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肠道微生物与自闭症研究进展

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摘要 自闭症是一种严重的神经发育障碍, 其患病率急剧上升, 不符合哈迪-温伯格平衡, 表明环境因素对其影响远大于遗传因素. 自闭症与肠道微生物失衡及肠-脑轴异常密切相关. 由于肠脑发育与头脑发育同步, 因而在婴幼儿发育的关键期肠道微生物发育异常可增加自闭症风险. 肠道微生物可通过代谢产物、免疫、神经内分泌以及迷走神经等途径影响自闭症. 特定有益微生物菌株主要通过微生物-肠-脑轴、调节微生态平衡和抗感染、调节宿主代谢和吸收、改善肠漏等方式改善和治疗自闭症. 益生菌以肠道菌群为靶点或可成为自闭症有效辅助治疗方法. 本文对近年来与肠道微生物相关的自闭症研究进行综述, 为我国今后全面防治自闭症提供人体共生微生物领域的参考.

关键词 自闭症(孤独症), 肠脑, 肠漏, 微生物-肠-脑轴, 益生菌

自闭症谱系障碍(autism spectrum disorders, ASD)也称广泛性发育障碍或孤独症, 被定义为以社会交往和沟通障碍、兴趣范围狭窄及重复刻板行为为主要特征的发育障碍[1]. 患者通常在6~24月开始表现出ASD症状, 也有患者早期发育正常, 24~36个月起出现退行性变化, 语言和社交技能丧失[2~5].

ASD在世界范围内已经成为最严重的神经发育障碍之一, 平均每150名儿童就有一人患病, 且发病率不断攀升[6]. 美国8岁的儿童中每68人中就有一人患有ASD[7]; 韩国儿童ASD患病率高达2.64%[8]; 据保守估计, 目前中国ASD患病率为1%, 0~14岁的儿童患者达200余万[9]. 该病给个人、家庭以及社会带来巨大困扰, 并大幅增加家庭及社会医疗负担, 且临床治疗中尚无切实的、针对性的治疗方法, ASD已经成为一个世界性难题.

ASD发病原因复杂, 是遗传与外部环境相互作用引起的疾病[10]. 遗传因素仅能解释5%~25%[11]的病因, 环境因素对ASD的影响可能远大于遗传因素. ASD发病率迅速增高现状不符合哈迪-温伯格平衡, 提示该病不是一个与特定基因或垂直传递密切相关的疾患, 更多是由后天环境因素导致[12]. 此外, 同卵双生子基因一般认为相同, 而研究显示同卵双生子之间ASD症状严重程度不同[13], 甚至存在一个患病而另一个正常情况[14], 说明环境因素可能在ASD中起重要作用.

影响ASD的环境因素主要包括出生方式、喂养方式、药物、饮食、疫苗、重金属、农药、幼年时期感染以及免疫交互作用等. 人们注意到生活方式的改变, 如居住环境、传统饮食习惯消失、食品工业化、药物滥用等因素直接造成肠道微生物的快速改变, 这些改变可能与ASD突然高发相关[15]. 肠道微生物可能在ASD的发生与发展过程中起核心作用.

1 自闭症影响因素

1.1 孕期影响因素与自闭症

临床研究表明, 肠道菌群的发育可能始于胎儿

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时期, 菌群在子宫内可转移且受孕期母体因素影响[16]. 孕期影响因素可能通过影响胎儿肠道微生物而增加胎儿ASD风险.

孕妇肥胖可增加后代患神经发育障碍(包括ASD)的风险. 2011年Dodds等人[17]发现, 母亲在怀孕前的体重超过90 kg就会增加自闭症儿童发病的可能性. 此外, 2012年Krakowiak等人[18]揭示了母亲孕期肥胖和后代自闭症的发生之间的明确关系. 2013年Bilder等人[19]和2014年Reynolds等人[20]分别报道了类似的研究结果, 他们发现母亲肥胖与后代语言技能延迟存在相关性. 2016年的研究进一步表明, 肥胖的女性怀孕期间体重年增加过多也会升高后代患自闭症的风险[21]. 动物实验研究进一步证明, 孕妇肥胖可能通过影响肠道微生物而增加后代患神经发育障碍风险. 孕鼠高脂饮食模型(modified high-fat diet, MHFD)中, 高脂饮食诱发孕鼠肥胖, 其后代肠道微生物改变和大脑发育异常, 表现出社交缺陷; 对无菌小鼠(germ free, GF)进行粪便移植的研究证明, 正是高脂饮食母鼠的后代小鼠肠道微生物的紊乱造成了它们社交行为障碍; 进一步研究发现肠道菌群中显著减少的罗伊氏乳杆菌(下降超过90%)是造成小鼠社交缺陷的关键[22].

暴露于炎症母体可增加胎儿ASD风险[23~26]. 母体免疫激活(MIA)的子代出现社交受损和刻板行为等行为症状, 还表现出与ASD相关神经病理学特征和胃肠道以及免疫症状[27~29]. 在MIA的小鼠研究中, 母体免疫细胞被激活时, 辅助性T细胞17 (Th17)会产生一种免疫效应分子IL-17, 从而促成胎儿大脑皮质和行为发育异常. 值得关注的是, 研究发现只有携带分节丝状菌(*Segmented filamentous* bacteria, SFB)的母鼠免疫激活后生产的后代小鼠会出现行为异常. 当用抗生素杀死母体肠道内这种细菌后, 出生的小鼠发育就正常. 感染或者自发炎症的母体如果拥有倾向于诱导Th17细胞分化的肠道微生物, 其胎儿患神经发育障碍的风险会增加[30].

孕期内毒素处理可诱发ASD. 脂多糖(lipopolysaccharides, LPS)为革兰氏阴性细菌细胞壁成分, 也称为内毒素, 可在革兰氏阴性菌死亡后释放出来. 动物实验中发现孕期给予怀孕母鼠LPS处理, LPS会透过胎盘屏障和血脑屏障影响胎儿大脑发育[31]. LPS诱发的神经炎症影响胎儿的小神经胶质细胞表型和早期神经发育, 后代大鼠表现出严重社交和认知损伤[32].

1.2 饮食与自闭症

ASD与饮食关系密切, 食物影响ASD可通过肠道微生物实现.

婴儿期, 母乳是最主要的食物. 母乳中含有多种共生微生物, 是婴儿获得乳酸菌和双歧杆菌等有益微生物的重要来源[33,34]. 母乳喂养少于2个月的婴儿与母乳喂养至少6个月的婴儿相比患ASD风险显著增加[35]. 母乳喂养的婴儿肠道内双歧杆菌丰度更高, 而配方奶粉喂养的婴儿肠道中, 双歧杆菌、拟杆菌、梭状芽孢杆菌和葡萄球菌比例接近[36], 并未显示出有益菌的优势. 随着婴儿成长和辅食添加, 幼儿肠道微生物也在多样化的饮食中逐步建立与发育. 高脂或高蛋白食物促进拟杆菌增殖, 而普雷沃氏菌属数量增加则与高碳水化合物饮食相关[37,38]. ASD患者辅食添加过程中营养过剩, 会扰乱正常肠道微生物发育进程. 在没有完善肠道微生物定植前, 过早接触不适当食物会增加幼儿食物不耐受以及过敏的风险.

