

42 Chitnis T, Najafian N, Benou C, Salama AD, Grusby MJ, Sayegh MH, *et al*. Effect of targeted disruption of STAT4 and STAT6 on the induction of experimental autoimmune encephalomyelitis. J Clin Invest 2001; 108(5): 739-47.

43 Wang Y, Evans JT, Rodriguez F, Fields P, Mueller C, Chitnis T, *et al*. A tale of two STAT6 knock out mice in the induction of experimental autoimmune encephalomyelitis. J Neuroimmunol 2009; 206(1/2): 76-85.

44 Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. J Immunol 2000; 165(9): 4773-7.

45 Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. J Exp Med 2002; 195(11): 1499-505.

46 Jetten AM. Retinoid-related orphan receptors (RORs): Critical roles in development, immunity, circadian rhythm, and cellular metabolism. Nucl Recept Signal 2009; 7: e003.

47 Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006; 24(2): 179-89.

48 Ogura H, Murakami M, Okuyama Y, Tsuruoka M, Kitabayashi C, Kanamoto M, *et al*. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. Immunity 2008; 29(4): 628-36.

49 Ghilardi N, Ouyang W. Targeting the development and effector functions of TH17 cells. Semin Immunol 2007; 19(6): 383-93.

50 Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, *et al*. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med 2007; 13(10): 1173-5.

51 Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, *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(1): 146-55.

52 Montes M, Zhang X, Berthelot L, Laplaud DA, Brouard S, Jin J, *et al*. Oligoclonal myelin-reactive T-cell infiltrates derived from multiple sclerosis lesions are enriched in Th17 cells. Clin Immunol 2009; 130(2): 133-44.

53 Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthausen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. Brain 2009; 132(Pt 12): 3329-41.

54 Chen Z, Laurence A, O'Shea JJ. Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. Semin Immunol 2007; 19(6): 400-8.

55 Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu BM, Tato C, *et al*. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci USA 2006; 103(21): 8137-42.

56 Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, *et al*. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem 2007; 282(13): 9358-63.

57 Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, *et al*. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol 2007; 178(8): 4901-7.

58 Harris TJ, Grosso JF, Yen HR, Xin H, Kortylewski M, Albesiano E, *et al*. Cutting edge: An *in vivo* requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. J Immunol 2007; 179(7): 4313-7.

59 Maier J, Kincaid C, Pagenstecher A, Campbell IL. Regulation of signal transducer and activator of transcription and suppressor of cytokine-signaling gene expression in the brain of mice with astrocyte-targeted production of interleukin-12 or experimental autoimmune encephalomyelitis. Am J Pathol 2002; 160(1): 271-88.

60 Frisullo G, Angelucci F, Caggiula M, Nociti V, Iorio R, Patanella AK, *et al*. pSTAT1, pSTAT3, and T-bet expression in peripheral blood mononuclear cells from relapsing-remitting multiple sclerosis patients correlates with disease activity. J Neurosci Res 2006; 84(5): 1027-36.

61 Iwakura Y, Nakae S, Saijo S, Ishigame H. The roles of IL-17A in inflammatory immune responses and host defense against patho-

