

肺部微生物调控免疫与肺移植稳态

刘天华, 董明*

(天津医科大学总医院肺部肿瘤外科, 天津 300052)

摘要: 肺部微生物作为影响机体病理生理过程的复杂微生态系统, 调控多种肺疾病的致病机制。肺部微生物不仅参与代谢、炎症和免疫稳态等多种生物学过程, 而且影响肺移植术后慢性肺同种异体移植物功能障碍(chronic lung allograft dysfunction, CLAD)的发生发展。肺移植作为终末期肺疾病的主要治疗手段, 其术后并发症一直是亟待解决的问题。本文主要介绍了机体健康和疾病状态下肺部微生物的组成, 重点探讨了肺部微生物与免疫及肺移植稳态之间的联系, 以期肺部微生态环境的临床调控提供理论依据。

关键词: 肺部微生物; 呼吸系统疾病; 免疫耐受; 肺移植

Lung microbes regulate immunity and homeostasis in lung transplantation

LIU Tianhua, DONG Ming*

(Department of Pulmonary Oncology, General Hospital of Tianjin Medical University, Tianjin 300052, China)

Abstract: As a complex micro-ecological system that affects the pathophysiological process of the body, lung microorganisms regulate the pathogenic mechanisms of various lung diseases. Studies have found that lung microbes not only participate in various biological processes such as metabolism, inflammation, and immune homeostasis, but also affect the occurrence and development of chronic lung allograft dysfunction (CLAD) after lung transplantation. Lung transplantation is the main treatment for end-stage lung disease, and its postoperative complications have always been an urgent problem to be solved. This article mainly introduces the composition of the lung microbiome in healthy and diseased states and focuses on the relationship between the lung microbiome, immunity, and lung transplant homeostasis, to provide a theoretical basis for the clinical regulation of the lung microecological environment.

Key Words: lung microbiome; respiratory diseases; immune tolerance; lung transplantation

基于人体强大的免疫能力, 人们曾一度认为健康人的肺是无菌的。近十年来, 通过改善分子测序方法证实了肺部存在生物多样性。肺部微生物的早期研究起步受限, 主要是由于下呼吸道采

样技术的高难度、健康肺内菌群的低密度和低稳定性^[1], 以及动物模型的缺乏^[2]。肺部微生物不仅与肺疾病有关, 而且影响移植领域中免疫耐受和感染易感性的平衡。为了阐明肺部微生物如何影

收稿日期: 2022-11-20

基金项目: 天津市教育委员会自然科学基金(2019KJ202); 天津市肺癌研究所面上基金(TJLCMS2021-05); 中央高校基础研究基金(3332018180)

第一作者: E-mail: 1243568139@qq.com

*通信作者: E-mail: tjydming@yeah.net

响肺稳态的建立, 本文集中探讨肺疾病中肺部微生物组成、肺部微生物模组和机体的相互作用, 以及关联于肺移植的肺部微生物。

1 肺部微生物

1.1 肺部微生物的来源与组成

肺部微生物群是一种结合微生物、遗传物质和代谢产物的综合体。肺部微生物自婴儿出生后形成, 受生产和环境的影响, 在数日或数周内分化为不同的族群^[3]。生命初期, 肺部微生物的定植尚未稳定, 容易受到室内外空气组分的干扰。有研究结果表明, 在室内条件下, 肺内有棒状杆菌、链球菌、葡萄球菌、丙酸杆菌、乳球菌和肠杆菌富集; 在户外, 则有假单胞菌、不动杆菌和鞘氨醇单胞菌富集^[4]。由此看来, 肺部微生物具有空间差异的特性。

肺部微生物的密度要比上呼吸道低几个数量级, 但由于二者持续地、动态地交流, 在群落组成上高度一致。通过比较健康人群的漱口水、鼻拭子和支气管肺泡灌洗液(bronchoalveolar lavage fluid, BALF)中的微生物群落, 发现肺和口腔存在

着如普雷沃菌、韦荣氏球菌、罗斯氏菌、链球菌和卟啉单胞菌等相似的微生物群^[5,6]。这表明上呼吸道的微生物很可能通过黏膜扩张和微吸入进入肺部^[7]。另外, Dickson等^[8]在脓毒症小鼠模型及急性呼吸窘迫综合征患者的肺部均发现了丰富的肠源细菌, 这提示了血行播散和肠道菌群易位也是肺部微生物的潜在来源途径^[7]。

1.2 不同状态下的肺部微生物

基于聚合酶链式反应(polymerase chain reaction, PCR)和宏基因组学的研究发现, 健康肺部的细菌群落组成相对稳定, 在门水平上主要由厚壁菌门、变形菌门、拟杆菌门和放线菌门组成^[9]。核心气道优势细菌属主要有假单胞菌属、链球菌属、普雷沃菌属、梭杆菌属、流感嗜血杆菌属、奈瑟菌属、葡萄球菌属和卟啉单胞菌属等^[10,11](表1)。此外, 健康肺部还包含真菌和病毒, 真菌包括曲霉属、青霉属、念珠菌属、枝孢菌属和链格孢属^[10](表1); 病毒则以指环病毒(anelloviridae)和噬菌体为主, 同时也有微量的流感病毒、副流感病毒、鼻病毒、呼吸道合胞病毒和腺病毒等^[12]。

表1 不同人群的肺部微生物组成

检测人群	样本	方法	主要发现	参考文献
健康人群	口咽冲洗液和BALF	16S rRNA基因测序、ITS鉴定	BALF与口咽样本有相似的细菌谱, 包括链球菌、普雷沃菌、韦荣氏球菌等; BALF真菌谱主要有枝孢菌和曲霉菌	[10]
肺移植受者	口咽冲洗液和BALF	16S rRNA基因测序、ITS鉴定	BALF中微生物丰度下降, 主要以铜绿假单胞菌、链球菌和念珠菌为主	[10]
囊性肺纤维化患者	痰液	蛋白质组学	发现了链球菌、铜绿假单胞菌、芽孢杆菌、梭菌、曲霉菌和青霉菌等微生物	[14]
哮喘患者	口咽冲洗液和支气管冲洗液	16S rRNA基因测序	发现梭杆菌、放线杆菌、嗜血杆菌、奈瑟菌和卟啉单胞菌与哮喘的发病有关	[18]
肺癌患者	痰液和支气管冲洗液	16S rRNA基因测序	在支气管冲洗液中, 微生物以普雷沃菌、链球菌、韦荣氏球菌和奈瑟菌为主; 韦荣氏球菌和罗斯氏菌有助于预测肺鳞癌转移状态; 链球菌有助于预测肺腺癌转移状态	[24]
慢性阻塞性肺病患者	口咽拭子、鼻咽拭子、支气管拭子、外周肺标本	16S rRNA基因测序、qPCR	下呼吸道中链球菌、棒状杆菌、普雷沃菌、韦荣氏球菌、罗斯氏菌常见, 多来自口咽部微生物	[30]
新冠患者	痰液、鼻拭子、咽拭子	宏转录组测序	重症新冠患者主要呼吸道类群是洋葱伯克霍尔德菌、表皮葡萄球菌和支原体	[36]
新冠患者	BALF	16S rRNA基因测序	重症新冠患者下呼吸道中产碱假单胞菌、鞘氨醇单胞菌、链球菌、肠杆菌和不动杆菌含量丰富	[38]