错误的饮食方式和不安全的食品可引起肠道微生物紊乱而引发ASD样行为. ASD儿童普遍存在严重的饮食问题, 主要表现为食物选择范围狭窄, 偏爱高脂肪高碳水化合物饮食以及加工类食品, 而拒绝水果蔬菜蛋以及蛋白质类食物[39,40]. 这种西方式饮食习惯可能引起肠道微生物高度西化, 进而促进ASD的发生[41,42], 由传统饮食方式的改变而造成肠道微生物的剧烈变化, 可能是ASD患病人数激增的重要原因. 因为肠道微生物能够影响宿主食欲和食物偏好[43], 所以错误的饮食方式导致ASD患者肠道微生物严重失衡可能会引起宿主饮食问题加剧而形成恶性循环. 现代加工食品中的添加剂威胁儿童的肠道健康. 聚山梨酯80、羧甲基纤维素等乳化剂可造成野生型小鼠肠道菌群改变, 低度炎症及肥胖/代谢综合征[44,45]. 高葡萄糖饮食可增加白色念珠菌生长[46]. 研究表明, 食物可在一天之内引起肠道微生物改变, 并且这种改变还可受食物上负载的共生微生物影响[47]. 因此, 肠道微生物在ASD患者饮食中的作用应得到更多关注.

Campbell-McBride[48]认为, 均衡营养的自然疗法可以治疗包括ASD、焦虑症、抑郁症、多动症乃至精神分裂等心理疾病. 安全有益的食物会提升有益肠道菌群, 抑制和减少有害肠道微生物, 而盲目补充维生素、生酮饮食等方式并不值得推荐, 因为人类所需的一切营养物和维生素都可以靠肠道菌群的代谢

物来得以完善. 补充微生物的代谢物有时会使肠道微生物不能更好发挥自己的作用, 甚至反倒降低肠道微生物的多样性. 关注儿童饮食均衡, 杜绝零食和甜饮料等影响肠脑发育的加工类食品, 降低神经发育障碍发生的风险.

1.3 肠漏与自闭症

肠漏可被列为ASD发病的重要生理机制之一. 肠漏综合征是指肠屏障功能紊乱或者损坏, 以大分子物质、细菌或其代谢毒物进入固有层的异位为特征, 是多种疾病的诱因[49]. ASD患者中普遍存在肠漏问题, 与正常对照儿童相比, ASD儿童肠道通透性增加[50], 甚至ASD患者的父母, 肠道通透性异常比率也明显升高[51]. 丙戊酸(VPA)诱导的ASD动物模型出现黏膜肌层厚度和肠动力下降[52]. 孕期病毒感染的母鼠, 其子代除了表现ASD的核心症状, 还会出现成年肠道通透性增加, 同时血液中肠道微生物代谢产物增加[29]. 白色念珠菌具有增加肠细胞间缝隙的功能, 使得正常情况下不容易通过肠壁的物质能够进入体内[53].

造成肠漏的因素很多, 肠道微生物及其代谢产物起核心作用. 研究表明, 在出生前后, 肠道微生物决定小鼠血脑屏障和肠道屏障的正确发育, 而肠道屏障功能的建立完全由肠道微生物控制[54]. 肠道微生物组成的改变可能会导致肠道通透性增加以及肠道屏障功能损伤. ASD患者肠道中促炎菌群与抗炎菌群比例失衡也导致肠漏, 致使肠道微生物有毒代谢产物进入血液循环[55]. ASD患者肠道微生物中革兰氏阴性细菌过多, 血浆内毒素水平高于对照儿童[56,57]. 革兰氏阴性细菌过多是造成肠漏的危险因素, 其产生内毒素的革兰氏阴性菌还可引发慢性炎症, 损害神经系统功能.

城市化的生活方式使人们获得共生微生物的有效途径越来越少, 加之抗生素、消毒剂、杀虫剂以及食品添加剂的广泛使用更造成肠道微生物种类和丰度大幅下降, 导致肠道屏障破损甚至肠漏, 破坏人体与微生物的共生关系. 这些因素可能致肠道微生物失衡进而成为ASD的发生和病理发展的推手.

2 自闭症与肠道微生物密切相关

2.1 自闭症患者肠道微生物失衡

研究发现, ASD患者肠道微生物失衡, 细菌和真菌群构成及比例发生改变.

ASD患者的肠道细菌改变. 与健康人群相比, ASD患者肠道梭菌属含量显著增加[5,58,59], 拟杆菌与厚壁菌比率下降, 乳酸菌属和脱硫弧菌属含量升高[60]. 其中梭状芽孢杆菌与神经毒素产生相关, 释放的神经毒素通过迷走神经传入CNS, 抑制神经递质释放, 从而引起ASD相关的行为表现[61]. 有研究揭示, ASD患者肠道中, 降解和代谢碳水化合物的重要菌属包括普氏菌属、粪球菌属和韦荣球菌科含量降低[62,63], 这些菌属能够调节黏膜和肠上皮细胞完整性[64,65]. 2017年Coretti等人[66]发现, ASD群体肠道菌群失衡, 其中拟杆菌属、副拟杆菌属、萨特菌属、*Dehalobacterium*和颤螺菌属是性别特异的肠道微生物群落, 且与异常行为表现, 肠道通透性增加及肠炎相关. 还有研究发现, *Alkaliflexus*以及萨特菌属只存在于ASD患者肠道[64,67].

患者肠道真菌发生改变. 临床研究报道过ASD儿童出现念珠菌感染相关症状[68]. 对肠道真菌群落分析结果表明ASD患者具有更多念珠菌属(尤其是白色念珠菌)[56,53], 可能与之前大量使用抗生素有关[56,69]. 白色念珠菌代谢产生氨与毒素[70], 其在肠道生长引起碳水化合物和矿物质吸收下降可引发ASD相关行为[60,71].

2.2 自闭症的生理和行为异常与肠道微生物有关

多数ASD患者受到胃肠疾病的困扰[56]. ASD患者的胃肠道症状主要包括腹泻、便秘、食道反流、腹痛、胀气和排泄物恶臭[72], 并且胃肠疾病症状与ASD的严重程度正相关[56,73]. 尽管不同研究中ASD伴随胃肠道症状的比率存在差异, 但这种重叠关系已为研究者们共识[74]: 有胃肠疾病的ASD患者还可能出现焦虑、自残和攻击他人等异常行为[71]; 功能性便秘与ASD儿童的刻板-强迫行为有关[75]. 胃肠道症状和行为异常均受肠道微生物影响[60], 提示ASD与肠道微生物改变有关[76].