gens. Immunol Rev 2008; 226: 57-79.
62 Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, *et al*. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005; 201(2): 233-40.
63 Komiyama Y, Nakae S, Matsuki T, Nambu A, Ishigame H, Kakuta S, *et al*. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. J Immunol 2006; 177(1): 566-73.
64 Ichiyama K, Yoshida H, Wakabayashi Y, Chinen T, Saeki K, Nakaya M, *et al*. Foxp3 inhibits RORgammat-mediated IL-17A mRNA transcription through direct interaction with RORgammat. J Biol Chem 2008; 283(25): 17003-8.
65 Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, *et al*. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006; 126(6): 1121-33.
66 Zhou L, Lopes JE, Chong MM, Ivanov, II, Min R, Victora GD, *et al*. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature 2008; 453(7192): 236-40.
67 Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, *et al*. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity 2008; 28(1): 29-39.
68 Du J, Huang C, Zhou B, Ziegler SF. Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3. J Immunol 2008; 180(7): 4785-92.
69 Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, *et al*. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003; 421(6924): 744-8.
70 Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, *et al*. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J Clin Invest 2006; 116(5): 1317-26.
71 Vaknin-Dembinsky A, Balashov K, Weiner HL. IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligos increases dendritic cell IL-10 production. J Immunol 2006; 176(12): 7768-74.
72 Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, *et al*. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; 6(11): 1123-32.
73 Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, *et al*. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005; 6(11): 1133-41.
74 McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, *et al*. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells *in vivo*. Nat Immunol 2009; 10(3): 314-24.
75 McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, *et al*. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol 2007; 8(12): 1390-7.
76 Zhou L, Ivanov, II, Spolski R, Min R, Shenderov K, Egawa T, *et al.* IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 2007; 8(9): 967-74.
77 Mus AM, Cornelissen F, Asmawidjaja PS, van Hamburg JP, Boon L, Hendriks RW, *et al*. Interleukin-23 promotes Th17 differentiation by inhibiting T-bet and FoxP3 and is required for elevation of interleukin-22, but not interleukin-21, in autoimmune experimental arthritis. Arthritis Rheum 2010; 62(4): 1043-50.
78 Ivanov II, Zhou L, Littman DR. Transcriptional regulation of Th17 cell differentiation. Semin Immunol 2007; 19(6): 409-17.
79 Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, *et al*. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity 1995; 3(6): 811-21.
80 Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, *et al*. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 2007; 448(7152): 484-7.
81 Rodriguez M, Pavelko KD, McKinney CW, Leibowitz JL. Recombinant human IL-6 suppresses demyelination in a viral model of multiple sclerosis. J Immunol 1994; 153(8): 3811-21.
82 Dhib-Jalbut S, Jiang H, Williams GJ. The effect of interferon beta-1b on lymphocyte-endothelial cell adhesion. J Neuroimmunol 1996; 71(1/2): 215-22.
83 de Jong BA, Huizinga TW, Bollen EL, Uitdehaag BM, Bosma GP, van Buchem MA, *et al*. Production of IL-1beta and IL-1Ra as risk factors for susceptibility and progression of relapse-onset multiple sclerosis. J Neuroimmunol 2002; 126(1/2): 172-9.
84 Cannella B, Raine CS. The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol 1995; 37(4): 424-35.
85 Badovinac V, Mostarica-Stojkovic M, Dinarello CA, Stosic-Grujicic S. Interleukin-1 receptor antagonist suppresses experimental autoimmune encephalomyelitis (EAE) in rats by influencing the activation and proliferation of encephalitogenic cells. J Neuroimmunol 1998; 85(1): 87-95.
86 Furlan R, Bergami A, Brambilla E, Butti E, de Simoni MG, Campagnoli M, *et al*. HSV-1-mediated IL-1 receptor antagonist gene therapy ameliorates MOG(35-55)-induced experimental autoimmune encephalomyelitis in C57BL/6 mice. Gene Ther 2007; 14(1): 93-8.
87 Carpintero R, Burger D. IFNbeta and glatiramer acetate trigger different signaling pathways to regulate the IL-1 system in multiple sclerosis. Commun Integr Biol 2011; 4(1): 112-4.
88 Hebel K, Rudolph M, Kosak B, Chang HD, Butzmann J, Brunner-Weinzierl MC. IL-1beta and TGF-beta act antagonistically in induction and differentially in propagation of human proinflammatory precursor CD4+ T cells. J Immunol 2011; 187(11): 5627-35.
89 Cho ML, Kang JW, Moon YM, Nam HJ, Jhun JY, Heo SB, *et al*. STAT3 and NF-kappaB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice. J Immunol 2006; 176(9): 5652-61.
90 Ganesh BB, Bhattacharya P, Gopisetty A, Sheng J, Vasu C, Prabhakar BS. IL-1beta promotes TGF-beta1 and IL-2 dependent Foxp3 expression in regulatory T cells. PLoS One 2011; 6(7): e21949.
91 Kovanen PE, Leonard WJ. Cytokines and immunodeficiency

diseases: Critical roles of the gamma(c)-dependent cytokines interleukins 2, 4, 7, 9, 15, and 21, and their signaling pathways. Immunol Rev 2004; 202: 67-83.

92 Suto A, Wurster AL, Reiner SL, Grusby MJ. IL-21 inhibits IFN-gamma production in developing Th1 cells through the repression of Eomesodermin expression. J Immunol 2006; 177(6): 3721-7.