16S rRNA(16S ribosomal ribo nucleic acid)是原核生物的核糖体中30S亚基的组成部分; 内部转录间隔区(internal transcribed spacer, ITS)鉴定是指对ITS序列进行脱氧核糖核酸(deoxyribo nucleic acid, DNA)测序, 通过将测序得到的ITS序列与已知真菌ITS序列比较, 从而获得待测真菌种属信息的一种方法

1.2.1 囊性肺纤维化(cystic pulmonary fibrosis, CPF)

CPF是一种常染色体隐性遗传病。有研究认为, CPF的发生与一种跨膜调节因子突变导致的菌群感染有关^[13]。CPF患者的肺部微生物主要由铜绿假单胞菌、芽孢杆菌、奈瑟菌、卟啉单胞菌、普雷沃菌、链球菌、韦荣氏球菌和曲霉菌组成^[14](表1)。其中, 铜绿假单胞菌和嗜麦芽窄食单胞菌的过敏原蛋白酶能够诱使肺上皮细胞产生炎症因子白介素-33(interleukin-33, IL-33), 进而造成功能紊乱^[15]。另外, Carmody等^[16]还发现, 厌氧菌的增多会导致CPF病情恶化。

1.2.2 哮喘

哮喘是一种气道慢性炎症性疾病, 主要特征包括气道高反应性、可逆性气流受限及气道重塑^[17]。哮喘患者肺部微生物由流感嗜血杆菌、放线杆菌、奈瑟菌、梭杆菌和卟啉单胞菌等组成^[18](表1)。此外, 肺孢子菌和呼吸道合胞病毒的失调也参与哮喘的发病机制^[19,20]。一项对重症哮喘患者的痰液细菌学分析表明, 流感嗜血杆菌、奈瑟菌和链球菌的增加与中性粒细胞、IL-8水平的增高和肺功能恶化有关, 可以作为哮喘发病的预测标志物^[21]。

1.2.3 肺癌

肺癌占有癌症死亡人数的四分之一, 肺部微生物与肺癌的发生、治疗和预后存在着复杂而矛盾的关系^[22]。采用保护性毛刷刷检方法, 发现肺癌患者肺部富含普雷沃菌、肠球菌、链球菌、韦荣氏球菌、巨球菌、嗜二氧化碳细胞菌和流感嗜血杆菌等菌群^[23-26](表1)。线性判别分析证实, 鳞癌比腺癌有着更丰富的多样性, 鳞癌与沙雷氏菌、克鲁维菌、摩根氏菌、无色杆菌和嗜二氧化碳细胞菌具有相关性; 腺癌则与不动杆菌、丙酸杆菌、葡萄球菌具有相关性^[27]。而在鳞癌和腺癌的远处转移性样本中, 分别发现了韦荣氏球菌、罗斯氏菌和链球菌的显著增加^[24](表1)。另外, 对比健康组织, 在晚期肺癌患者的肿瘤组织中还发现了丰富的栖热菌和军团菌^[28]。

1.2.4 慢性阻塞性肺病(chronic obstructive pulmonary disease, COPD)

COPD是以气流持续受限、慢性支气管炎和阻

塞性肺气肿为特征的肺疾病^[29]。稳定期COPD患者的肺部微生物主要是链球菌、普雷沃菌、罗斯氏菌、流感嗜血杆菌、卡他莫拉菌和奈瑟菌^[30,31](表1)。急性期的肺部微生物多样性减少, 主要由流感嗜血杆菌、肺炎链球菌和卡他莫拉菌组成^[32]。其中, 流感嗜血杆菌的增加通常预示着病情恶化^[33,34]。另一项横断面研究发现, 假单胞菌和密螺旋体的比例降低会加重COPD患者气道阻塞程度^[35]。

1.2.5 新型冠状病毒肺炎(corona virus disease 20-19, COVID-19)

COVID-19是一种呼吸道病毒感染疾病, 病毒感染可能通过改变肺部微生物稳态从而加重疾病严重程度。通过对多种类临床标本进行元转录组分析, Zhong等^[36]在重症COVID-19感染者中鉴定出洋葱伯克霍尔德菌、表皮葡萄球菌和支原体等微生物(表1)。Dickson等^[37]使用支气管镜对COVID-19重症患者下呼吸道进行采样, 发现病毒体的高载量和一种口腔共生菌——唾液支原体的富集, 通常预示着不良的临床结果。Gaibani等^[38]将COVID-19重症和阴性患者的BALF进行对比, 结果显示, 重症患者肺部产碱假单胞菌、鞘氨醇单胞菌、链球菌、肠杆菌和不动杆菌含量明显增多(表1)。

由此可见, 肺部微生物的失调与不同的肺疾病相关, 为了更好的预防及治疗, 确定参与不同疾病的关键微生物至关重要。

1.3 肺部微生物独特的生态位

1.3.1 适应性岛屿模型

从微生物富集的上呼吸道延伸到微生物稀疏的肺泡, 存在着微生物迁移与消除的动态平衡。“适应性岛屿模型”将微生物群落比喻成一座岛^[39,40]。模型解释了如何平衡微吸入迁移机制和黏液纤毛、咳嗽反射以及噬菌体等消除机制, 从而形成具有稳态的肺部微生物群落。该模型也可作为探讨肺疾病的基石, 当呼吸系统受到损害时, 肺部微生物的暴露条件发生变化, 将会导致生态失衡和发病机制紊乱。

1.3.2 气道特性选择

呼吸道是一个具有空间异质性的生态系统, 时刻处于动态的环境中, 不同的温度、湿度、酸

碱度、氧分压及营养可用性等因素都会对不同解剖学部位的微生物定植产生影响^[41]。比如, 高黏液区域能为铜绿假单胞菌提供适宜的生态环境^[42]; 黏膜纤毛会选择不同黏附特性的微生物^[43]; 杯状细胞和黏膜下腺的分泌物能作为营养源诱使微生物聚集等^[43]。此外, 呼吸道还分泌多种抗菌肽, 如表面活性蛋白A、乳铁蛋白和防御素等^[44], 能够依据抗菌特性筛选合适的微生物。