2.3 改变肠道微生物可影响自闭症相关症状

无菌小鼠表现出明显社交障碍, 特别是雄性小鼠, 出现社交回避和刻板行为, 不对同类表现出明显兴趣, 也不会对陌生个体有更多的探究行为, 同时表现出一些重复性行为. 在补充微生物后, 社交回避和重复行为可得到改善, 但社会认知障碍并未得以改

变[77]. 由抗生素或者益生菌引起的菌群改变可导致ASD相关症状发生变化. ASD症状与抗生素使用密切相关. Sandler等人[78]发现, 11名幼儿(研究入组标准: 倒退型ASD儿童, 即发病迟于一岁后, 由于服用一段时间抗生素后出现原有技能退化, 且临床诊断为自闭症), 这些患者使用万古霉素干预期间出现ASD症状改善, 但是停用后行为又出现退步, 这项研究进一步提示ASD与肠道微生物有关, 前期抗生素的使用破坏了肠道菌群可能是自闭症发病的一个诱因, 但是试验中万古霉素的使用可能是通过降低自闭症儿童中增多的梭菌数量和比例达到了改善症状的效果, 这提示我们不同的抗生素改变肠道微生物即可对ASD症状造成不同的影响, 也进一步说明了肠道微生物在自闭症发病和治疗中的潜在影响力. 其他个案研究也发现抗生素使用与ASD症状和共病改善相关联[79]. 氧霉素和二甲胺四环素在临床研究和动物模型研究中显示出对于ASD症状的治疗作用[80~82]. 补充益生菌调节肠道微生物能够改善ASD患者的行为和胃肠症状, 也提示肠道微生物在ASD中发挥重要作用[78]. 整体替换患者的肠道微生物, 比如通过粪菌移植(fecal microbiota transplantation, FMT)也可改善ASD患者的胃肠道以及行为症状[83]. 但是FMT尚无对于小龄ASD患者的干预案例, 效果的持续时间追踪以及可能存在风险, 仍需大量动物实验以及临床研究提供证据.

基于调整肠道微生物干预方法改善了患者的ASD患者症状, 提示ASD有类似于感染症的特征, 更加证实肠道微生物与ASD的密切联系. 研究者们正在逐步揭示肠道微生物失衡与ASD症状的关联性, 同时致力于发现和探究肠道微生物在ASD相关症状中的作用机制.

3 肠道微生物如何影响自闭症

3.1 肠道微生物是肠脑的重要组成部分

肠道是人体最大的消化器官、免疫器官和内分泌器官. 肠道微生物是人体第二大代谢器官, 人体的新陈代谢多数由微生物完成[84]. 健康人体可承载2000 g左右的共生微生物, 已发现人体微生物组编码基因数量超过人体自身基因的500倍, 被称为人体的“第二基因组”或“被遗忘的器官”[85~88]. 肠道微生物包含细菌、古细菌、病毒、真菌及其他真核微生物. 肠共生微生物与消化道复合系统被称为第二脑或肠脑, 它对机体稳态和大脑功能的维护发挥重要作用. 肠道微生物是肠脑发挥功能的重要组成部分, 肠道微生物不仅能够帮助人体消化和吸收营养物质, 还能通过分泌各种酶类, 合成某些维生素和生物活性物质影响人体代谢, 控制体重, 帮助抵御病源微生物的侵入以及塑造人体免疫系统[5,89,90]. 血液中大约70%的物质来自于肠道[91], 其中36%的小分子物质是由肠道微生物产生的[92]. 肠道微生物不仅影响人体的生理健康, 还可通过神经化学物质的变化[93], 影响人的心理和行为[94,95].

3.2 肠脑发育与大脑发育同步

类似于人体生长发育, 肠道微生物也有其发育规律, 肠道微生物经历由简单到复杂最终趋于稳定的发展过程. 如果菌群正常发育过程被打破而导致肠道微生态失衡, 很可能伴随出现大脑功能与行为异常, 菌群异常得不到及时纠正, 就可能会发生ASD、多动症、抽动症、甚至青春期会出现精神分裂症[96~98]. 微生物在精神疾病, 特别是在ASD中的作用, 已经处于临床精神病学和神经科学研究的最前沿.

肠道微生物发育与儿童大脑发育同步[99], ASD发生时间与肠道微生物的发育节点具有时间相似性. ASD发病时间通常是3岁以内, 关键期为出生之前、期间或出生后不久. ASD发病的关键期同样也是肠道微生物发育的关键期, 极易受到外界环境的影响. 人体肠道微生物的发育始于胚胎期, 出生方式、哺乳方式以及辅食的添加是影响肠道微生物发育的关键因素, 直到3岁菌群建立趋向稳定, 接近成人水平. 婴儿大脑的发育可能伴随着肠道微生物发育完成[100]. 肠道微生物在婴儿早期发育异常可能导致ASD的发生, 目前正在积累的生命科学研究证据支持这一推论. 肠道微生物为大脑小神经胶质细胞发育和正常功能所必需[101]. 大量无菌动物研究证实, 共生微生物在个体正常发育, 特别是大脑发育与行为中起重要作用. 与常规小鼠或SPF小鼠相比, GF小鼠表现出大脑基因表达和神经生理方面异常: 大脑前额叶、纹状体、海马和下丘脑转录组谱改变, 特别是海马区外显子表达和RNA编辑发生改变, 而此区域是大脑调节社交刺激的关键情感中枢[94,102]; 突触长时程增强、甾体类激素代谢和神经元传递改变, 脑源性神经营养因子和突触蛋白的改变, 五羟色胺(5-hydroxy-

tryptamine, 5-HT)能、多巴胺能和谷氨酸能系统信号等均与正常小鼠存在差异[76,93,94,103~105]; GF小鼠海马神经发生增加[106]. 在三箱实验中, GF小鼠表现出社交减少, 更加喜欢非社会性的物体而非同类小鼠, 更加偏好与熟悉小鼠社交而非陌生小鼠[77]. GF小鼠与抗生素处理的小鼠均表现出学习记忆能力缺陷[93]. GF小鼠下丘脑-垂体-肾上腺(hypothalamus-pituitary-adrenocortical, HPA)轴反应过强, 应对压力会释放更多的皮质酮和肾上腺皮质激素[106], 补充婴儿双歧杆菌可改善这种异常[76].

在发育关键期, 肠道微生物定植影响大脑发育及行为[94]. 因而在幼年及时纠正异常菌群对于ASD患儿延缓病理发展, 恢复正常脑发育以及纠正异常行为有至关重要的意义. 断奶时给予GF小鼠定植野生型小鼠的肠道微生物能够逆转GF小鼠的社交行为异常[103]. 啮齿类动物的未成年期大约在出生后的28~42 d, 小鼠出生6周内重建正常肠道菌群对于纠正GF小鼠表现出的脑发育、免疫、代谢和行为异常, 以及HPA轴功能异常有作用[94], 超过6周重建菌群虽有改善, 但无法恢复到对照水平, 提示成年大脑功能和心理健康与幼年期菌群定植状态密切相关[15].

3.3 肠道微生物通过微生物-肠-脑轴影响大脑和行为

胃肠道与大脑之间存在多条途径连接的双向交流系统, 称为肠-脑轴. 肠道微生物可通过血液循环系统、内分泌系统和神经系统影响大脑和行为, 它们之间相互影响构成了微生物-肠-脑轴[107,108]. 大量研究表明, 肠道微生物改变主要通过影响免疫系统、神经系统和代谢通路, 直接或间接诱发ASD[96].