93 Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, *et al.* Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 2007; 448(7152): 480-3.

94 Vollmer TL, Liu R, Price M, Rhodes S, La Cava A, Shi FD. Differential effects of IL-21 during initiation and progression of autoimmunity against neuroantigen. J Immunol 2005; 174(5): 2696-701.

95 Wei L, Laurence A, Elias KM, O'Shea JJ. IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. J Biol Chem 2007; 282(48): 34605-10.

96 Dienz O, Eaton SM, Bond JP, Neveu W, Moquin D, Noubade R, *et al*. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. J Exp Med 2009; 206(1): 69-78.

97 Brustle A, Heink S, Huber M, Rosenplanter C, Stadelmann C, Yu P, *et al*. The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. Nat Immunol 2007; 8(9): 958-66.

98 Huber M, Brustle A, Reinhard K, Guralnik A, Walter G, Mahiny A, *et al*. IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. Proc Natl Acad Sci USA 2008; 105(52): 20846-51.

99 Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, *et al*. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med 2006; 203(10): 2271-9.

100 Chung Y, Yang X, Chang SH, Ma L, Tian Q, Dong C. Expression and regulation of IL-22 in the IL-17-producing CD4+ T lymphocytes. Cell Res 2006; 16(11): 902-7.

101 Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, *et al*. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol 2007; 179(12): 8098-104.

102 Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, *et al*. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007; 445(7128): 648-51.

103 Pols TW, Bonta PI, de Vries CJ. NR4A nuclear orphan receptors: Protective in vascular disease? Curr Opin Lipidol 2007; 18(5): 515-20.

104 Baecher-Allan C, Viglietta V, Hafler DA. Human CD4+CD25+ regulatory T cells. Semin Immunol 2004; 16(2): 89-98.

105 Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004; 22: 531-62.

106 Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. J Immunol 2004; 172(9): 5213-21.

107 Horwitz DA, Zheng SG, Gray JD. Natural and TGF-beta-induced Foxp3(+)CD4(+) CD25(+) regulatory T cells are not mirror images of each other. Trends Immunol 2008; 29(9): 429-35.

108 Pyzik M, Piccirillo CA. TGF-beta1 modulates Foxp3 expression and regulatory activity in distinct CD4+ T cell subsets. J Leukoc Biol 2007; 82(2): 335-46.

109 Tsuji-Takayama K, Suzuki M, Yamamoto M, Harashima A, Okochi A, Otani T, *et al*. The production of IL-10 by human regulatory T cells is enhanced by IL-2 through a STAT5-responsive intronic enhancer in the IL-10 locus. J Immunol 2008; 181(6): 3897-905.

110 Zhang L, Zhao Y. The regulation of Foxp3 expression in regulatory CD4(+)CD25(+)T cells: Multiple pathways on the road. J Cell Physiol 2007; 211(3): 590-7.

111 Toda A, Piccirillo CA. Development and function of naturally occurring CD4+CD25+ regulatory T cells. J Leukoc Biol 2006; 80(3): 458-70.

112 Murai M, Turovskaya O, Kim G, Madan R, Karp CL, Cheroutre H, *et al*. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. Nat Immunol 2009; 10(11): 1178-84.

113 McGeachy MJ, Stephens LA, Anderton SM. Natural recovery and protection from autoimmune encephalomyelitis: Contribution of CD4+CD25+ regulatory cells within the central nervous system. J Immunol 2005; 175(5): 3025-32.

114 Gartner D, Hoff H, Gimsa U, Burmester GR, Brunner-Weinzierl MC. CD25 regulatory T cells determine secondary but not primary remission in EAE: Impact on long-term disease progression. J Neuroimmunol 2006; 172(1/2): 73-84.

115 Reddy J, Illes Z, Zhang X, Encinas J, Pyrdol J, Nicholson L, *et al*. Myelin proteolipid protein-specific CD4+CD25+ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. Proc Natl Acad Sci USA 2004; 101(43): 15434-9.

116 Feger U, Luther C, Poeschel S, Melms A, Tolosa E, Wiendl H. Increased frequency of CD4+ CD25+ regulatory T cells in the cerebrospinal fluid but not in the blood of multiple sclerosis patients. Clin Exp Immunol 2007; 147(3): 412-8.

