T细胞亚群参与白塞病炎症应答和治疗靶向的研究进展

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【摘要】 白塞病(Behcet's disease,BD)是一种系统性炎症性疾病,临床上以口腔、外阴溃疡和葡萄膜炎为主要表现。病理表现为累及各种血管管径的血管炎,有血栓倾向。BD发病机制不明,除环境和遗传因素外,T细胞免疫应答异常也参与发病,涉及固有免疫的γδ T细胞、特性抗原的识别、抗原呈递及以CD4+、CD8+ T细胞为代表的适应性免疫。T细胞稳态失衡主要表现为Th1、Th17辅助T细胞的活化、增殖和Treg细胞损伤。上述免疫应答异常诱导和维持BD的促炎环境。本文围绕T细胞亚群参与BD的免疫应答特征、T细胞亚群参与免疫介导炎症反应以及治疗的转化和未来的靶向进行综述。

【关键词】 γδ T细胞; CD4+ T细胞; CD8+ T细胞; 白塞病(BD)
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Research progress in the roles of T cell subsets in immune-responses in Behcet's disease

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【Abstract】 Behcet's disease (BD) is a systemic inflammatory disorder characterized by orogenital ulcerations and uveitis. Pathology of BD suggests a vasculitis with mixed-cellular perivascular infiltrates and thrombotic tendency. Its pathogenesis is obscure. Environmental agent, genetic predisposition and immune-dysregulation involving T cells are reported to have a role in the pathogenesis of BD, which includes γδT cells featuring innate immunity, recognition of specific antigens, antigen presenting, CD4+ T cells and CD8+ T cells featuring adaptive immunity. Th1/Th17 expansion and Treg impairment are the central futures of altered T-cell homeostasis. All of those have been suggested to be responsible for inducing and/or maintaining the proinflammatory environment characteristic of BD. Here, we review the features of the phenotypes of T cell subsets in BD, by which modify the immune conducted inflammation, and the transformation of treatment and future target therapy.

【Key words】 γδ T cell; CD4+ T cell; CD8+ T cell; Behcet's disease (BD)
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白塞病(Behcet's disease,BD)是主要累及口、眼、生殖器和皮肤的慢性系统性炎症性疾病[1],若累及消化道、心血管、中枢神经系统,则提示预后不佳。病理特征为可累及大、中、小血管的血管炎。病因包括遗传因素、感染因素和免疫因素。发病机制为:具有一定遗传易感性的个体在感染等因素的作用下,引起固有免疫和适应性免疫应答异常(包括T细胞稳态改变),最终导致慢性炎症[2]。T细胞

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稳态改变主要表现为Th1/Th17辅助T细胞的增殖和Treg损伤[3]，可能导致固有免疫过度激活，也是组织损伤的基础，患者会出现相应症状和体征。本文系统性综述T细胞亚群参与BD的免疫发病机制，有助于深入理解T细胞亚群参与免疫炎症的调控机制。

Th17细胞和IL-23-IL-17通路　活动期BD患者的循环Th17细胞比率增加，产生的IL-17A增多。循环Th17细胞数量与疾病活动呈正相关，血浆IL-17与C反应蛋白、红细胞沉降率正相关[4]。活动期BD患者外周血Th17/Th1比值明显高于健康对照[5]，葡萄球膜炎或毛囊炎患者的Th17/Th1比值尤其高[6]。缓解期BD患者Th17水平较低[4]。BD患者中分泌IL-17和IFN-γ的CD4+ T细胞数量明显高于健康对照[7]。活动期BD患者血清中IL-17、IL-23、IL-12/23p40、IFN-γ水平显著升高[7]，分泌IFN-γ的Th17细胞增加[7]。因此，混合性Th1/Th17细胞因子极化是BD的免疫应答特点。IL-17细胞的产生主要通过G-CSF途径促进中性粒细胞聚集并介导炎症。这些细胞可能在BD的发病机制中起关键作用。除CD4+T细胞外，γδT细胞也能产生IL-17。

IL-23是维持Th17细胞分化的必要条件。BD患者结节性红斑灶中IL-23 p19 mRNA表达上调[8]。研究提示，IL-23R、IL-12RB2、IL-10为BD易感性位点[9]；IL-23-IL-17轴在BD炎症反应中起重要作用[4,6]。BD患者外周血单个核细胞（peripheral blood mononuclear cell，PBMC）中IL-17[4,7]和IL-23水平较高[4,6]。IL-23轴是肠道稳态的核心，体外用重组IL-23刺激BD患者的CD4+ T细胞，可产生IL-17[4,6]。在炎症性肠病患者和肠炎模型中发现固有淋巴细胞（innate lymphoid cell，ILC），证实IL-23通路作用于ILC细胞产生IL17、IFNγ、GM-CSF等促炎细胞因子[10]。