3.3.1 肠道微生物通过代谢产物影响自闭症

胃肠道的一个重要功能是将营养物质分解为小分子, 这些小分子通过多种转运机制入位于肠道黏膜细胞的另一面的血液或毛细淋巴管. 肠道菌群可将饮食中的多糖代谢生成短链脂肪酸(short-chain fatty acids, SCFA; 如丙酸、丁酸、醋酸)、鞘脂、甘油磷脂等; 将饮食中的蛋白质代谢生成异丁酸盐、异戊酸盐等; 并可代谢菌群自身的生物活性物质, 例如将异黄酮代转化为雌马酚, 将鞣花单宁代谢成为尿石素等; 同时可转化菌群-宿主的共同代谢产物, 例如将共轭初级胆汁酸代谢为游离胆汁酸, 再将游离胆汁酸代谢为次级胆汁酸等[109]. 如果肠道微生物紊乱, 会导致消化过程异常, 同时会产生影响人体健康的有毒代谢物.

微生物有害代谢产物积累可损害神经系统, 引起情绪和行为异常[29]. ASD患者与对照的尿液与粪便的代谢组研究揭示了与神经发育相关的微生物代谢产物的变化. 例如, 3-(3-羟苯基)-3羟基苯酸(3-(3-hydroxyphenyl)-3-hydroxypropionic acid, HPHPA)是一种酪氨酸代谢物, 它能够消耗儿茶酚胺, 可引起如刻板行为、极度活跃高度-反应性的ASD样行为[110]. 在ASD小鼠研究中发现, MIA小鼠多种血清代谢物含量升高, 其中最为主要的是细菌代谢产物4-乙基苯基硫酸酯(4-ethylphenylsulfate, 4EPS). 通过脆弱拟杆菌干预改变幼年MIA小鼠肠道微生物, 可显著改善ASD症状态[29].

肠道微生物代谢产生的SCFA、酚类化合物、游离氨基酸等, 可通过迷走神经传递至大脑, 从而引发ASD, 比如丙酸与丁酸. ASD患者脂肪酸代谢存在问题, 粪便中总SCFA浓度显著高, 除了已酸之外, 乙酸、丁酸、异丁酸、草酸、异戊酸都显著高于健康对照组[111]. 丙酸是常用的食物保鲜剂, 可由许多肠道微生物代谢产生. ASD与丙酸有关, 丙酸可通过血脑屏障(blood-brain barrier, BBB)[112], 诱发ASD行为. 动物研究发现, 脑室内注射丙酸可引起大鼠ASD样行为(如刻板行为、社会交互和玩耍行为减少等)和神经炎性反应[113,114]. ASD儿童粪便中丙酸含量升高[115], 可能与患者肠道梭状芽孢杆菌、拟杆菌和脱硫弧菌增加有关, 这些细菌都能产生丙酸[116].

这些研究线索值得充分关注, 并通过实验探究其内在机制. 尽管肠道微生物代谢物影响ASD相关行为已经得到多个实验描述, 但在肠道微生物如何相互作用导致这些物质含量异常改变, 以及肠道微生物与宿主相互作用的模式还没有更加明晰的证据. 受到菌群-宿主共同调节的菌群产生的代谢产物可调节宿主健康及疾病状态, 是药物的潜在靶点.

3.3.2 肠道微生物通过免疫途径影响自闭症

肠道微生物与免疫系统存在双向调节[117]. 肠道微生物调控骨髓细胞的发育和分化[118~120]. 免疫系统通过对菌群代谢产生的毒素进行免疫应答, 释放炎症细胞因子, 产生炎症[4]. 调节性T细胞(Treg), 为大脑正常发育所必需, 可释放免疫调节因子[121]. 与正常对照相比, ASD患者Treg和Th亚群减少[119].

幼年肠道微生物发育影响儿童免疫系统的发育.

免疫系统通过细胞因子和传入神经向大脑传递信号[122]. 某些肠道微生物群落改变会诱发促炎症状态, 导致自身免疫疾病的发生, 而另一些共生微生物及其抗原成分出现会保护宿主对抗炎症, 包括CNS炎症[123]. 肠道微生物还通过代谢产物影响免疫细胞. 例如色氨酸代谢产物通过结合芳基碳氢化合物来调节肠道细胞因子的产生[124]. Treg能够被大量与微生物相关信号激活, 如细菌代谢产物以及细菌成分[125]. 肠道细菌通过发酵产生的SCFA可通过G蛋白偶联受体维持和激活Treg[126], SCFA可以提高无菌动物肠道结肠Treg数目及其分泌IL-10的能力, 并且在SPF小鼠中也有同样作用, SCFA通过作用于结肠Treg细胞表面的Ffar2受体以抑制组蛋白去乙酰化酶, 从而促进Treg增殖与功能[127]. 在小肠分节丝状菌积累可激活Th17细胞和炎症环境, 在大肠抗炎Treg被梭菌和拟杆菌激活[128]. 炎症小体信号通路参与肠道菌群-宿主互作, 它的缺陷扰乱抗菌肽平衡, 驱动肠道生态失调的发展. 修复代谢物-炎症小体-抗菌肽轴, 可恢复正常菌群并改善结肠炎[129]. 真菌有毒代谢产物对免疫的影响不容忽视. 胶霉毒素(GLIO)是烟曲霉产生的真菌毒素, 对哺乳动物细胞具有免疫抑制及促凋亡作用, 可通过抑制NAPDH氧化酶活性进而抑制粒细胞抗微生物作用. GLIO对在组胺和炎症介质产生中发挥重要作用的肥大细胞也具有抑制作用. 食物特定的IgG抗体与GLIO协同作用破坏炎症调节, 造成肠道炎症恶化[130].

炎症在ASD发生中发挥重要作用, 包括神经炎症和外周炎症. 对幼年以及成年ASD患者大脑检测发现激活的星形胶质细胞和小胶质细胞等免疫细胞以及细胞因子的存在, 暗示长期的神经炎症[131,132]. 围产期精神压力与肠道菌群、HPA轴及免疫系统之间存在复杂联系. 炎症调控能力不足会导致应对心理压力会出现炎症反应增多[133]. ASD患者往往存在神经炎症, 脑和脑脊液中促炎症细胞因子水平上升; 存在外周炎症, 除易对食物产生过敏反应外, ASD患者血浆中促炎症细胞因子水平也上升, 而抗炎症细胞因子水平降低, 虽然不同研究中数量增加的促炎症细胞因子有种类差别, 但大部分研究都认为ASD患者存在外周炎症[134]. 最近研究发现, ASD儿童的血浆IL-1β, IL-6, IL-14, P40等细胞因子水平显著高于正常发育儿童, 特别是其中具有退行性的ASD患者. 细胞因子水平上升可能与ASD的某些异常行为增加有关[4]. 除此之外, ASD患者的固有免疫系统似乎也发生变化, 如肠道黏膜免疫的过度激活[134,135]. 早期肠道菌群破坏与过敏性疾病发生成正相关, 肠道菌群可以诱导产生, RORγt$^+$ Treg细胞通过CTLA4调控树突状细胞(DC)表面的共刺激分子CD80和CD86的表达抑制Th2反应, 在GF小鼠或抗生素处理过的小鼠的肠道中, RORγt$^+$ Treg细胞数目大大降低[136].