1.3.3 免疫系统选择

免疫功能障碍会影响肺部微生物的定植。例如在抗逆转录病毒治疗后, 肺部惠普尔养障体的含量骤降^[45]。抗逆转录病毒治疗1年后, 肺部微生物多样性下降, 但链球菌、普雷沃菌和韦荣氏球菌含量总体增多^[46]。这提示了在发育和进化中避开机体免疫反应的微生物才更有可能长期存在。

2 肺部微生物与免疫

肺部微生物和机体免疫系统之间的双向串扰在保持免疫稳态方面发挥着重要作用。肺部微生物的失调可能是肺疾病易感性、进展和慢性化的基础。合理利用微生物预防与治疗可以最大程度地减少失调的负面影响。

2.1 肺部微生物调节免疫系统

2.1.1 肺部微生物的宏观调控

肺部微生物能够促进免疫系统的发育和成熟。Gollwitzer等^[47]研究认为, 肺部微生物的存在可以促进新生小鼠体内调节性T细胞(regulatory T cell, Treg)的发育和程序性细胞死亡配体1(programmed cell death-ligand 1, PD-L1)的成熟, 增加对过敏原的耐受性。Jang等^[48]收集了84名肺癌患者的BALF进行分析, 结果显示, 殊异韦荣球菌和奈瑟菌的含量分别在高、低PD-L1表达组里占据优势, 并证实殊异韦荣球菌具备更强的免疫应答能力。这些研究结果能够为免疫治疗提供新的思路。

肺部微生物在调节宿主病理生理过程中也发挥关键作用。研究发现, 尼泊尔葡萄球菌能够分泌一种高盐依赖性促凋亡肽corisin, 会大幅诱导中性粒细胞浸润, 导致肺上皮细胞凋亡和胶原蛋白沉积, 加重肺疾病^[49]。

除了肺部微生物本身, 其代谢产物也具有调

控免疫的作用。微生物衍生的维生素B代谢物抗原, 通过主要组织相容性复合体 I 类相关基因蛋白-T细胞抗原受体激活黏膜相关恒定T细胞, 释放颗粒酶B、穿孔素、 γ 干扰素(interferon- γ , IFN- γ)、肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)、IL-17和粒细胞-巨噬细胞集落刺激因子(granulocyte-macrophage colony stimulating factor, GM-CSF)等杀伤感染细胞^[50]。在某些情况下, 微生物产生的基因毒素和代谢产物会破坏宿主DNA, 通过活性氧(reactive oxygen species, ROS)、含氮物质或自然杀伤免疫受体诱导其损伤, 并促进细胞增殖和血管生成, 导致癌症相关特异性改变^[51,52]。

2.1.2 肺部微生物调节Th1/Th2和Treg/Th17平衡

分化决定簇抗原4⁺(cluster of differentiation 4⁺, CD4⁺)T细胞包含三种主要分化亚型: 辅助性T细胞1(helper T cell 1, Th1)、Th2、Th17。Th1能产生IL-4和IFN- γ 增强机体对病原体感染的免疫防御, 而Th2被认为是“免疫缺陷”, Th1/Th2在正常体内处于平衡状态^[53]。在移植研究中发现, Th1/Th2细胞相对水平的变化是产生排斥反应或免疫耐受的重要原因^[54]。Th17能够分泌IL-17、IL-6和TNF- α 等炎症因子, 可巩固机体防御杀伤病原体, 也可增强炎症反应引起组织损伤^[53]。此外, Treg通过细胞间的直接接触及分泌抑制性细胞因子实现免疫抑制^[55], 和Th17相互制约, 维持机体稳态。肺部微生物可以调节Th1/Th2以及Treg/Th17之间的平衡。

丝裂原活化蛋白激酶(mitogen activated protein kinase, MAPK)/细胞外调节蛋白激酶(extracellular regulated protein kinase, ERK)和磷脂酰肌醇-3-激酶(phosphatidylinositol-3-kinase, PI3K)/蛋白激酶B(protein kinase B, PKB)的信号传递途径涉及调节细胞生长、发育及组织侵袭^[56,57]。Tsai等^[26]发现, 肺癌患者下呼吸道中韦荣氏球菌和链球菌的富集具有影响ERK和PI3K通路的作用。该团队在晚期肺癌小鼠模型中证实, 韦荣氏球菌引起的生态失调会激活MAPK/ERK和PI3K/PKB通路并上调Th1、Th17, 释放炎症因子, 影响肿瘤进展和预后^[23]。同样, 在非小细胞肺癌中, 唾液链球菌和B族链球菌也被证实能够增强Th1和Th17反应^[58]。

博来霉素是一种具有抗肿瘤活性的碱性糖肽类抗生素,其毒副作用之一是引起肺纤维化^[59]。Yang等^[59]研究指出,失调的肺部微生物参与了其纤维化过程,扩增的拟杆菌和普雷沃菌借助细菌外膜囊泡驱动Toll样受体(Toll-like receptor, TLR)-髓样分化因子88(myeloid differentiation factor 88, MyD88)信号诱导促纤维化基因*TNF- α* 增多和肺泡巨噬细胞中IL-17B的表达,形成炎症网络并上调Th17,参与肺纤维化。此外,韦荣氏球菌和链球菌的异常增多也会引起肺内MyD88依赖性Th17炎症反应增加^[60]。

转化生长因子- β (transforming growth factor- β , TGF- β)可使成熟的T细胞转化为Treg,避免自身免疫疾病的发生。而在大量IL-6的作用下,TGF- β 促进成熟T细胞向Th17的分化,导致自身免疫或炎症^[61]。Ren等^[62]将COPD患者和健康人群的肺部微生物分成3个亚组,发现以链球菌和罗斯氏菌为主的亚组中与Th17分化的炎性因子,包括IL-6、IL-17、TGF- β 、STAT3和RORC的表达水平显著增加,说明肺部微生物可能调控IL-6和TGF- β 影响Th17的形成。

铜绿假单胞菌是CPF患者肺部最常见的细菌。Collin等^[63]报道了该细菌通过Th17炎症反应途径间接促进CPF患者体内免疫球蛋白A表达上调,启动体液免疫防御机制。Yadava等^[64]发现,经脂多糖(lipopolysaccharide, LPS)和弹性蛋白酶处理的小鼠,体内假单胞菌富集,并伴有IL-17A、IL-6和

