细菌外膜囊泡在脓毒症中的作用机制及应对策略

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摘要 脓毒症是以全身性炎症反应及多器官功能障碍为特征的一种致命性疾病, 革兰氏阴性菌感染是其最常见的病因. 外膜囊泡(outer membrane vesicles, OMVs)是革兰氏阴性菌在一定机制作用下, 通过细菌外膜膨出、释放的球形囊泡(10~300 nm), 其介导细菌-细菌及细菌-宿主相互作用, 并提升细菌的生存及致病能力. 在脓毒症发生过程中, OMVs作用于多种宿主细胞(如上皮细胞、免疫细胞、血管内皮细胞、血小板等), 诱导宿主对感染的免疫应答失控及炎症-凝血正反馈环路, 最终导致过激的炎症反应及器官功能障碍. 本文简要介绍OMVs的结构组分、分泌机制及生物学功能, 重点讨论了OMVs在脓毒症发生中的作用及应对策略(例如利用阳离子抗菌聚合物吸附清除OMVs), 旨在为脓毒症的临床防治提供理论基础.

关键词 脓毒症, 外膜囊泡, 脂多糖, 革兰氏阴性菌, 阳离子抗菌聚合物

脓毒症是感染引起的全身炎症反应失控所致的严重疾病, 如得不到及时控制, 可引起多个脏器功能障碍. 脓毒症的发生率逐年上升, 已成为导致危重患者死亡的主要原因. 细菌是造成感染的直接原因, 一旦局部感染的致病菌及其毒素进入体内, 便可导致脓毒症及相关疾病的发生^[1]. 随着抗生素的广泛使用, 细菌耐药问题成为全球性的威胁和挑战. 2017年世界卫生组织(WHO)公布的耐药细菌中危害最严重的包括: 鲍曼不动杆菌、绿脓杆菌、大肠杆菌等革兰氏阴性菌^[2].

革兰氏阴性菌不仅易产生耐药, 且其外膜均包含共同的特征毒性成分——脂多糖(lipopolysaccharide, LPS), 即内毒素. LPS是毒性很强的致炎和热原物质, 可诱导机体炎症及免疫反应, 导致脓毒症和内毒素性休克等严重疾病. 最近研究发现: 除了通过经典的细胞膜表面Toll样受体, 由核转录因子- κ B(NF- κ B)通路激活细

胞内炎症反应, LPS还存在于革兰氏阴性菌分泌的外膜囊泡(out membrane vesicles, OMVs)上, 随着OMVs在体内传输, 由胞浆受体促发Caspase-11依赖的炎症通路, 构成了宿主抵御革兰氏阴性菌感染的重要机制^[3].

OMVs来源于革兰氏阴性菌外膜, 富含病原体相关分子模式(pathogen-associated molecular patterns, PAMPs), 如LPS、肽聚糖、细菌DNA等, 一旦进入机体, 便可与宿主细胞的模式识别受体(pattern recognition receptors, PRRs)结合, 进而激活免疫系统, 促发炎症反应. OMVs相关的PRRs包括: 细胞表面的Toll样受体(TLRs), 如由LPS激活的TLR4; 细胞内的NOD样受体(NLRs), 如NOD1和NOD2, 可由肽聚糖组分激活; 作为LPS的胞内受体的含半胱氨酸的天冬氨酸蛋白水解酶(Caspases), 如Caspase-11可介导OMVs胞内炎症通路的激活^[4]. 鉴于此, OMVs在脓毒症等感染性疾病进程中

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的作用正引起人们越来越广泛的关注。

本文将简要介绍OMVs的结构组分、分泌机制及影响因素,再通过从上皮细胞侵入、免疫细胞激活,到血管内皮细胞、血小板活化形成炎症-凝血正反馈,对革兰氏阴性菌OMVs在脓毒症发生中的致病作用及应对策略进行综述,旨在为脓毒症的源头防治提供理论支持与方向引导。

1 革兰氏阴性菌OMVs的结构组成

OMVs是革兰氏阴性菌在一定机制作用下由外膜膨出、释放的囊泡状结构,大多为球形,直径大约10~300 nm。OMVs从外到内主要包括: LPS、外膜和周浆间隙的蛋白质和脂质、肽聚糖、核酸、酶和毒力因子等^[5]。OMVs的分泌是一个主动过程,其构成组分具有选择性、菌种特异性及应变性^[6]。