过去将免疫分为特异性免疫和非特异性免疫, 但是事实远非如此. 现代化生活方式导致幼儿肠道微生物缺失和免疫系统发育异常, 造成幼儿对共生微生物无法形成免疫耐受, 因而持续存在低水平炎症状态, 通常表现为C反应蛋白(一种非特异性炎症标记物)增高, 却可能没有明显临床症状[15]. 微生物具有免疫调节作用[137], 已经应用于治疗动物模型的慢性炎症疾病[138], 其机理在于微生物能增加Treg的数量[139].

因此, 微生物可能通过刺激宿主的免疫系统, 通过细胞因子及一些重要物质如色氨酸的代谢进而直接或间接地影响宿主的CNS, 从而引起认知行为及心理状态或精神状态的改变.

3.3.3 肠道微生物通过神经内分泌途径影响自闭症

神经递质是神经信号传递过程中的信使, 可以将神经冲动从一个神经细胞传递到另一个神经细胞, 是神经系统功能正常运行的关键. 肠道产生人体所需95%的5-HT和50%的多巴胺, 以及神经肽、脑啡肽等影响精神的生物活性物质, 肠道微生物显然在其中发挥重要作用.

肠内分泌细胞通过内分泌和旁分泌方式影响CNS活动. 内分泌传递是指肠内分泌细胞释放的神经内分泌物质进入循环系统, 并最终作用于下丘脑和其他相关脑区; 旁分泌传递则是神经内分泌物通过作用于迷走神经进而影响CNS的活动. 内分泌传递中一个重要组成部分是HPA轴. 当受到应激时, HPA轴释放皮质醇, 皮质醇能够调控肠道中免疫细胞活动和细胞因子的释放, 影响肠道渗透性和屏障功能, 改变肠道菌群组成. 相反, 肠道菌群也能够调节HPA轴活动, 对脑活动产生影响. 事实上, 肠内分泌细胞是第一个对肠腔内的化学和机械刺激做出反应的. 肠内分泌细胞的微绒毛上分布着机械敏感型离子通道, 肠道内容物运动产生的剪切力使这些离子通道去极化从而激活肠内分泌细胞[140].

肠道菌群在HPA轴正常活动的维护中发挥重要的作用[76]. 无菌动物对束缚应激表现出HPA轴的过度反应, 释放过多的促肾上腺皮质激素和皮质酮. 而正常肠道菌群的定植能够恢复无菌动物的HPA轴活动. 肠道菌群能够通过TLR受体调控肠内分泌细胞的分泌活动[141]. 肠道还有一类特殊肠内分泌细胞——肠嗜铬细胞, 能产生5-HT, 通过迷走神经传递调节大脑的情绪活动[140]. 此外, 肠道菌群还通过影响色氨酸的代谢调控中枢活动. 色氨酸是5-HT合成的前体物质, 对5-HT合成和产生至关重要. 色氨酸还能通过吲哚胺2,3加双氧酶(IDO)转化为犬尿氨酸. 肠道细菌可能通过影响促炎症细胞因子和皮质醇, 调节IDO活性, 改变色氨酸代谢活动[142,143]. 而ASD者普遍存在促炎症细胞因子和皮质醇水平增高.

ASD患者行为异常与5-HT能系统功能失调相关. 受5-HT调控的神经-免疫协同作用可影响神经系统与免疫系统功能, 在ASD的发生与发展过程中有重要作用. 5-HT在社交功能、刻板行为以及感觉发展中都有重要作用. 血液总5-HT含量升高是ASD中确定的第一个生物学指标, 出现在超过25%的ASD儿童中. 血液5-HT水平与ASD病儿童胃肠道症状呈正相关[144]. 一些研究发现ASD患者血液中色氨酸水平下降, 提示5-HT合成改变, 同时也可能反映出患者饮食或者其他代谢改变[145]. 目前还没有研究报道血小板5-HT和肠道5-HT的关系. 怀孕期外周5-HT产生量会增加, 可影响胎儿大脑5-HT水平[146]. 布劳特氏菌(*Blautia*)通过增加色氨酸羟化酶在肠道的表达, 激活5-HT合成, 在宿主5-HT代谢中起重要作用[147]. BTBR小鼠表现出ASD样行为表型[148], 其肠道5-HT以及色氨酸羟化酶水平与*Blautia*丰度显著正相关, *Blautia*水平降低可造成BTBR小鼠肠道5-HT合成不足[149].

催产素(Oxytocin, OT)是由下丘脑室旁核与视上核神经细胞合成的一种高度保守的九肽, 其广为人知的作用是分娩时控制宫缩和调节泌乳. OT对学习记忆、社会交往、摄食消化、血压心率、痛觉等生理现象的调节作用逐步为人们所关注[150]. 啮齿类大脑OT与非语言社会行为缺陷、亲和行为、社会认知和社交方式有关[151]. 鼻腔施用OT是一种潜在而存在争议的ASD治疗方法[152]. 对ASD群体研究发现ASD儿童血液OT水平下降[153], 但与对照相比OT前体水平升高[154,155]. 小鼠动物模型显示伴随OT和OT受体(OTR)系统改变表现出社交缺陷[156]. 微生物能够影响宿主OT水平, 例如罗伊氏乳杆菌能够提升mHFD自闭样小鼠血浆的OT水平和逆转其社交缺陷[22].

5-HT与OT系统相互作用影响与ASD相关的社交、攻击和焦虑行为[157]. 5-HT通过影响OT释放影响社交行为. 值得注意的是, 5-HT水平与年龄和OT水平呈负相关. 缺少OT受体的青少年小鼠与野生型相比5-HT水平较高, OT系统影响周围5-HT系统[158]. 这一结果也验证了在对ASD人群研究中发现幼年ASD患者血浆OT值低[153], 而成年患者OT值高[155]. 正常状态下血液中5-HT不会通过BBB进入大脑, 但是当BBB被破坏时, 一些细胞因子与5-HT可以进入大脑[159]. 它们参与多种神经元成熟, 在发育阶段影响神经元输出和行为. 用内毒素、IL1-β或者绵羊红细胞激发大鼠免疫会导致海马5-HT水平改变[160]. 反之, 给予新生个体注射抗5-HT IgG, 可导致成年大脑5-HT水平下降[161].