IL-1B等炎症因子明显增加,猜测假单胞菌很可能是LPS引起炎症反应的介质之一。

脆弱拟杆菌是一种革兰氏阴性厌氧菌,其表面的荚膜多糖A是由四糖重复单元组成的两性离子免疫调节多糖。在小鼠模型中,多糖A通过TLR2激活CD11c⁺树突状细胞(dendritic cell, DC),DC则将多糖A呈递给主要组织相容性复合体-II(major histocompatibility complex-II, MHC-II),并结合共刺激分子CD86,以此激活CD4⁺T细胞。多糖A还诱导DC细胞分泌IL-12,结合CD4⁺T细胞表面的IL-12受体,激活Th1特异性转录因子STAT4,驱动Th1细胞活化,产生IFN- γ 介导免疫平衡^[65](图1)。

金黄色葡萄球菌是人类呼吸道常见的共生细菌之一。金黄色葡萄球菌和表皮葡萄球菌的分子信号可以通过IL-17A启动GM-CSF活性,进而释放ERK刺激肺泡巨噬细胞通过ROS杀灭病原体(肺炎链球菌和肺炎克雷伯杆菌)^[66],增强呼吸系统免疫。金黄色葡萄球菌还能增强免疫细胞表面的TLR2表达,促进IL-13释放,营造肺泡诱导环境,然后通过CC类趋化因子配体2(C-C motif chemokine ligand 2, CCL2)介导的趋化作用募集外周血CC类趋化因子受体2(C-C motif chemokine receptor 2, CCR2)阳性的巨噬细胞亚群进入肺泡,在诱导环境中将其极化为M2型巨噬细胞,进而促进Th2分化并释放IL-10和TGF- β 减轻肺损伤^[67]。CCL24是由M2a巨噬细胞分泌的一种趋化因子,结合CCR3受体募集嗜酸性粒细胞、嗜碱性粒细胞等,在肿瘤

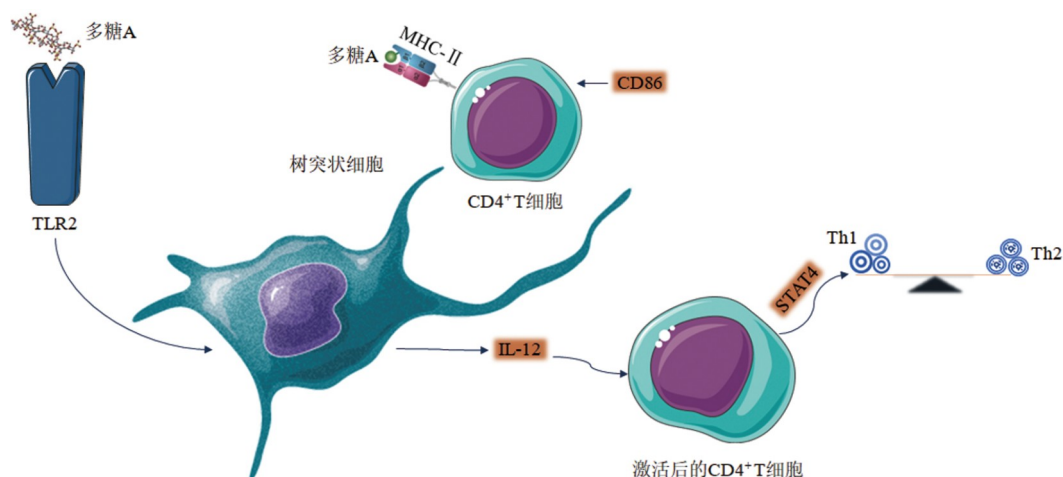


图1 脆弱拟杆菌多糖A驱动Th1分化

进展中发挥作用。通过小鼠模型,发现肺部共生细菌能够通过维持低水平的CCL24,促进肺部免疫细胞IL-17A⁺γδT浸润,参与抗肿瘤反应^[68]。

综上,微生物的抗炎和促炎机制的研究可用于设计合理的微生物疗法。微生物与免疫之间的复杂的相互作用已成为疾病治疗的一个新方向。

2.2 免疫系统对肺部微生物的反馈

免疫系统通过免疫细胞表面的模式识别受体(pattern recognition receptor, PRR)结合微生物表面的病原体相关分子模式(pathogen-associated molecular pattern, PAMP)启动固有免疫应答。PRRs包括TLR、核苷酸结合寡聚化结构域样受体、黏附分子和C型凝集素受体(C-type lectin receptor, CLR)^[69]。TLR2识别的配体包括革兰氏阳性细菌、分枝杆菌、酵母菌的某些成分,如脂蛋白、脂磷壁酸、肽聚糖等^[70],TLR4特异识别革兰氏阴性细菌的LPS^[71],TLR7、TLR8识别单链RNA病毒^[72,73],CLRs能够识别致病真菌^[74]。这些受体协调先天免疫反应,促进炎症因子、趋化因子和IFN的产生,同时把具有免疫原性的小分子抗原肽,借助MHC提供给T细胞,启动获得性免疫应答(图2)。

机体气道黏膜能够识别肺部致病微生物,进而释放抗菌肽和炎症因子,先天免疫细胞(如吞噬细胞、中性粒细胞、巨噬细胞、肥大细胞等)参与反应。其中,巨噬细胞可以结合致病微生物表面的LPS,通过核因子kappa-B或c-Jun氨基末端激酶/

应激活化蛋白激酶信号通路启动细胞内的信号转导,分泌多种细胞因子如TNF-α、IL-8和IL-1B,引起自噬反应的发生^[75,76]。

3 肺部微生物与肺移植

肺移植作为终末期肺病患者的首选治疗,其术后的死亡率和免疫排斥率仍高于其他所有实体器官移植受者。而随着对肺部微生物认知的丰富,也渐渐明晰了移植免疫浸润细胞在肺移植中所扮演的独特作用。

3.1 肺移植受者的肺部微生物

肺移植术后,肺部微生物发生了紊乱。Das等^[77]对64名肺移植受者的234份纵向BALF样本进行分析,发现移植肺和健康肺的微生物组成有很大的重叠,主要由普雷沃菌、链球菌、韦荣氏球菌、奈瑟菌、假单胞菌、葡萄球菌和颗粒菌组成。该团队还依据BALF样本的细菌群落差异,应用机器学习方法将其分为4种亚型,其中以金黄色葡萄球菌和铜绿假单胞菌为主的2种亚型,病毒和细菌载量最多,术后发生感染的几率最大^[77]。瑞典的前瞻性研究对移植受者1年后的BALF样本进行了评估,发现最常见的微生物是白色念珠菌、假单胞菌和凝固酶阴性葡萄球菌^[78]。Beaume等^[79]证实,在CPF患者进行肺移植后,假单胞菌会首先占据受体肺部微生物群,并发生从黏液表型到非黏液表型的转变。而在移植95 d后,放线杆菌取代假单胞菌占据优势地位。这种生物过程的转变很