OMVs最外侧包含的LPS是革兰氏阴性菌共有的重要毒性成分,由内到外分3层: (1) 脂质A(lipid A)是LPS毒性的主要来源,带负电荷; (2) 核心多糖层(core OS): 内侧2个带负电的2-酮基-3-脱氧辛酸(KDO)与脂质A的N-乙酰氨基葡萄糖(GlcNAc)共价结合,组成LPS内核; 外侧的8个糖单位构成外核,亦带负电荷; (3) O抗原(O antigen),由不同的单糖组成; 绿脓杆菌有两种O抗原: 中性的CPA(A带)与带负电荷的OSA(B带)。研究结果表明: 绿脓杆菌OMVs富集B带LPS。推测这可能因为相邻B带LPS分子的负电荷排斥力,促进外膜膨出形成OMVs^[7]。

2 OMVs的分泌机制及影响因素

革兰氏阴性菌OMVs的分泌机制迄今尚未完全明了。目前,已提出的模型主要包括: 脂蛋白模型、双层耦合模型、机械模型、生物力学模型、肽聚糖碎片模型等。上述模型的作用结局均为: 解离细胞壁的稳定交

联、拉大细菌内外膜之间的距离,使外膜局部膨出、断裂,最终脱落生成OMVs^[8]。

OMVs是细菌包膜对各种外部应激的反应产物^[9,10]。OMVs在细菌生长的各个阶段都能产生,但其构成组分、产率受环境的影响。研究表明,缺氧、温度变化、铁缺乏、过氧化氢、多黏菌素、庆大霉素等因素,均可影响革兰氏阴性菌分泌OMVs。OMVs的生物合成还受基因调控,编码交联蛋白、周质蛋白、脂蛋白的基因突变均可影响其产量^[11,12]。在感染过程中,OMVs分泌与宿主反应相互影响、密切相关。细菌一旦侵入,机体会发生体温升高、氧化应激等改变,加之抗生素的使用,以期杀灭细菌、维持内环境稳态;反过来,这些因素又可刺激存活细菌分泌更多的OMVs,增强细菌防御、攻击能力^[13]。

OMVs作为一种新型的细菌分泌机制,与经典的I-VI型分泌系统不同^[14]。OMVs不需要细菌接触宿主细胞,便可传输高浓度的毒力因子至机体远处,加重感染;即使细菌被杀灭,OMVs依旧可停留在体内,继续发挥致病作用。显然,在细菌-宿主的反复较量过程中,都离不开OMVs。

3 OMVs的生物学功能

OMVs介导细菌-细菌及细菌-宿主相互作用(图1),其有利于细菌在宿主体内存活,同时可向宿主运输多种病原体相关分子、调节宿主免疫反应,在感染发生中发挥重要致病的作用。

3.1 OMVs介导的细菌-细菌相互作用

(i) 促进细菌获取营养物质。OMVs可优化细菌的营养摄取机制,其通过酶解宿主体内分子为细菌生长提供所需的营养物质,伯氏疏螺旋体OMVs携带的烯醇化酶可降解宿主的基质蛋白作为营养分子,这也有利

图1 (网络版彩色)细菌OMVs的生物学功能

Figure 1 (Color online) Biological functions of bacterial OMVs

于病原体扩散^[15]. 部分细菌可直接利用OMVs内的DNA、蛋白质, 作为碳、氮营养元素的来源^[16]. 此外, OMVs还参与摄取、储存细菌生长所需的金属元素^[17,18].

(ii) 促进生物膜形成. 生物膜是细菌在应激条件下产生的保护性结构, 可使菌体免受脱水、饥饿、抗菌药物等的负面影响, 而OMVs与生物膜形成及稳定密切相关^[19]. OMVs传输与生物膜发生相关的信号分子, 通过胞外多糖的成核作用, 直接诱导生物膜的形成. Murphy等人^[20]指出, 绿脓杆菌OMVs中O抗原对生物膜形成至关重要. 此外, OMVs还可防御生物膜的破坏、介导生物膜内外分子交流^[21].