某些具有神经递质功能的气体被叫做气体神经递质, 如一氧化氮(NO)、一氧化碳(CO)、氨(NH_3)、硫化氢(H_2S)、氢气(H_2)、甲烷(CH_4)和二氧化碳(CO_2)等. 这些气体神经递质多由肠道微生物产生, 可调控微生物之间和微生物与宿主间的信息交流, 影响产生它们的细胞和邻近的细胞及远端组织或器官, 甚至影响人的大脑、情绪和行为[162]. H_2S和NH_3是两类与ASD密切相关的重要气体神经递质, 它们在浓度低时作为信号分子参与正常的生理活动, 而在浓度高时会产生神经毒性. NH_3和H_2S极易溶于水, 能够顺浓度梯度跨过细胞膜. ASD可能与H_2S含量增加有关, 患者肠道中异常增高的脱硫弧菌导致更多的H_2S产生, 对宿主产生毒害作用[163]. 正常生理浓度下, H_2S维持神经细胞氧化还原电势、5-HT能神经元活性和诱导促肾上腺皮质释放激素的释放. 星形胶质细胞中H_2S影响细胞内钙水平, 钙在细胞交流过程中起重要作用[164]. H_2S与许多神经退行性疾病相关. ASD可能与高血氨有关, 高血氨能破坏屏障结构, 损伤大脑, 导致认知和行为异常. 成人体每日约产氨4 g, 其中90%以上来自肠道, 主要由肠道细菌分解尿素产生. ASD患者肠道异常升高的梭菌属以及白色念珠菌属也是NH_3的重要产生菌属[165,166]. 当NH_3超过生理浓度会引发谷氨酸盐从神经元快速释放直接产生神经毒, 造成出现易激惹、攻击性行为、过度兴奋和运动障碍. 高浓度NH_3可造成BBB通透性增加. ASD患

者排便恶臭, 特别是幼儿如果体味和便味异常应引起家长的足够重视, 异常的气体神经递质会毒害儿童神经系统, 导致神经发育障碍. 通常气味异常可能先于行为异常, 关注儿童的气味有助于预防和治疗疾病.

3.3.4 肠道微生物通过迷走神经影响自闭症

迷走神经是第10对脑神经, 是脑神经中最长, 分布最广的一对, 含有感觉、运动和中间神经元. 迷走神经连接肠道内肠神经系统的5亿个神经元[112], 在髓质与大脑最底端相连. 迷走神经负责身体和大脑信号的联络.

ASD与迷走神经功能异常有关. Porges和Furman[167]认为, ASD患者的异常行为与髓鞘迷走神经发育不足有关. ASD患者迷走神经张力降低[168], ASD行为异常和语言障碍与迷走神经活动减弱有关[169]. 迷走神经刺激能够改善ASD[170].

肠道微生物及代谢物通过迷走神经影响大脑的功能与行为. ASD患者促炎症细胞因子水平高, 促炎症细胞因子通过激活迷走传入神经直接影响CNS, 刺激特定脑区[171], 比如胃肠道感染破伤风梭菌后释放的神经毒素可通过迷走神经传入CNS[172]. 当切断迷走神经, 很多微生物的调节功能消失, 说明迷走神经在此过程中起关键作用. 在抑郁症动物模型中, 迷走神经切断导致鼠李糖乳杆菌(JB-1)对实验动物的神经化学物质和行为影响消失[173]; 在葡聚糖硫酸钠模型的小鼠肠炎中, 切断迷走神经使得长双歧杆菌NCC3001株的抗焦虑作用消失[174].

综上所述, ASD与肠道微生物异常密切相关, 患者肠道微生物失衡, 肠脑和大脑发育受损, 微生物-肠-脑轴功能异常. 联系肠脑和大脑的各条通路, 如神经内分泌、免疫和迷走神经途径等途径均出现异常, 这些异常均可由肠道微生物异常导致(图1).

4 补充益生菌对自闭症的作用

食品农业组织/世界卫生组织定义益生菌为“当补充一定量能够对宿主健康有益的活性微生物”[175]. 不同的益生菌在人消化道中定植位置不同, 生物学功能不同, 代谢产物不同, 这些决定了它们的作用和对健康的影响不同. 菌种和菌株之间的生物学功能也有显著不同. 有些益生菌对于心理疾病的治疗和改善有效, 因而被称为益心菌(psychobiotics)[176].

益生菌调节肠道菌群平衡, 有利于宿主身心健康, 被认为是治疗微生物-肠-脑轴疾病的安全有效的生物制剂. 很多研究报道了益生菌改善情绪和调节宿主行为的有益作用[173,177~181], 且临床研究显示肠易激综合征(IBS)[177,182]、炎症性肠病(IBD)[183,184]和肝性脑病(HE)患者[185,186]服用益生菌后消化道症状、焦虑抑郁情绪和认知能力得到改善, 生活质量得到提高. 已有研究人员评估了嗜酸乳杆菌(*Lactobacillus acidophilus*)、鼠李糖乳杆菌(*Mactobacillus rhamnosus*)和长双歧杆菌(*Bifidobacterium longum*)ASD临床研究效果, 补充以上益生菌3个月后, ASD儿童的自闭症疗效评估量表(autism treatment evaluation checklist, ATEC)总分数显著降低, 表明ASD症状严重程度降低, ASD儿童的表达/语言沟通能力、社交能力、感知/认知能力和健康/生理/行为改善, 此外ASD儿童总六项胃肠道症状严重程度指数问卷(six item gastrointestinal severity index, 6-GSI)得分明显改善, 便秘、排便状况、胀气和腹痛症状均得到明显改善, 而腹泻便秘以及大便气味症状也明显下降, 表明益生菌或可作为儿童ASD患者的非药理学和低风险辅助治疗手段[187].

4.1 调节微生态平衡和抗感染

益生菌重建菌群平衡、改善代谢, 降低异常代谢产物, 从而改善和治疗ASD. 一项研究在给予益生菌之后, ASD儿童体内拟杆菌门/厚壁菌门的比例升高, 脱硫弧菌属和双歧杆菌属细菌比例均得以改善[60]. 在MIA ASD动物模型中, 脆弱拟杆菌干预能够改变肠道微生物组成, 降低神经毒性物质4EPS, 从而改善ASD症状. 虽然已经明确对于ASD发生关联的一些菌群存在, 但不宜采取过激方法清除, 肠道应该是一个平衡的生态系统, 药物干预导致总体失衡而引发严重后果. 然而益生菌, 特别是乳酸菌在人类出生之时最早定植肠道, 在整个肠道微生物逐步建立平衡过程中发挥不可替代的作用.

益生菌帮助宿主对抗感染. 补充益生菌, 可改善ASD患者的念珠菌和梭菌感染情况. 乳酸菌产生大量SCFA, 例如丁酸能有效阻止白色念珠菌菌丝转化, 而发生菌丝转化是白色念珠菌由共生菌转化为病原菌的第一步, 导致菌丝入侵和系统感染[188,189]. 此外, 益生菌能够显著降低ASD患者体内异常真菌, 明显改善ASD样行为. D-阿拉伯糖醇作为真菌感染的诊断指标, 检测体内真菌状况. ASD患者尿液中D-阿拉伯