γδ T细胞　γδ T细胞占总体T细胞的0.5%～5%，表达T细胞受体γ、δ链，对T细胞功能有重要作用。γδ T细胞参与固有免疫，具有防御感染、肿瘤免疫及免疫调节的作用。γδ T细胞通过抗原识别，调节针对不同特异抗原的反应。基于功能的差异和可塑性，γδ T细胞在BD发病机制中发挥重要的作用。

γδ T细胞的表达与转录特征　Fortune等[11]最先报道BD患者PBMC中存在高水平γδ T细胞，其在关节炎型患者中升高明显，而在葡萄膜炎和皮肤黏膜患者中升高不显著。后续研究发现，γδ T细胞的平均活化比例较活化细胞总数的意义更大[12]。对活动期BD患者外周血γδ T细胞进一步分析发现，Vδ1、Vδ2 T细胞比例降低[13]。活化的γδ T细胞分泌IFNγ和TNFα，继而造成促炎症环境[13-14]。随着病情缓解，Vδ1 T细胞比例恢复正常[15]。已知至少有8种功能性Vγ基因和Vδ转录基因，其中Vγ9Vδ2是人类γδ T细胞的主要亚型，BD患者PBMC中Vγ9Vδ2增加[16]。高度保守的Vδ3存在于BD患者的PBMC[17]，活动期BD患者脑脊液中Vδ1 T细胞显著增加[18]。

γδ T细胞的激活与炎症应答　Vδ1 T细胞是人类γδ T细胞的第二大亚群，主要位于上皮细胞，通过自然杀伤细胞组2 D（natural killer group 2 member D，NKG2D）激活受体，与表达MHC Ⅰ类多肽相关序列A（MHC class Ⅰ polypeptide-related sequences A，MICA）和MICB的细胞相互作用[19]。γδ T细胞通过识别病原相关分子模式（pathogen-associated molecule pattern，PAMP）分子，启动固有免疫应答，进而分泌IL-4和IFNγ，分别作用于Th2和Th1 CD4+T细胞来影响适应性免疫。Vδ1 T细胞可分泌生长因子维持黏膜稳态。BD患者口腔溃疡组织中存在3种Vδ链，提示BD患者间存在明显异质性，即Vδ T细胞产生多克隆活化[20]。

γδ T细胞可能被BD患者口腔溃疡中的病原微生物所激活并增殖，如致病性链球菌属的*S. sanguinis*和*S. mitis*在内的口腔微生物激活Vδ2细胞亚群[21]。γδ T细胞的T细胞受体通过识别热休克蛋白（heat shock protein，HSP）启动免疫应答。HSP是应激诱导蛋白的自身决定簇，口腔黏膜和微生物抗原之间存在交叉反应性。微生物HSP和人HSP存在基因序列同源性，提示它们可能触发炎症反应[22]。BD患者能对4种HSP肽产生免疫应答，包括与*S. sanguinis*相关的、存在于血浆和黏膜溃疡中的HSP65[23]。诱导对HSP的特异性免疫耐受可减轻BD相关症状[21]。再次应答后γδ T细胞异常激活，随后迁徙至PBMC或淋巴系统[24]。外周Vδ2细胞可迁徙至黏膜，针对口腔暴露抗原产生免疫反应，进而激活远处其他炎症细胞[25]。当发生急性牙科疾病和扁桃体炎时，BD患者的临床症状可能加

重[26],提示黏膜免疫异常。γδ T细胞的分布提示其在黏膜免疫防御中起重要作用。约90%的BD患者首发症状为口腔溃疡,随后其他症状陆续出现,口腔感染及活动期BD患者Vγ9Vδ2细胞显著增多。HLA-B51阳性患者的Vδ1细胞亚群活化较Vδ2细胞亚群更为普遍[15]。BD样动物模型中,γδ T细胞可诱导产生Th1和Th17应答[27]。微生物感染时,γδ T细胞活化,并与中性粒细胞、单核细胞相互作用。中性粒细胞吞噬病原体,γδ T细胞识别细菌终产物,分泌促炎症因子(如TNFα、Th17)。组织局部γδ T细胞增殖,分泌趋化因子IL-8,趋化中性粒细胞。活化的γδ T细胞持续释放生存和活化信号,并招募中性粒细胞、单核细胞至炎症部位,最终形成临床上反复、持续的炎症状态。Toll样受体(2、3、4、7、8、9)表达会进一步增强γδ T细胞的免疫功能[23]。BD患者单核细胞和口腔黏膜中TLR2、4表达均上调。中性粒细胞上活化的TLR受体提供γδ T细胞额外激活信号。BD患者的抑制性细胞因子信号异常,负向调节JAK-STAT信号通路受损[28]。