(iii) 传播抗生素耐药性. OMVs通过复杂的机制诱导细菌形成抗生素耐药性. 首先, OMVs可在同种或异种细菌间水平传播耐药基因, 促进耐药性形成^[22], 如绿脓杆菌OMVs可转移 β -内酰胺酶而使对 β -内酰胺酶敏感的细菌耐药^[23]. 其次, OMVs可结合、吸附多种抗菌物质并使其失去活性, 增强耐药性, 如大肠杆菌的超囊泡突变株或与OMVs共培养的野生型菌株均表现出显著的多黏菌素抗性^[24]. 此外, OMVs内含的多种抗生素水解酶、药物外排系统, 以及OMVs促成的生物膜共同干扰了抗生素的杀菌作用, 促使细菌形成耐药性^[25]. OMVs可携带核酸(如质粒DNA、染色体DNA及RNA)并完成水平基因传播^[26], 为进一步解释细菌胞质中核酸进入囊泡腔的具体机制, 有学者提出一种新的细胞外囊泡模型, 即内外膜囊泡(outer-inner membrane vesicles, O-IMVs)^[27], 其以外膜及内膜突出并将胞质成分装配入囊泡腔为特征. 该模型具有革兰氏阴性菌保守性并可解释膜囊泡中DNA、ATP、胞质蛋白等成分的存在.

(iv) 杀灭竞争性微生物. Vasilyeva等人^[28]发现溶菌杆菌XL1分泌携带溶菌酶的OMVs, 可降解竞争的革兰氏阴性菌. 黄色黏球菌OMVs内的碱性磷酸酶可裂解大肠杆菌, 同时释放利于自身菌落生长的磷酸盐. 这表明细菌OMVs可杀灭竞争性微生物, 争夺有限的营养, 使其在体内成为优势菌群. 此外, OMVs对于肽聚糖组分与供体相似的细菌具有显著的杀灭效果^[29].

3.2 OMVs介导的细菌-宿主相互作用

(i) 黏附侵入宿主细胞. OMVs携带的黏附分子(如Ata、BabA、SabA、OmpA)介导细菌黏附于宿主细胞^[30], 随后OMVs可通过巨胞饮、内吞(包括网格蛋

白、小窝蛋白及脂筏介导的内吞)及膜融合作用进入宿主细胞^[31].

(ii) 输送毒力因子及PAMPs. 致病菌可利用OMVs将毒力因子及PAMPs输送至宿主细胞, 进而破坏宿主防御系统、调控免疫反应, 引发感染性疾病^[32]. 肠出血性大肠杆菌(EHEC) O157的OMVs携带多种毒力因子, 包括志贺毒素、细胞致死性膨大毒素V、EHEC溶血素及鞭毛蛋白. 这些毒素在OMVs被细胞内化并在胞内运输过程中被释放, 激活DNA损伤反应, 最终引起细胞死亡^[33]. 绿脓杆菌OMVs可将碱性磷酸酶、磷脂酶-C、 β -内酰胺酶及Cif直接输送至细胞质中、介导细胞毒性^[34].

此外, 一些OMVs已被鉴定为基因毒素. 例如, 幽门螺杆菌OMVs可导致染色体错误分布及胃上皮细胞基因组损伤相关的铁代谢和氧化应激的改变^[35].

(iii) 促发宿主炎症免疫反应. OMVs携带的PAMPs可与宿主细胞的PRRs结合, 通过信号级联放大及炎症通路激活, 导致炎症介质合成增多、炎症细胞募集, 促发炎症反应, 并诱导OMVs特异性的细胞和体液适应性免疫反应^[36].