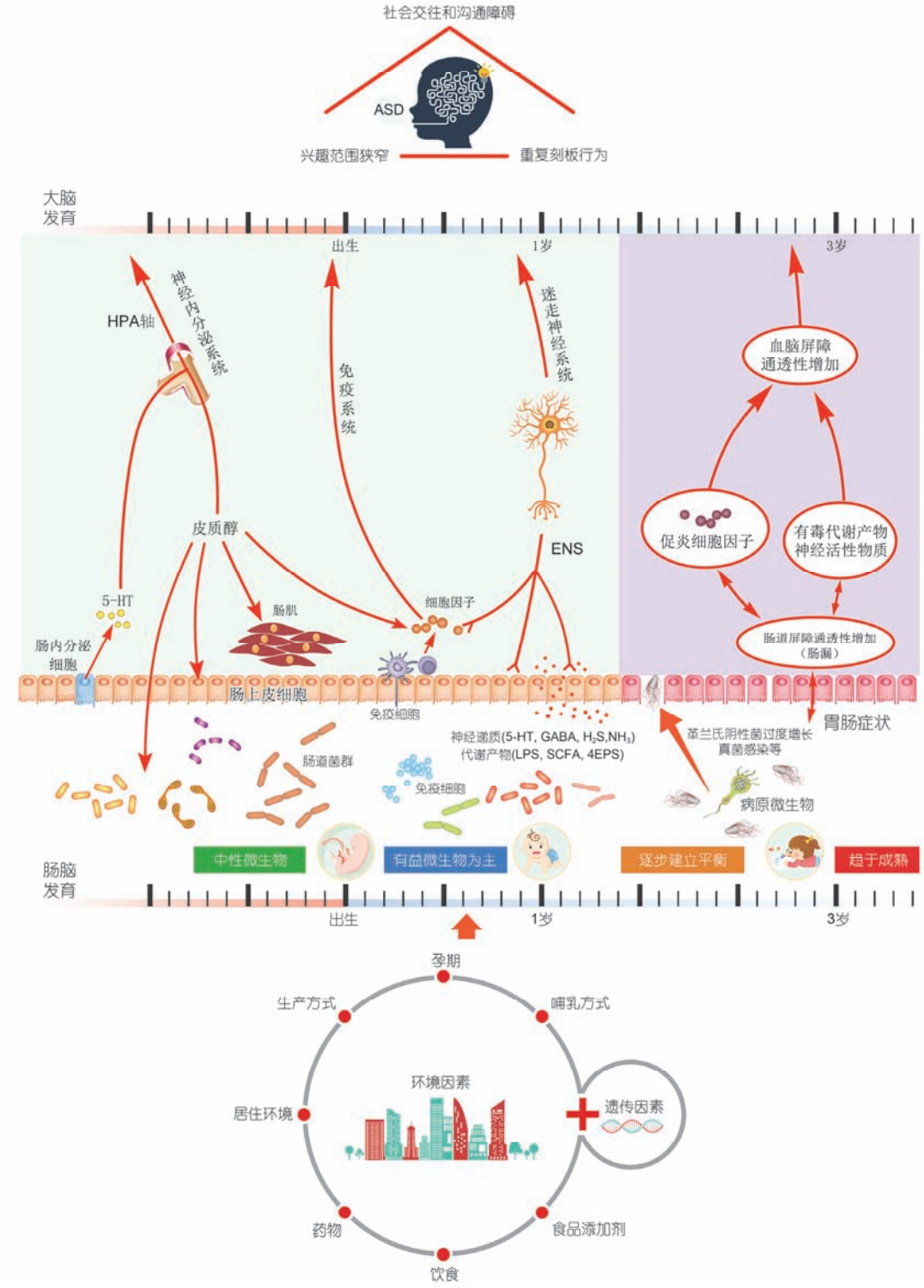

图 1 孤独症与肠道菌群失衡及肠-脑轴异常相关. 肠脑与大脑对称协同发育, 成长关键期多种影响肠道菌群的因素均可增加孤独症风险. 肠道微生物可通过代谢产物、免疫、神经内分泌以及迷走神经等途径影响孤独症. 异常的肠道微生物造成肠漏与血脑屏障通透性增加也在孤独症中起重要作用

Figure 1 ASD closely associated with imbalanced intestinal flora and abnormal gut-brain axis. Gut brain has collaborative development with the great brain, while variety of factors in the critical period may damage the intestinal flora and increase the risk of ASD. Microorganisms in gut may influence ASD through metabolites, immunity, neuroendocrine, and vagal nerves. Abnormal intestinal microbiota causes leaky gut and blood-brain barrier permeability which act an important role in ASD

糖醇含量高于正常人, 研究人员发现给22位ASD孩子服用嗜酸乳杆菌后, 尿液中真菌感染的标志物质D-阿拉伯糖醇(D-DA)数量明显降低, 并且D-阿拉伯糖醇与L-阿拉伯糖醇比例(DA/LA)也显著降低, 同时, 目光回避、社会交往以及反馈行为等ASD行为也明显改善[190]. 益生菌能够促进肠道菌群平衡, 抑制有害菌生长[191], 特别是抑制梭状芽孢杆菌生长, 减少有害菌产生的有毒物质, 并已经应用于临床治疗艰难梭菌感染[191,192], 也提示益生菌可用于治疗艰难梭菌感染的ASD患者.

有益微生物通过自凝集和共凝集作用清除有害微生物. 共生微生物表面包被着大量的IgA, 研究发现IgA具有多反应性, 并不针对单个细菌类群有特异性, 在初始B细胞中, IgA出现频率较低, 需依赖于在淋巴结的再循环而被选择, 且选择进程不依赖于菌群或饮食抗原[193].

4.2 调节宿主代谢与吸收

益生菌能够促进宿主的营养吸收, 帮助宿主代谢药物或重金属, 对消化代谢有关的疾病有治疗效果[73,191,194]. 益生菌帮助ASD患者改善谷蛋白和酪蛋白不耐受的问题, 蛋白水解能力强的微生物在这一过程中起重要作用. 瑞士乳杆菌能减轻婴儿对牛奶过敏反应, 乳杆菌蛋白酶对αS1-和β-酪蛋白的水解降低人体免疫球蛋白IgE特异性识别[195], 改善ASD患者的蛋白代谢问题.

肠道微生物影响肠道屏障完整性、上皮细胞再生、黏液产生和肠动力[196]. 补充特定益生菌可改善ASD患者和ASD模型动物的肠漏和生理行为异常[197]. 例如, 脆弱拟杆菌处理ASD样小鼠, 结果小鼠的肠道通透性得到明显改善, 并伴随着ASD样行为的改善[29]. 瑞士乳杆菌R0052和长双歧乳杆菌R0175能够抑制肠漏, 防止慢性应激引起大脑突触可塑性异常和神经形成减少的发生[198]. 鼠李糖乳杆菌LOCK0900、鼠李糖乳杆菌LOCK0908和罗伊氏乳杆菌LOCK0919可提升GF小鼠肠道黏膜屏障完整性, 减轻过敏症状[199].

4.3 通过微生物-肠-脑轴治疗自闭症

益生菌干预不仅能调节菌群异常, 还能改善微生物-肠-脑轴功能异常, 从而治疗ASD.

益生菌可通过迷走神经改善ASD. 长双歧杆菌NCC3001能通过迷走神经防止小鼠的焦虑行为以及海马脑源性神经营养因子(brain-derived neurotrophic factor, BDNF) mRNA的表达异常[200,201,239]. 十二指肠约氏乳杆菌定植可通过组胺影响肾脏交感神经和胃迷走神经的活动[202]. 鼠李糖乳杆菌也通过迷走神经影响大脑功能[173]. 复合益生菌(乳酸双歧杆菌CNCM I-2494、保加利亚乳杆菌、嗜热链球菌和乳酸乳杆菌)干预能通过迷走神经改变健康志愿者在接受情绪反应测试时脑岛的活动[203].

