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◇综述◇

儿童慢性肉芽肿病移植及基因治疗研究进展

疏恒,叶同生,赵钰玮,戴立英,刘光辉

作者单位:安徽省儿童医院新生儿科,安徽 合肥230051

通信作者:刘光辉,男,教授,硕士生导师,研究方向为新生儿危重症疾病及脑损伤,Email:lgh508@sina.com

摘要 慢性肉芽肿疾病是一种罕见的原发性免疫缺陷病,当常规护理和治疗失败时,造血干细胞移植可以是一种治愈方式,年龄越轻,效果越佳。然而,随着病毒载体、核酸内切酶及新的基因编辑技术的出现,基因治疗成为另一种有前途的方法,显示了巨大前景。该文重点综述了近年来移植及基因治疗的相关进展。

关键词 肉芽肿病,慢性; 造血干细胞移植; 基因治疗

Advances in transplantation and gene therapy of chronic granulomatous disease in children

SHU Heng,YE Tongsheng,ZHAO Yuwei,DAI Liying,LIU Guanghui

Author Affiliation:Department of Neonatology, Anhui Children's Hospital, Hefei, Anhui 230051,*China*

Abstract Chronic granulomatous disease is a rare primary immune deficiency disease. When routine nursing and treatment fail, hematopoietic stem cell transplantation can be a cure. The earlier the age, the better the effect. However, with the emergence of viral vectors, endonucleases and new gene editing technologies, gene therapy has become another promising method, showing great prospects. This article focuses on the recent advances in transplantation and gene therapy.

Keywords Granulomatous disease,chronic; Hematopoietic stem cell transplantation; Gene therapy

慢性肉芽肿病(chronic granulomatous disease, CGD)最早是在20世纪50年代被Janeway发现,由于编码烟酰胺腺嘌呤二核苷酸磷酸(nicotinamide adenine dinucleotide phosphate oxidase complex, NADPH)氧化酶复合物亚单位的基因缺陷引起的吞噬细胞功能异常的原发性免疫缺陷病(primary immune deficiencies, PID)[1],遗传方式有以蛋白$gp91^{phox}$缺陷的X连锁隐性遗传和以$p47^{Phox}$缺陷为主的常染色体隐性遗传,发病率约为1/25万~1/13万,临床可反复出现严重细菌或真菌感染威胁病儿生命,以及过度炎症性疾病严重影响病儿生长发育及生活质量[2-3]。目前传统预防及治疗并没有重大突破及更新,本文主要阐述近年来关于移植及基因治疗相关的研究进展。

1 造血干细胞移植治疗

自1977年首例造血干细胞移植(hematopoietic stem cell transplantation, HSCT)治疗CGD成功,40多年来HSCT已被认为是严重CGD的治愈性方式,

成功移植后几乎可以达到无病存活[4-5]。最近一项多中心研究证实了这些观察结果,3年中位生存期为85.7%,无事件生存率(event free survival,EFS)为75.8%[6];当嵌合度超过90%,炎症性肠病(inflammatory bowel disease,IBD)病人症状也可以完全消失[7]。但因为移植同样需要面临供体短缺、预处理相关毒性、植入失败、移植物抗宿主病(graft versus host disease,GVHD)和移植后感染等问题,且无法充分评估晚期影响,关于HSCT仍有争论。

1.1 HSCT移植时机及指征 研究显示经过合理有效的传统预防和治疗,大约90%的CGD病人将会存活到成年[8],部分研究也显示移植和非移植病人总生存率(overall survival,OS)方面并无明显差异[9],已经不再盲目认为任何能获得人类白细胞抗原(human leukocyte antigen,HLA)相合供者的CGD病儿均应尽早进行HSCT,而是将是否能在发生器官损害前获得比传统治疗更好效果作为移植适应证的核心原则,对于长期没有曲霉菌病或结肠炎等并发症的病儿来说,持续观察和等待可能是更好的选择[10]。来自一项跨度60多年的回顾性研究发现≤14岁时移植与移植相关生存率(transplant related survival,TRS)改善相关(*HR*=4.51;*P*=0.035),诊断后30年的存活率估计为65%[11],因此,建议对于所有HLA配型≥9/10的NADPH氧化酶活性缺失的年轻CGD病人才应该考虑进行HSCT,特别是小于14岁、无活动性并发症的病人效果极好,而且确诊后越早越好,8岁前更佳[9,11],2年OS可达90%以上[12],与传统治疗相比病儿生理及心理状况显著改善[11,13]。