117 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; 4(4): 330-6.

118 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299(5609): 1057-61.

119 Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol 2007; 8(3): 277-84.

120 Huan J, Culbertson N, Spencer L, Bartholomew R, Burrows GG, Chou YK, *et al*. Decreased FOXP3 levels in multiple sclerosis patients. J Neurosci Res 2005; 81(1): 45-52.

121 Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature 2007; 445(7129): 766-70.

122 Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, *et al*. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity 2011; 34(4): 566-78.

123 Yao Z, Kanno Y, Kerenyi M, Stephens G, Durant L, Watford WT, *et al*. Nonredundant roles for Stat5a/b in directly regulating

Foxp3. Blood 2007; 109(10): 4368-75.
124 Heo YJ, Joo YB, Oh HJ, Park MK, Heo YM, Cho ML, *et al*. IL-10 suppresses Th17 cells and promotes regulatory T cells in the CD4+ T cell population of rheumatoid arthritis patients. Immunol Lett 2010; 127(2): 150-6.
125 Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001; 19: 683-765.
126 Bettelli E, Das MP, Howard ED, Weiner HL, Sobel RA, Kuchroo VK. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. J Immunol 1998; 161(7): 3299-306.
127 Kennedy MK, Torrance DS, Picha KS, Mohler KM. Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. J Immunol 1992; 149(7): 2496-505.
128 Jander S, Pohl J, D'Urso D, Gillen C, Stoll G. Time course and cellular localization of interleukin-10 mRNA and protein expression in autoimmune inflammation of the rat central nervous system. Am J Pathol 1998; 152(4): 975-82.
129 Cua DJ, Groux H, Hinton DR, Stohlman SA, Coffman RL. Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis. J Exp Med 1999; 189(6): 1005-10.
130 Cua DJ, Hutchins B, LaFace DM, Stohlman SA, Coffman RL. Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. J Immunol 2001; 166(1): 602-8.
131 Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998; 16: 137-61.
132 Zhang X, Reddy J, Ochi H, Frenkel D, Kuchroo VK, Weiner HL. Recovery from experimental allergic encephalomyelitis is TGF-beta dependent and associated with increases in CD4+LAP+ and CD4+CD25+ T cells. Int Immunol 2006; 18(4): 495-503.
133 Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. J Exp Med 2005; 201(7): 1061-7.
134 Lalive PH, Paglinawan R, Biollaz G, Kappos EA, Leone DP, Malipiero U, *et al*. TGF-beta-treated microglia induce oligodendrocyte precursor cell chemotaxis through the HGF-c-Met pathway. Eur J Immunol 2005; 35(3): 727-37.

Chemokines and Transcription Factors in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

Cai Yingying, Du Changsheng*

(*Laboratory of Receptor-based Bio-medicine, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China*)

Abstract Multiple sclerosis (MS) is a demyelinating inflammatory disorder of the central nervous system. The experimental autoimmune encephalomyelitis (EAE) shares clinical and pathological features with MS and is widely used as the animal model for MS. The Pathogenesis of MS is still unknown, but it is widely accepted that MS is a $CD4^+$ T cell-mediated autoimmune disease of the central nervous system which is based on susceptibility genes and triggered by environmental factors. Upon T-cell receptor (TCR)-mediated cell activation, naive $CD4^+$ T cells can differentiate into at least four major lineages, T_H1, T_H2, T_H17 and iT_{reg} cells, which participate in different types of immune responses. Networks of cytokines and transcription factors are critical for $CD4^+$ T cell differentiation and effector cytokine production. This article will review the collaboration and cross-regulation between various essential cytokines and transcription factors during the process of $CD4^+$ T cell differentiation towards distinct lineages, as well as in the process of MS/EAE. Understanding the roles of key cytokines and transcription factors in these processes will help to understand disease pathogenesis and supply indications for disease therapy.

Key words multiple sclerosis; experimental autoimmune encephalomyelitis; cytokine; transcription factor

Received: March 23, 2012 Accepted: May 25, 2012

This work was supported by the National Natural Science Foundation of China (No.31000399, No.31171348) and the National Basic Research Program of China (973 Program) (No.2012CB910404)

*Corresponding author. Tel/Fax: 86-21-65986852, E-mail: duchangsheng@gmail.com