益生菌可通过免疫途径改善ASD. 益生菌能够增强机体的免疫调节机能, 促进Treg形成, 增加抗炎症细胞因子水平, 降低促炎症细胞因子水平[204,205], 从而抑制ASD患者免疫系统的长期激活状态. 瑞士乳杆菌NS8能促进抗炎症细胞因子IL-10释放, 调节免疫[206]. 婴儿双歧杆菌能够降低血浆中促炎症细胞因子的浓度和色氨酸代谢水平[143,207]. 益生菌能降低炎症水平, 改善生理和认知异常[208].

益生菌可通过神经内分泌途径改善ASD. 瑞士乳杆菌R0052和长双歧乳杆菌R0175能改善HPA轴功能[198]. 罗伊氏乳杆菌可提升下丘脑OT水平, 激发中脑腹侧被盖区神经元[22]. 瑞士乳杆菌NS8可降低血浆皮质酮(CORT)和促肾上腺皮质激素(ACTH)水平, 恢复海马5-HT和去甲肾上腺素(NE)水平, 提升海马BDNF mRNA的表达[209]. 发酵乳杆菌NS9可提升海马盐皮质激素和天冬氨酸受体水平[210]. 鼠李糖乳杆菌可以改变抑制性神经递质GABA的神经传导和在特定脑区的表达[173]. 动物双歧杆菌可作为抗氧化剂减少氧化应激反应, 抑制神经炎症, 减少单胺氧化酶的酶活性使得神经递质(5-HT、多巴胺、NE)能在突触间隙停留较长的时间[211]. 某些益生菌还能降低毒性气体神经信号水平, 如氨水平[162].

事实上, 益生菌干预并不直接作用大脑和行为, 而是针对肠道. 通过纠正肠道微生物失衡, 重建或恢复肠脑与大脑的正常信息交流, 从而改善微生物-肠-脑轴功能更可能是ASD治疗的最佳选择.

5 展望

肠道微生物通过微生物-肠-脑轴影响大脑功能与行为, 与ASD的发生和发展密切相关. ASD患者存在肠道微生物异常和微生物-肠-脑轴功能异常, 肠道微生物可能是ASD治疗的良好靶点. 然而, 由于ASD患者的饮食、生活习惯、药物使用情况以及个体遗传

因素致使肠道微生物组差异极大, 目前尚难以确定ASD患者特定的肠道微生物图谱.

ASD可能为感染症, 针对微生物的干预方法能够显著改善ASD症状. 虽然临床研究显示抗生素对ASD具有显著的干预效果, 但是一旦停药患者会出现退步, 因而抗生素不是理想的干预手段. FMT是一种新的改变患者肠道微生物的方法, 然而应用于ASD的治疗还缺乏临床评估和安全性评价. 补充有益微生物帮助幼儿逐步建立与微生物共生的关系, 可能是帮助人体重建微生态平衡最为安全有效的方法.

益生菌干预可改变肠道微生物群落和微生物-肠-脑轴功能, 从而治疗ASD. 在诸多益生菌中, 具有积极的行为效应的多为乳酸杆菌和双歧杆菌[180,187,209,210]. 通过补充益生菌干预ASD存在最佳关键期, 而错过关键期干预效果会大打折扣. 目前有益微生物在心理疾病中的应用研究如火如荼地在世界范围开展, 并有望今后在ASD的治疗中成为主流. 但是由于益生菌种类繁多, 不同的菌类具有不同的功能, 在应用于改善疾病状态时必须明确到菌株而不是菌种. 大量动物研究以及临床研究已经揭示益生菌在ASD治疗的作用以及良好的前景, 但是临床的安全性深入研究十分必要, 益生菌应用于改善和治疗ASD也需进一步研究和规范化. 尽管任重道远, 微生物干预治疗ASD已经开始初现曙光.

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Summary for “肠道微生物与自闭症研究进展”

Gut microbiota and autism

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Autism spectrum disorder (ASD) is one of the most severe neurodevelopmental disorders in the world, and it has brought tremendous burden for family and society. However, there is still no effective method clinically to cure this disorder. The morbidity of the disorder has increased rapidly in recent decades, which does not correspond with Hardy-Weinberg Equilibrium, indicating the disorder is more involved in some environmental factors than genes. Maternal disturbances, poor diet, and leaky gut are remarkable risk factors for autism, all of which can induce gut microbiota dysbiosis. More and more studies have indicated that autism is closely related to imbalanced gut microbiota and abnormal gut-brain axis. Autistic children have different gut microbiota including bacteria and fungi from healthy children, and gut microbiota modulation using either antibiotics, probiotics, or fecal microbiota transplantation may change autistic symptoms. Due to the synchronization of gut brain development and brain development, factors that affect the gut microbiota development of infants during the critical period will increase the incidence rate of autism.

Gut microbiota can influence brain development and brain function like behavior and cognition through gut-brain axis/microbiota-gut-brain axis. The microbiota-gut-brain axis mainly include four pathways, which are metabolism, immune system, neuroendocrine system, and vagus nervous system. Abnormal microbiota can increase the harmful metabolites including 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) and 4-ethylphenylsulfate (4EPS) to induce autistic-like behaviors. Gut microbiota regulates the development and function of immune system, and microbiota dysbiosis can result in chronic inflammation to impair the normal development of brain and mentality. Abnormal microbiota can induce autistic symptoms through neuroendocrine pathway, it probably disturbs the development and function of hypothalamus-pituitary-adrenal (HPA) axis, influence the activity of serotonergic system and oxytocin system, and increase the content of harmful gas neurotransmitters such as hydrogen sulfide (H_2S) and ammonia (NH_3). Microbiota dysbiosis can also lead to autistic-like behaviors through vagus nervous system.

Gut microbiota dysbiosis will possibly be the effective target of autism treatment. Increasing studies have shown that gut microbiota restoration including probiotics supplementation alleviates autism symptoms. Certain beneficial microbial strains can recover normal microbiota, prevent pathogen infection, alleviate gut barrier leakiness, prompt gastrointestinal function, and improve behavior and cognition development. These beneficial bacteria probably alleviate autism through regulating the microbiota-gut-brain axis function. They can improve brain function via vagus nervous systems, alleviate brain inflammation through immune response regulation, and restore normal brain neurotransmitters and neurogenesis through HPA function recovery and neurotransmitters metabolism regulation.

The new intervention is different from traditional interventions which usually focus on one or two symptoms of autism, it not only restores normal gut microbiota but also improve the whole microbiota-gut-brain axis function including the gut brain and the great brain. Certain symbiotic microorganism intervention will probably become promising auxiliary treatment for autism. In the present paper, we review and summarize some valuable related researches between autism and gut microbiota, to provide an important reference for comprehensive prevention and treatment of autism in China.

autism, gut brain, leaky gut, microbiota-gut-brain axis, probiotics

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