不同种类细菌的OMVs激活的PRR信号通路及炎症反应机制也存在差异^[3,4,37,38]. 大肠杆菌和绿脓杆菌OMVs作用于细胞膜表面受体TLR4, 诱导NF- κ B、髓样分化原代反应蛋白88(MyD88)、IL-1 β 及IL-8等表达增多, 介导炎症发生. 幽门螺杆菌、绿脓杆菌、淋病奈瑟球菌OMVs可通过肽聚糖与细胞内的NOD1受体结合, 激活NF- κ B及NOD1依赖的炎症反应. 细胞质中Caspase-11通路的过度激活是脓毒性休克的重要分子机制, LPS经OMVs输送进入胞浆中与Caspase-11受体结合, 进而活化NLRP3炎症小体并诱导IL-1 β 、IL-18表达增多, 同时, Caspase-11直接导致细胞热凋亡. 此外, 幽门螺杆菌OMVs在胃上皮细胞中还可启动促分裂原活化蛋白激酶(MAPK)和活化蛋白1(AP-1)信号传导.

(iv) 利于细菌在宿主体内存活. 细菌进入机体后, 宿主体内温度变化、氧化应激反应及抗生素的应用, 可刺激细菌迅速产生大量OMVs, 将抗生素、细菌毒力因子等包裹排出. 这可消除细菌细胞内的有害物质, 维持细菌包膜的稳定性, 保护细菌^[10,39]. 溶菌性噬菌体可识别细菌胞膜上的靶受体并裂解细菌, OMVs可充当结合、吸收溶菌性噬菌体及抗菌物质的诱饵靶点, 发挥即时保护细菌的作用^[24], 这些逃逸的细菌可介导难治性感染.

4 OMVs在脓毒症发生中的作用

感染进程中, 细菌OMVs首先黏附定植于多种上皮细胞, 损害上皮屏障侵入皮下组织, 进而激活免疫细胞及其他组织细胞. 随着炎症进展, OMVs扩散进入循环系统, 活化血管内皮细胞及血小板、白细胞等血细胞, 促进炎症-凝血正反馈形成. 一旦宿主免疫应答失控, 局部感染便可引起全身性炎症, 最终导致脓毒症等严重的感染性疾病. OMVs与各种宿主细胞的相互作用, 引发脓毒症的主要作用机制(图2), 具体详述如下.

4.1 OMVs与上皮细胞的相互作用

上皮屏障是机体抵御病原体的第一道免疫防线. 上皮屏障一旦受损, 细菌便可侵入引起局部感染. 细菌分泌的OMVs与皮肤、呼吸道、消化道上皮细胞相互作用, 可破坏上皮的完整性、诱导炎症发生、妨碍上皮修复与再生. 此外, OMVs还可诱导合成抗菌肽、抑炎介质, 介导免疫抑制. 细菌感染的临床表现多样, 可为急性或慢性病程、局部感染灶或全身性炎症反应甚至脓毒性休克等.

(i) OMVs与皮肤上皮细胞. 皮肤上皮屏障受损的患者, 如创伤、烧伤、手术伤口、留置管道等, 容易发生伤口感染. 绿脓杆菌是伤口感染最常见的病原菌之一^[40]. 细菌附着于皮肤表面后, 在OMVs协助下形成生物膜以隔离抗生素的打击. OMVs还可直接导致皮肤上皮细胞死亡、破坏细胞间紧密连接、抑制上皮细胞增生、迁移与修复, 破坏皮肤的完整性, 使细菌深入扩散. 同时OMVs还能激活皮肤免疫应答, 在初期表现为促炎

反应, 随后上皮细胞合成抗菌肽、诱导抑炎反应^[41,42]. 可见, 绿脓杆菌OMVs可使细菌在皮肤伤口中持久存在且难以清除, 这不仅导致伤口延迟愈合, 还可诱导机体持续、失控的炎症反应, 甚至脓毒症的发生^[43].

(ii) OMVs与呼吸道上皮细胞. 研究发现, 绿脓杆菌OMVs可刺激支气管上皮细胞分泌炎症因子和趋化分子, 它们迅速招募炎症细胞、促进呼吸道炎症发生^[44]. OMVs还可调控宿主对绿脓杆菌的免疫反应, 一方面, OMVs可结合气道上皮细胞受体TLR4、上调炎性蛋白表达(如MyD88、IL-1 β 、NF- κ B)^[45]. 另一方面, OMVs在细胞内释放毒力因子Cif, 进而抑制气道的免疫应答. 这主要与Cif抑制氯离子分泌及气道水合作用、减弱气道黏液对病原菌的清除, 以及减少气道上皮细胞中MHC I类抗原表达、抑制抗原呈递有关^[46].