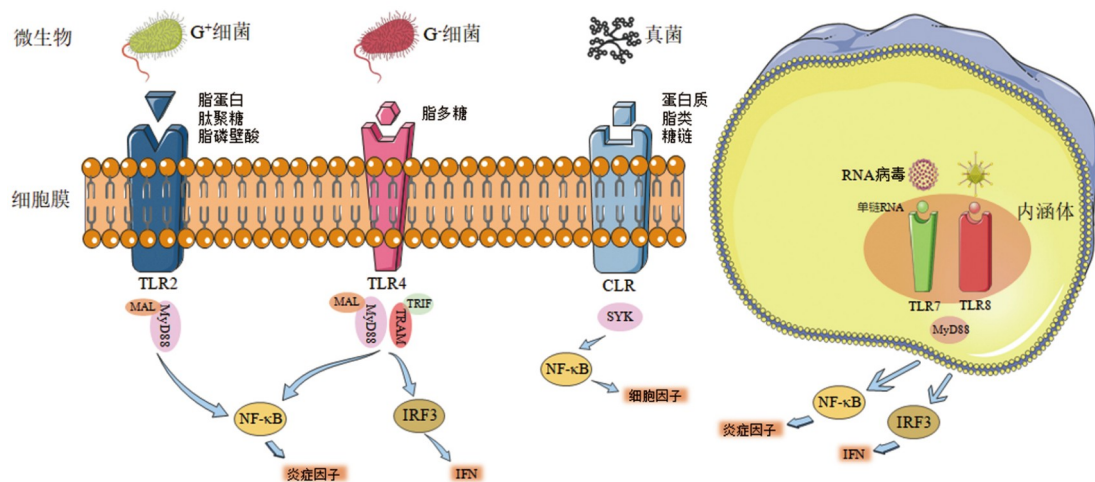


图2 免疫细胞对微生物的特异识别

可能导致了受体肺部微生物的失调和多样性的下降。

一项更精细的研究表明,肺移植受者BALF中假单胞菌、肠杆菌和葡萄球菌含量丰富,而普雷沃菌、韦荣氏球菌和链球菌的含量相对较低。假单胞菌又可分为铜绿假单胞菌和荧光假单胞菌。铜绿假单胞菌与肺移植术后肺部微生物多样性降低、细菌DNA富集、中性粒细胞增多以及呼吸道感染有关^[80]。

Van等^[81]收集了6年内286名肺移植受者的临床数据,分析发现,肠杆菌和铜绿假单胞菌是引起移植后感染最常见的细菌。除细菌外,还有30%的感染是由于病毒所致,其中最常见病原体是呼吸道病毒(主要是细小核糖核酸病毒、流感病毒和呼吸道合胞病毒)和疱疹病毒(包括巨细胞病毒、单纯疱疹病毒和水痘-带状疱疹病毒)。anelloviridae是广泛存在于人体的共生病毒。有研究表明,在肺移植受者中不仅检测到供体肺的anelloviridae谱系,还在供体肺中追溯到受体血清中的anelloviridae谱系,表明病毒在受体和供体之间存在双向转移^[82]。移植后可能导致肺部微生物紊乱的其他因素包括:(1)术后机械通气引起移植肺内源性微生物紊乱^[82];(2)受者天然肺及原发疾病或者生存环境中的微生物串扰;(3)移植受者术后黏膜纤毛清除功能障碍、咳嗽反射失调、淋巴回流障碍;(4)免疫抑制剂或者抗菌药物对微生物群的影响。

3.2 肺部微生物与CLAD

肺移植受者生存率低,主要是由于CLAD的限制。通过分析134名肺移植受者的BALF样本,Combs等^[83]发现,肺部微生物的载量增多会引起慢性排斥反应和死亡事件的发生,强调了肺部微生物是诱发CLAD的危险因素。Mouraux等^[84]也证实了肺部微生物是炎症的潜在驱动因素和移植后存活状态的预测因子,他们发现,以葡萄球菌、假单胞菌和棒状杆菌为主的促炎细菌不仅能够促进分解代谢和基质降解改善重塑,还直接影响成纤维细胞活化和细胞外基质沉积,从而导致CLAD功能紊乱。

闭塞性细支气管炎综合征(bronchiolitis obliterans syndrome, BOS)是引起CLAD的重要因素,超过75%的肺移植受者在10年内会患有

BOS^[85]。研究表明,以放线杆菌为主的革兰氏阳性菌群与早期BOS肺移植受者的中性粒细胞表达呈负相关,并降低其发生急性排斥反应和重型BOS的几率^[86]。而铜绿假单胞菌的定植是BOS发展的危险因素。在肺移植受体中,铜绿假单胞菌能够诱导中性粒细胞通过共刺激分子CD80/CD86,促进CD4⁺T细胞活化以及IL-17⁺CD4⁺和IFN- γ ⁺CD8⁺T细胞的产生,并阻碍CD154/CD40和CD28/共刺激分子B7相互作用建立的移植免疫耐受^[87]。Borthwick等^[88]研究表明,铜绿假单胞菌感染气道上皮会导致细胞损伤和死亡,并且释放IL-1A诱导成纤维细胞促进炎症发展。

骨髓源性抑制性细胞(myeloid-derived suppressor cell, MDSC)是某些免疫细胞的前体,可分为促炎表型和免疫抑制型。远端气道中的促炎表型MDSC比例高,近端气道免疫抑制型MDSC更为常见,两者比例失衡会导致BOS并引起移植排斥^[89]。而肺移植后高水平的假单胞菌和葡萄球菌偏向于诱导免疫抑制性MDSCs,进而引发BOS^[90,91]。

在对单肺移植受者进行移植肺和天然肺的代谢组学分析中,Sharma等^[92]发现,移植肺微生物中高水平的血管内皮生长因子(vascular endothelial growth factor, VEGF)和乙酰化脯氨酸-甘氨酸-脯氨酸(acetyl Proline-Glycine-Proline, AC-PGP)与不动杆菌和假单胞菌的富集有显著相关性。AC-PGP是一种胶原蛋白降解产生的趋化因子,能够作用于中性粒细胞表面的CXC趋化因子受体1/2来趋化中性粒细胞并介导肺部炎症以及CLAD^[92]。而VEGF则能够通过缺氧诱导因子-1 α /VEGF通路参与BOS的致病过程^[93]。