1.2 如何选择合适的供体 找到合适的供体是HSCT的前提条件。HSCT的最理想供体是HLA相合的同胞供体(matched sibling donor,MSD),OS最高,移植失败(graft failure,GF)、GVHD和移植相关死亡率(transplant related mortality,TRM)的风险更低,但是总体来讲这样的供体不足30%[14],而我国可能还不到10%,其余的则需要一个替代的供体,那么在没有MSD的情况下,HLA相合的非血缘供体(matched unrelated donor,MUD)是首选;而因为脐血更快的可获得性以及更低的急慢性GVHD,通常用于急需移植的婴儿或幼儿[15];因为HLA-单倍体相合的供体与更高的GF率、严重GVHD和免疫延迟重建风险相关,在以前被认为是在其他替代供体不可用时的最后手段,然而,随着移植物操作策略的最新进展,如移植后环磷酰胺和选择性去除T细胞受体,使其几乎等同于来自其他替代供体的HSCT[16],我国唐湘凤团队运用单倍体HSCT联合第三方脐血移植治疗CGD及其他PID均取得了良好的效果,植入成功率、供体嵌合度高、GVHD发生率低,TRM为11.9%,53/60例无病存活,5年的无失败OS和整体生存率分别为83.9%和88.1%[17],该方法既发挥了单倍体移植供体来源稳定、造血和免疫重建快的特点,同时又发挥了脐带血免疫调节、促进植入、降低排异的优点,疗效显著,特别适合我国国情。而随着HLA分型技术、GVHD预防和并发症处理方面的进步,替代供体的HSCT也越来越安全和可行。

1.3 预处理的方式 预处理是影响移植结果的重要环节,需综合考虑病儿原发疾病、感染程度、供体类型及合并症等因素,鉴于传统的清髓性预处理(MAC)方案较大的毒副作用,特别是在婴儿和儿童中可引起生长障碍、性腺功能障碍和继发性恶性肿瘤等晚期副作用,近年研究表明降低强度/降低毒性的预处理方案(reduced intensity conditioning,RIC/reduced toxicity conditioning,RTC)对CGD病人(包括IBD病人)是安全有效的,已成为降低TRM和晚期不良事件的首选[18-19],在非恶性疾病中已证实可提高儿童HSCT生存率,特别是对于合并严重程度感染的病人时,即使出现混合嵌合体,也并不影响OS和EFS[20],但对于出现混合嵌合体的女性CGD携带者仍还需进行长期随访,以评估自身免疫疾病的可能性[21],目前已被欧洲血液和骨髓移植学会(european society for blood and marrow transplantation,EBMT)/欧洲免疫缺陷学会(european society for immunodeficiencies,ESID)指南推荐的RIC方案由氟达拉滨和烷化剂组成,如环磷酰胺、塞替派和曲奥舒凡等[18],但因清髓效应不彻底,也需同时面临混合嵌合及植入失败的风险,因此对于具有较高GF风险的病人,MAC的疗效优于RIC[22]。当然预处理方式的选择和供体类型也密切相关,EBMT/ESID指南建议对于脐带血或单倍体相合的供体建议采用MAC,其他供体来源则采用RIC/RTC的预处理方案。国内因以单倍体相合供体为多,且缺乏一些新药如Alemtuzumab和Treosulfan,主要还是采用MAC方案,病儿不仅能耐受预处理,也能获得稳定的完全嵌合体[17]。