(iii) OMVs与消化道上皮细胞. 在口腔, 牙周疾病的常见病原体(如牙龈卟啉单胞菌、密闭螺旋体)分泌的OMVs包含可降解细胞间的连接、破坏口腔上皮屏障的分子(如牙龈蛋白酶或牙本质素)^[47], 还含有如脂寡糖、外膜蛋白等毒性分子^[48]. 牙龈卟啉单胞菌OMVs可释放黏附素, 聚集细胞、促进牙龈菌斑生物膜形成. 此外, OMVs结合口腔上皮PRRs, 使细胞活化并释放炎症因子(如IL-6、IL-8、MCP-1), 进而招募巨噬细胞、中性粒细胞浸润牙周组织, 最终可导致牙周组织的破坏及慢性牙周炎的发生^[49].

在胃肠道, OMVs可穿透黏液层后侵入胃肠上皮细胞, 通过释放毒素、免疫调节分子介导炎症反应、加剧疾病进展. 幽门螺杆菌OMVs的肽聚糖组分通过结合NOD1受体, 激活NF- κ B通路, 促使胃上皮细胞合成大

图2 (网络版彩色)细菌OMVs入侵宿主细胞引发脓毒症的主要作用机制

Figure 2 (Color online) Main mechanism of bacterial OMVs invading host cells and causing sepsis

量趋化因子(尤其是IL-8). 大肠杆菌OMVs通过TLR4受体, 刺激肠上皮细胞分泌炎症因子, 其携带的肠毒素还可断裂肠上皮细胞的DNA双链, 成为细菌引发肠道疾病的新机制^[49-51]. 此外, 肠道内非致病性的共生菌、益生菌也可合成OMVs, 它们有益于肠道菌群稳态, 对机体健康起保护作用^[52].

4.2 OMVs与免疫细胞的相互作用

OMVs穿透上皮屏障后, 在黏膜下层与多种免疫细胞(如中性粒细胞、单核巨噬细胞、树突状细胞、淋巴细胞)相互作用, OMVs对免疫细胞的影响因菌种而异.

(i) OMVs与中性粒细胞. 脑膜炎奈瑟球菌OMVs刺激人中性粒细胞产生炎症介质、诱发炎症反应, IFN- γ 可增强这一促炎效应^[53]. 相反, 非致病性大肠杆菌OMVs含有1型细胞毒性坏死因子, 可损害中性粒细胞的吞噬和趋化功能^[54]. 脑膜炎奈瑟球菌OMVs还可诱导中性粒细胞胞外捕获网(NETs)的形成, 这是细胞死亡的标志, 但NETs内含DNA、抗菌肽和组蛋白, 有利于捕获和杀死细菌^[55].

(ii) OMVs与单核巨噬细胞. 通过外周静脉注射, 发现大肠杆菌OMVs主要分布于小鼠的肝、肺、肾、小肠、脾, 而心脏中基本无分布. 该研究结果提示: OMVs在体内器官的分布与单核巨噬细胞系统的摄取作用及免疫应答密切相关^[56]. OMVs与巨噬细胞相互作用, 可表现为促炎或抗炎反应. 脑膜炎奈瑟球菌OMVs促使巨噬细胞表达HLA-DR、CD80、CD86以及ICAM-1, 可驱动适应性免疫反应. 沙门氏菌、牙龈卟啉单胞菌OMVs可活化小鼠巨噬细胞、介导TNF、NO、iNOS等促炎介质合成^[34]. 除有促炎作用外, 幽门螺杆菌OMVs还可诱导人外周血单核细胞产生抑炎因子^[57]. 牙龈卟啉单胞菌OMVs可抑制巨噬细胞表面CD14的表达^[58], 使细胞对二次感染的抗原刺激无反应, 这种免疫抑制状态将加重炎症反应.

(iii) OMVs与树突状细胞. OMVs也可作用于树突状细胞(DCs), 诱导DCs成熟并参与免疫调节. 脑膜炎奈瑟球菌OMVs可促进DCs成熟, 并促进炎症性介质产生^[59]. 沙门氏菌属OMVs诱导DCs表达CD80、CD86和MHC II类分子, 介导保护性B细胞和T细胞的免疫应答^[60].