4 展望

虽然先前的研究已经详细阐述了肺部微生物的总体特征,提出了微生物群与免疫反应稳态之间存在重要相关性,但仍缺乏充分的证据。随着人们对微生物学和免疫学日益了解,我们可以通过更优的动物模型和高质量临床研究来识别潜在的炎症标志物和微生物靶点,探索疾病的新机制和新治疗。

肺部微生物研究的未来方向需要关注个体微

生物功能和微生物的相互作用。例如, 精细使用抗生素或探针调控微生物的代谢途径, 以改变肺疾病的发展过程。确定微生物与气道驻留细胞之间的串扰以及不同生态位下肺部微生物的特征将为创新疗法奠定基础。

参考文献

- [1] O'Dwyer DN, Ashley SL, Gurczynski SJ, et al. Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *Am J Respir Crit Care Med*, 2019, 199(9): 1127-1138
- [2] Miller AJ, Spence JR. *In vitro* models to study human lung development, disease and homeostasis. *Physiology*, 2017, 32(3): 246-260
- [3] Santacroce L, Charitos IA, Ballini A, et al. The human respiratory system and its microbiome at a glimpse. *Biology*, 2020, 9(10): 318
- [4] Adams RI, Bateman AC, Bik HM, et al. Microbiota of the indoor environment: a meta-analysis. *Microbiome*, 2015, 3(1): 49
- [5] Bassis CM, Erb-Downward JR, Dickson RP, et al. Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. *mBio*, 2015, 6(2): e00037
- [6] Segal LN, Clemente JC, Tsay JCJ, et al. Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat Microbiol*, 2016, 1(5): 16031
- [7] Wu BG, Segal LN. Lung Microbiota and its impact on the mucosal immune phenotype. *Microbiol Spectr*, 2017, 5(3). doi: 10.1128/microbiolspec.BAD-0005-2016
- [8] Dickson RP, Singer BH, Newstead MW, et al. Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nat Microbiol*, 2016, 1(10): 16113
- [9] Dickson RP, Erb-Downward JR, Martinez FJ, et al. The microbiome and the respiratory tract. *Annu Rev Physiol*, 2016, 78(1): 481-504
- [10] Charlson ES, Diamond JM, Bittinger K, et al. Lung-enriched organisms and aberrant bacterial and fungal respiratory microbiota after lung transplant. *Am J Respir Crit Care Med*, 2012, 186(6): 536-545
- [11] Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS One*, 2010, 5(1): e8578
- [12] Jankauskait L, Miseviiien V, Vaidelien L, et al. Lower airway virology in health and disease—from invaders to symbionts. *Medicina*, 2018, 54(5): 72
- [13] Glasgow AMA, Greene CM. Epithelial damage in the cystic fibrosis lung: the role of host and microbial factors. *Expert Rev Respiratory Med*, 2022, 16(7): 737-748
- [14] Hardouin P, Pible O, Marchand H, et al. Quick and wide-range taxonomical repertoire establishment of the cystic fibrosis lung microbiota by tandem mass spectrometry on sputum samples. *Front Microbiol*, 2022, 13: 975883
- [15] Chen W, Chen S, Yan C, et al. Allergen protease-activated stress granule assembly and gasdermin D fragmentation control interleukin-33 secretion. *Nat Immunol*, 2022, 23(7): 1021-1030
- [16] Carmody LA, Caverly LJ, Foster BK, et al. Fluctuations in airway bacterial communities associated with clinical states and disease stages in cystic fibrosis. *PLoS One*, 2018, 13(3): e0194060
- [17] Cazzola M, Rogliani P, Ora J, et al. Asthma and comorbidities: recent advances. *Polish Arch Internal Med*, 2022, 132(4): 16250
- [18] Durack J, Lynch SV, Nariya S, et al. Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. *J Allergy Clin Immunol*, 2017, 140(1): 63-75
- [19] Rojas DA, Iturra PA, Méndez A, et al. Increase in secreted airway mucins and partial MUC5B STAT6/FoxA2 regulation during pneumocystis primary infection. *Sci Rep*, 2019, 9(1): 2078
- [20] Sabogal Piñeros YS, Bal SM, Dijkhuis A, et al. Eosinophils capture viruses, a capacity that is defective in asthma. *Allergy*, 2019, 74(10): 1898-1909
- [21] Son JH, Kim JH, Chang HS, et al. Relationship of microbial profile with airway immune response in eosinophilic or neutrophilic inflammation of asthmatics. *Allergy Asthma Immunol Res*, 2020, 12(3): 412-429
- [22] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. *CA-Cancer J Clin*, 2007, 57(1): 43-66
- [23] Tsay JCJ, Wu BG, Sulaiman I, et al. Lower airway dysbiosis affects lung cancer progression. *Cancer Discov*, 2021, 11(2): 293-307
- [24] Huang D, Su X, Yuan M, et al. The characterization of lung microbiome in lung cancer patients with different clinicopathology. *Am J Cancer Res*, 2019, 9(9): 2047-2063
- [25] Liu HX, Tao LL, Zhang J, et al. Difference of lower airway microbiome in bilateral protected specimen brush between lung cancer patients with unilateral lobar masses and control subjects. *Int J Cancer*, 2018, 142(4): 769-778
- [26] Tsay JCJ, Wu BG, Badri MH, et al. Airway microbiota is associated with upregulation of the PI3K pathway in lung cancer. *Am J Respir Crit Care Med*, 2018, 198(9): 1188-