1.4 GF的预防 HSCT术后良好的结果取决于对原发性和继发性移植物衰竭的预防(包括骨髓供体嵌合体下降至10%以下,以及GVHD)[23]。作为最严重的并发症,防治GVHD至关重要,尤其是对于单倍体及无关供者的移植,目前采用的方法有体外去T细胞、回输干细胞后给予环磷酰胺、血清疗法(抗胸腺细胞球蛋白、抗淋巴细胞球蛋白和抗CD52单克隆抗体阿仑单抗)及低剂量辐射[24],有学者在此基础上联合富含内调节T淋巴细胞和间充质干细

胞的脐血,研究发现它们在防治GVHD中发挥重要作用[25];另外对于其他免疫缺陷疾病目前已采取一种新型的选择性去T细胞(TCRαβ T细胞)的HSCT应用于PID,留存TCRγδ T细胞、NK细胞、树突状细胞、调节性T细胞,取得了良好的效果[26-27],成为后期研究的一个方向。

2 基因治疗

自体基因治疗(gene therapy,GT)作为一种新的治疗方式,因可避免GVHD和其他同种异体反应性并发症[28-29],以及降低预处理方案的复杂性,在没有遗传毒性证据及供体的情况下,成为更有前途的治疗方式。

GT的临床试验始于1900年末,起初由γ-逆转录病毒为载体,但不幸的是因为持续的增强子活性引导了原癌基因的插入激活,导致骨髓异常增生综合征的发生,或只是显示短期的临床收益,自2000年以来,设计了一种具有嵌合内部启动子的自失活慢病毒载体[30],该启动子优先驱动gp91^{phox}在吞噬细胞中表达,利用富含HSC和祖细胞的自体CD34^{+}治疗X连锁CGD,69例病人随访中表现出稳定的载体拷贝数和持久的氧化酶活性[28],基于这一有希望的结果,FDA已于2020年1月授予这种基因治疗药物(OTL-102)指定用于治疗X连锁CGD。尽管载体研究有所突破,但基于传统基因治疗带来了不可避免的插入突变风险,新的基因组编辑技术则可以在离体或体内细胞上介导基因添加、切除、校正和其他高度靶向的基因组修饰,通过位点特异性核酸内切酶如锌指核酸酶、转录激活因子样效应核酸酶或CRISPR/Cas9核酸酶进行的基因编辑显示了巨大的潜力。通过核酸内切酶诱导的双链断裂(double-stranded break,DSB)的产生来启动靶向DNA改变,但因锌指核酸酶或转录激活因子样效应核酸酶需要为每个新的DNA靶标设计一对特定的核酸酶[31-32],加上CGD病人基因突变的独特性,需要为每位病人单独设计,增加了实际应用难度及成本,而其中CRISPR/Cas9核酸酶最具发展前景[33],CRISPR/Cas9核酸酶由一个设计好的单向引导的RNA的指引下与感兴趣的靶位点互补,可以实现有效的DSB,其通过导致插入或缺失的非同源末端连接途径或允许供体DNA掺入的同源定向修复途径进行修复[34],并已经被研究证实[35-36]。使用靶向基因编辑技术对来自CGD病人的诱导多能干细胞或祖细胞进行同源定向基因修复的临床前研究已经实现了分化的中性粒细胞中NADPH氧化酶基因表达及其活性的功能性恢复[37],但目前该领域仍有许多挑战需要克服,包括来自细胞的来源和培养、核酸酶和校正模板的递送、脱靶基因组编辑的基因毒性、核酸酶活性和基因校正的效率,对核酸酶的免疫反应、移植物衰竭以及高昂的价格[33,38]。预期效果如何,还需要进一步地研究,以达到临床应用的目标。

3 小结

有效的感染预防方案和积极的干预措施的发展大大改善了CGD病儿预后。我们认为,HSCT治疗因个体化,提供给没有其他治疗方案且患有严重感染和/或自身炎症并发症的年轻、高危CGD病人,对于没有匹配供体的高危CGD病人应该开展更多单倍体相合的HSCT和GT的前瞻性试验研究。

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