(iv) OMVs与淋巴细胞. OMVs对T细胞免疫有抑制作用, 其可抑制T细胞增殖及细胞因子分泌. 研究发现, 幽门螺杆菌OMVs的抑制机制并非直接作用于T细胞, 而是通过单核细胞过表达COX-2, 进而实现免疫调

节作用. 此外, 幽门螺杆菌OMVs内的VacA毒素可进一步增强其免疫抑制效应^[57,61]. 研究还发现, OMVs可诱导B细胞激活及抗体生成. 卡他莫拉菌和流感嗜血杆菌的OMVs可与免疫球蛋白IgD BCR交联或结合TLR9受体, 促进B细胞增殖, 上调其HLA-DR、CD64、CD86等表面标记物, 并增加B细胞IgM、IgG的分泌^[62].

4.3 OMVs与血管内皮细胞的相互作用

一旦OMVs侵入血管内皮细胞, 可进一步促使炎症反应的发生及凝血系统稳态的破坏. 大肠杆菌OMVs通过激活NF- κ B通路, 可增加人脐静脉内皮细胞表达的炎性细胞因子(如IL-6、TNF- α)与黏附分子(如ICAM-1、E-选择素、VCAM-1)^[63,64], 导致炎症反应. 同时, OMVs介导的内皮细胞活化还可调节组织因子、血栓调节蛋白的表达水平, 共同诱导血管舒缩张力改变, 并促进血液向高凝状态转化及血管内微血栓形成^[65]. 此外, P-选择素、内皮细胞来源的膜囊泡也可促进了血小板活化, 增多单核细胞-血小板聚集并形成微血栓^[66].

另外, OMVs也可负调节内皮细胞的免疫反应. 牙龈卟啉单胞菌OMVs减少内皮细胞表达HLA-DR、MHC-II类分子, 抑制抗原呈递及适应性免疫反应, 还可抑制毛细血管形成^[49].

4.4 OMVs与血小板的相互作用

研究发现, OMVs可促进血小板聚集或增加血小板-白细胞聚集而致微血栓形成^[67]. 微血栓将堵塞器官的微血管, 导致微循环障碍、组织灌注不足及缺氧, 最终发生器官的功能障碍, 引起脓毒症. 此外, 血小板活化与内皮活化可相互促进, 形成了炎症-凝血正反馈环路^[68]. 过度的炎症反应将破坏血管内皮, 增加血管的通透性, 导致血浆渗漏、组织水肿及器官低灌注. 而血小板过度活化及循环中血小板耗竭, 还将引发弥散性血管内凝血等脓毒症的严重临床表现.

4.5 OMVs与其他组织细胞的相互作用

牙龈卟啉单胞菌、密闭螺旋体的OMVs可诱导牙龈成纤维细胞分泌IL-8、MCP-1、NO、基质金属蛋白酶、前列腺素E等炎症介质, 产生局部强烈的炎症反应及牙周组织破坏^[48,69]. 此外, OMVs与成骨细胞、滑膜细胞相互作用, 可导致骨关节的炎症^[70]. 金氏杆菌OMVs可从口咽道扩散至骨骼系统, 通过内化进入成骨细胞和滑膜细胞, 促使细胞产生GM-CSF、IL-6等因子,

诱导骨组织的炎症及破坏。

5 针对OMVs的防治策略

显然,在革兰氏阴性细菌-宿主的反复较量过程中,离不开OMVs。这些研究结果提示我们:在选择抗菌手段时,除了考虑对细菌的杀灭性能,还必须考虑不同抗菌条件下,细菌分泌OMVs的产率及其组成的影响因素与调控机制。这不仅有助于阐明抗菌及耐药机理,还对控制临床革兰氏阴性菌感染具有重要意义。因此,调控OMVs的生成,并探寻OMVs的有效清除方法成为脓毒症防治领域的新挑战。