- 1198
- [27] Gomes S, Cavadas B, Ferreira JC, et al. Profiling of lung microbiota discloses differences in adenocarcinoma and squamous cell carcinoma. *Sci Rep*, 2019, 9(1): 12838
- [28] Yu G, Gail MH, Consonni D, et al. Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. *Genome Biol*, 2016, 17(1): 163
- [29] Scaramuzza G, Ottaviani I, Volta CA, et al. Mechanical ventilation and COPD: from pathophysiology to ventilatory management. *Minerva Med*, 2022, 113(3): 460-470
- [30] Pragman AA, Lyu T, Baller JA, et al. The lung tissue microbiota of mild and moderate chronic obstructive pulmonary disease. *Microbiome*, 2018, 6(1): 7
- [31] Yip W, Li X, Koelwyn GJ, et al. Inhaled corticosteroids selectively alter the microbiome and host transcriptome in the small airways of patients with chronic obstructive pulmonary disease. *Biomedicines*, 2022, 10(5): 1110
- [32] Ko FW, Chan KP, Hui DS, et al. Acute exacerbation of COPD. *Respirology*, 2016, 21(7): 1152-1165
- [33] Mayhew D, Devos N, Lambert C, et al. Longitudinal profiling of the lung microbiome in the AERIS study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations. *Thorax*, 2018, 73(5): 422-430
- [34] Dicker AJ, Huang J TJ, Loneragan M, et al. The sputum microbiome, airway inflammation, and mortality in chronic obstructive pulmonary disease. *J Allergy Clin Immunol*, 2021, 147(1): 158-167
- [35] Millares L, Pascual S, Montón C, et al. Relationship between the respiratory microbiome and the severity of airflow limitation, history of exacerbations and circulating eosinophils in COPD patients. *BMC Pulm Med*, 2019, 19(1): 112
- [36] Zhong H, Wang Y, Shi Z, et al. Characterization of respiratory microbial dysbiosis in hospitalized COVID-19 patients. *Cell Discov*, 2021, 7(1): 23
- [37] Dickson RP. Lung microbiota and COVID-19 severity. *Nat Microbiol*, 2021, 6(10): 1217-1218
- [38] Gaibani P, Viciani E, Bartoletti M, et al. The lower respiratory tract microbiome of critically ill patients with COVID-19. *Sci Rep*, 2021, 11(1): 10103
- [39] Dickson RP, Erb-Downward JR, Freeman CM, et al. Spatial variation in the healthy human lung microbiome and the adapted island model of lung biogeography. *Ann ATS*, 2015, 12(6): 821-830
- [40] Dickson RP, Erb-Downward JR, Huffnagle GB. Towards an ecology of the lung: new conceptual models of pulmonary microbiology and pneumonia pathogenesis. *Lancet Respiratory Med*, 2014, 2(3): 238-246
- [41] Pai S, Muruganandah V, Kupz A. What lies beneath the airway mucosal barrier? Throwing the spotlight on antigen-presenting cell function in the lower respiratory tract. *Clin Transl Immunol*, 2020, 9(7): e1158
- [42] Mirghani CR, Saba CT, Khaliq CH, et al. Biofilms: formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiol*, 2022, 8(3): 239-277
- [43] Hewitt RJ, Lloyd CM. Regulation of immune responses by the airway epithelial cell landscape. *Nat Rev Immunol*, 2021, 21(6): 347-362
- [44] Rogan MP, Geraghty P, Greene CM, et al. Antimicrobial proteins and polypeptides in pulmonary innate defence. *Respir Res*, 2006, 7(1): 29
- [45] Lozupone C, Cota-Gomez A, Palmer BE, et al. Widespread colonization of the lung by *Tropheryma whipplei* in HIV infection. *Am J Respir Crit Care Med*, 2013, 187(10): 1110-1117
- [46] Alexandrova Y, Costiniuk CT, Jenabian MA. Pulmonary immune dysregulation and viral persistence during HIV infection. *Front Immunol*, 2021, 12: 808722
- [47] Gollwitzer ES, Saglani S, Trompette A, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat Med*, 2014, 20(6): 642-647
- [48] Jang HJ, Choi JY, Kim K, et al. Relationship of the lung microbiome with PD-L1 expression and immunotherapy response in lung cancer. *Respir Res*, 2021, 22(1): 322
- [49] D'Alessandro-Gabazza CN, Kobayashi T, Yasuma T, et al. A staphylococcus pro-apoptotic peptide induces acute exacerbation of pulmonary fibrosis. *Nat Commun*, 2020, 11(1): 1539
- [50] Legoux F, Salou M, Lantz O. MAIT cell development and functions: the microbial connection. *Immunity*, 2020, 53(4): 710-723
- [51] Espinoza JL, Minami M. Sensing bacterial-induced DNA damaging effects via natural killer group 2 member D immune receptor: from dysbiosis to autoimmunity and carcinogenesis. *Front Immunol*, 2018, 9: 52
- [52] Maddi A, Sabharwal A, Violante T, et al. The microbiome and lung cancer. *J Thorac Dis*, 2019, 11(1): 280-291
- [53] Ruterbusch M, Pruner KB, Shehata L, et al. *In vivo* CD4⁺ T cell differentiation and function: revisiting the Th1/Th2 paradigm. *Annu Rev Immunol*, 2020, 38(1): 705-725
- [54] Jiang H, Fu D, Bidgoli A, et al. T cell subsets in graft versus host disease and graft versus tumor. *Front Immunol*, 2021, 12: 761448
- [55] Lee G. The balance of Th17 versus Treg cells in autoimmunity. *Int J Mol Sci*, 2018, 19(3): 730
- [56] Liang N, Bing Z, Wang Y, et al. Clinical implications of EGFR-associated MAPK/ERK pathway in multiple primary lung cancer. *Clin Transl Med*, 2022, 12(5): e847
- [57] Wang J, Hu K, Cai X, et al. Targeting PI3K/AKT signaling for treatment of idiopathic pulmonary fibrosis. *Acta*