CD8+ T细胞 CD8+ T细胞作为经典Ⅰ类MHC分子抗原肽呈递细胞,在BD发病机制中起重要作用。活动期BD患者外周血CD8+ T细胞比例显著升高,治疗控制后CD8+ T细胞显著下降[15]。外周CD8+CD146+ T细胞在健康成人中比例低,而在BD患者中显著升高[29],该群细胞分泌IL-17,表达内皮黏附分子CD146。流式细胞术检测衰老相关β牛乳糖活动提示,活动期BD患者外周血衰老CD8+T细胞显著增加,表达更多IL-6、IL-10、IFNγ、TNF-α、穿孔素和颗粒酶。慢性病毒感染针对HSP产生抗原特异性T细胞免疫应答及炎症复发时,都会产生CD8+ T细胞。BD相关皮肤活检中发现,CD8+ T细胞水平高于CD4+ T细胞,CD8+ T细胞表达IL-4强于IFN-γ[30]。穿孔素、颗粒酶主要由CD8+ T细胞表达。BD患者滑膜液中大量表达穿孔素[31];而口腔溃疡、生殖器溃疡和痤疮样皮疹标本中均可见颗粒酶阳性的CD4+、CD8+ T细胞,但结节样红斑病变未见上述特征T细胞[32]。与健康对照组相比,活动期BD患者外周血白细胞和粒细胞的HLA-DQ频率显著降低;而单核细胞群HLA-DR阳性细胞比例显著增加。活动期BD患者CD4+CCR7+和CD8+CCR7+细胞比例在全PBL中显著增高[33]。

治疗转归和靶点 环孢素(cyclosporine,CsA)的主要作用机制是抑制参与调控T细胞活化的细胞因子,尤其抑制IL-2转录。一项RCT研究提示,CsA应用6个月内能改善BD葡萄膜炎患者的最佳矫正视力[34]。霉酚酸酯(mycophenolate mofetil,MMF)是霉酚酸的前体药物,可消耗T细胞和B细胞中的鸟苷核苷酸并抑制其增殖,从而抑制细胞介导的免疫反应和抗体形成。小样本研究提示,4例BD患者接受2 g/天的MMF治疗,随访3~7年无复发[35]。TNF-α是导致BD炎症通路的重要组成部分,也是重要的治疗靶点。TNF抑制剂(TNFi)适用于难治性黏膜皮肤损害、关节炎及眼、心血管、肠道和中枢神经受累[36]。IL-23/IL-17通路的激活是产生致病性Th17细胞及其扩增的必要环节,可作为治疗的重要靶点[37]。乌司奴单抗(Ustekinumab)是一种人源化的抗IL-12和IL-23共享p40单元的单克隆抗体,与Th1和Th17激活相关。前瞻性研究提示,乌司奴单抗对秋水仙碱耐药的BD患者口腔溃疡有效[38]。苏金单抗(Secukinumab)是IL-17阻断剂,可用于治疗银屑病、脊柱关节炎和银屑病关节炎。苏金单抗治疗BD患者口腔溃疡、周围型关节炎安全有效,可能对生殖器溃疡、关节炎和肠道症状产生有益影响[39]。由于BD易感性与*STAT*基因的遗传突变相关[40],且在BD患者的CD4+ T细胞和单核细胞中观察到JAK/STAT活化表达[41],因此JAKi可能是BD的治疗靶点。小样本研究提示托法替布治疗葡萄膜炎[42]、血管型和关节型BD有效[43]。由于BD为罕见病,上述研究的研究队列规模较小,有效性有待进一步验证。

结语 BD具有自身炎症性疾病特征,包括反复发作和自限性临床表现。有显著遗传易感性的个体由感染等环境因素触发BD,出现炎症相关免疫应答异常。固有免疫应答异常及适应性免疫失衡都提示复杂且相互作用的免疫应答体系,抗原特异性T细胞在BD发病机制中起主导作用。对相关免疫应答的研究有助于深入理解BD的发病机制,以寻找潜在的治疗靶点。

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