- [Pharmaceutica Sin B](#), 2022, 12(1): 18-32
- [58] Ma QY, Huang DY, Zhang HJ, et al. Upregulation of bacterial-specific Th1 and Th17 responses that are enriched in CXCR5⁺ CD4⁺ T cells in non-small cell lung cancer. [Int Immunopharmacol](#), 2017, 52: 305-309
- [59] Yang D, Chen X, Wang J, et al. Dysregulated lung commensal bacteria drive interleukin-17B production to promote pulmonary fibrosis through their outer membrane vesicles. [Immunity](#), 2019, 50(3): 692-706
- [60] Wu BG, Sulaiman I, Tsay JCJ, et al. Episodic aspiration with oral commensals induces a MyD88-dependent, pulmonary T-helper cell type 17 response that mitigates susceptibility to *Streptococcus pneumoniae*. [Am J Respir Crit Care Med](#), 2021, 203(9): 1099-1111
- [61] Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. [Eur J Immunol](#), 2010, 40(7): 1830-1835
- [62] Ren L, Zhang R, Rao J, et al. Transcriptionally active lung microbiome and its association with bacterial biomass and host inflammatory status. [mSystems](#), 2018, 3(5): e00199-18
- [63] Collin AM, Lecocq M, Noel S, et al. Lung immunoglobulin A immunity dysregulation in cystic fibrosis. [EBioMedicine](#), 2020, 60: 102974
- [64] Yadava K, Pattaroni C, Sichelstiel AK, et al. Microbiota promotes chronic pulmonary inflammation by enhancing IL-17A and autoantibodies. [Am J Respir Crit Care Med](#), 2016, 193(9): 975-987
- [65] Troy EB. Beneficial effects of *Bacteroides fragilis* polysaccharides on the immune system. [Front Biosci](#), 2010, 15(1): 25-34
- [66] Brown RL, Sequeira RP, Clarke TB. The microbiota protects against respiratory infection via GM-CSF signaling. [Nat Commun](#), 2017, 8(1): 1512
- [67] Wang J, Li F, Sun R, et al. Bacterial colonization dampens influenza-mediated acute lung injury via induction of M2 alveolar macrophages. [Nat Commun](#), 2013, 4(1): 2106
- [68] Cheng M, Chen Y, Wang L, et al. Commensal microbiota maintains alveolar macrophages with a low level of CCL24 production to generate anti-metastatic tumor activity. [Sci Rep](#), 2017, 7(1): 7471
- [69] Gulati A, Kaur D, Krishna Prasad GVR, et al. PRR function of innate immune receptors in recognition of bacteria or bacterial ligands. [Adv Exp Med Biol](#), 2018, 1112: 255-280
- [70] Fan V, Gharib S, Martin T, et al. COPD disease severity and innate immune response to pathogen-associated molecular patterns. [Int J Chron Obstruct Pulmon Dis](#), 2016, 11: 467-477
- [71] Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. [Cell Mol Life Sci](#), 2021, 78(4): 1233-1261
- [72] de Marcken M, Dhaliwal K, Danielsen AC, et al. TLR7 and TLR8 activate distinct pathways in monocytes during RNA virus infection. [Sci Signal](#), 2019, 12(605): eaaw1347
- [73] Guo-Parke H, Linden D, Weldon S, et al. Mechanisms of virus-induced airway immunity dysfunction in the pathogenesis of COPD disease, progression, and exacerbation. [Front Immunol](#), 2020, 11: 1205
- [74] Underhill DM, Iliev ID. The mycobiota: interactions between commensal fungi and the host immune system. [Nat Rev Immunol](#), 2014, 14(6): 405-416
- [75] Ju MJ, Liu BF, He HY, et al. MicroRNA-27a alleviates LPS-induced acute lung injury in mice via inhibiting inflammation and apoptosis through modulating TLR4/MyD88/NF- κ B pathway. [Cell Cycle](#), 2018, 17(16): 2001-2018
- [76] Hiemstra PS. Parallel activities and interactions between antimicrobial peptides and complement in host defense at the airway epithelial surface. [Mol Immunol](#), 2015, 68(1): 28-30
- [77] Das S, Bernasconi E, Koutsokera A, et al. A prevalent and culturable microbiota links ecological balance to clinical stability of the human lung after transplantation. [Nat Commun](#), 2021, 12(1): 2126
- [78] Stjärne Aspelund A, Hammarström H, Inghammar M, et al. Microbiological findings in bronchoalveolar lavage fluid from lung transplant patients in Sweden. [Transpl Infect Dis](#), 2018, 20(6): e12973
- [79] Beaume M, Köhler T, Greub G, et al. Rapid adaptation drives invasion of airway donor microbiota by *Pseudomonas* after lung transplantation. [Sci Rep](#), 2017, 7(1): 40309
- [80] Dickson RP, Erb-Downward JR, Freeman CM, et al. Changes in the lung microbiome following lung transplantation include the emergence of two distinct *Pseudomonas* species with distinct clinical associations. [PLoS One](#), 2014, 9(5): e97214
- [81] van Delden C, Stampf S, Hirsch HH, et al. Burden and timeline of infectious diseases in the first year after solid organ transplantation in the swiss transplant cohort study. [Clin Infect Dis](#), 2020, 71(7): e159-e169
- [82] Abbas AA, Young JC, Clarke EL, et al. Bidirectional transfer of Anelloviridae lineages between graft and host during lung transplantation. [Am J Transplant](#), 2019, 19(4): 1086-1097
- [83] Combs MP, Wheeler DS, Luth JE, et al. Lung microbiota predict chronic rejection in healthy lung transplant recipients: a prospective cohort study. [Lancet Respiratory Med](#), 2021, 9(6): 601-612
- [84] Mouraux S, Bernasconi E, Pattaroni C, et al. Airway

- microbiota signals anabolic and catabolic remodeling in the transplanted lung. *J Allergy Clin Immunol*, 2018, 141(2): 718-729
- [85] Khush KK, Potena L, Cherikh WS, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: 37th adult heart transplantation report-2020; focus on deceased donor characteristics. *J Heart Lung Transplant*, 2020, 39(10): 1003-1015
- [86] Schott C, Weigt SS, Turturice BA, et al. Bronchiolitis obliterans syndrome susceptibility and the pulmonary microbiome. *J Heart Lung Transplant*, 2018, 37(9): 1131-1140
- [87] Yamamoto S, Nava RG, Zhu J, et al. Cutting edge: *Pseudomonas aeruginosa* abolishes established lung transplant tolerance by stimulating B7 expression on neutrophils. *J Immunol*, 2012, 189(9): 4221-4225
- [88] Borthwick LA, Suwara MI, Carnell SC, et al. *Pseudomonas aeruginosa* induced airway epithelial injury drives fibroblast activation: a mechanism in chronic lung allograft dysfunction. *Am J Transplant*, 2016, 16(6): 1751-1765
- [89] Sharma NS, Wille KM, Athira S, et al. Distal airway microbiome is associated with immunoregulatory myeloid cell responses in lung transplant recipients. *J Heart Lung Transplant*, 2018, 37(2): 206-216
- [90] Heim CE, Vidlak D, Scherr TD, et al. Myeloid-derived suppressor cells contribute to *Staphylococcus aureus* orthopedic biofilm infection. *J Immunol*, 2014, 192(8): 3778-3792
- [91] Rieber N, Brand A, Hector A, et al. Flagellin induces myeloid-derived suppressor cells: implications for *Pseudomonas aeruginosa* infection in cystic fibrosis lung disease. *J Immunol*, 2013, 190(3): 1276-1284
- [92] Sharma NS, Vestal G, Wille K, et al. Differences in airway microbiome and metabolome of single lung transplant recipients. *Respir Res*, 2020, 21(1): 104
- [93] Xu H, Abuduwufuer A, Lv W, et al. The role of HIF-1 α -VEGF pathway in bronchiolitis obliterans after lung transplantation. *J Cardiothorac Surg*, 2019, 14(1): 27