5.1 OMVs疫苗

疫苗的开发,可以预防细菌感染,从来源上减少细菌产生OMVs,预防脓毒症的发生。传统疫苗的生产均需添加佐剂,但后者具有潜在的毒性,且对细胞免疫及黏膜免疫的诱导能力差。与传统疫苗相比,OMVs疫苗具有多种优势,天然的自佐剂性质、优异的免疫刺激能力、无自我复制能力使OMVs成为高效、安全的疫苗平台^[71]。现有的一系列研究证实:OMVs疫苗在小鼠体内可驱动高效的先天性和适应性免疫应答,对感染的预防作用更佳^[72,73]。

随着蛋白组学和基因工程的进展,重组OMVs疫苗显著优化了预防效果。将异源性抗原蛋白与OMVs结合而成的多价重组疫苗,既保留了OMVs的天然构象及靶向抗原呈递功能,又使疫苗的覆盖面更加广泛^[71]。鲍曼不动杆菌外膜蛋白Omp22与大肠杆菌OMVs结合而成的重组疫苗Omp22-OMV在脓毒症小鼠模型中诱导了高滴度的抗体、提高了存活率^[74]。对OMVs进行基因修饰有望在LPS的免疫原性及细胞毒性间达到最佳平衡,使OMVs疫苗能够在规避LPS所致风险的同时,保留最佳的免疫激活能力^[71]。

目前,OMVs疫苗在脑膜炎、牙周炎、百日咳、肠道感染、结核病、脓毒症等疾病的动物模型中获得了可靠的防治效果。脑膜炎奈瑟球菌B血清群的OMVs疫苗Bexsero是首个应用于临床的OMVs疫苗,该疫苗成功控制了脑膜炎的爆发,降低了脓毒症的发生率。鲍曼不动杆菌OMVs作为免疫疗法,可抵抗多重耐药的鲍曼不动杆菌感染,减少了脓毒症的发生^[75-77]。

5.2 针对OMVs的脓毒症治疗分子

LPS是OMVs诱发脓毒症的关键毒性分子,研究发

现抗菌肽如内源性肽LL-37、合成肽Pep19-2.5、多黏菌素B可直接中和LPS,抑制非典型炎性小体活化,将减少细胞热凋亡^[3,78]。此外,阻断LPS激活的炎症通路也有一定的治疗意义,TLR4和Caspase-4/5/11均可作为治疗靶标^[79],现有针对这些靶标的药物对于小鼠脓毒症模型均有良好的保护效果,但由于信号通路存在物种间差异,这些药物在临床试验阶段并未表现出明显有益的疗效。

Kim等人^[80]以OMVs作为新型药物筛选系统的核心,重新评估现有脓毒症治疗药物的疗效,最终选定了沙丁胺醇作为最佳候选药物,沙丁胺醇可剂量依赖性地阻断OMVs诱导的巨噬细胞炎性因子释放,缓解脓毒症的临床症状,且无明显的细胞毒性。

最近的研究发现,间充质基质干细胞来源的纳米囊泡(NVs)可作用于活化的巨噬细胞,促使其合成IL-10,在体内外实验中均减轻了OMVs诱导的全身性炎症,在脓毒症小鼠模型中还可改善体重及体温的变化^[81]。

5.3 清除OMVs的抗菌材料

OMVs在细菌感染相关的疾病中发挥着重要作用,因而研制具有吸附、清除OMVs功能的抗菌材料有助于预防脓毒症发生。

阳离子聚合物的抗菌机理以带正电荷的官能团吸附于带负电荷的细菌表面为基础,辅以亲脂烷基链插入磷脂双分子层,破坏细菌膜结构,最终杀死细菌^[82,83]。基于OMVs的外膜结构特点(带负电荷的LPS外叶、蛋白质毒性组分及疏水性的磷脂内叶等),以阳离子聚合物为平台开发抗菌材料具有可行性,能够实现去除内毒素及OMVs的目标。此外,阳离子聚合物还具有良好的生物相容性和化学稳定性、重复使用率高、不易产生耐药性等优点^[84]。最近,我们团队设计合成了一系列阳离子抗菌聚合物,通过扫描电子显微镜观察不同聚合物表面对绿脓杆菌OMVs吸附能力,结果证实了阳离子聚合物膜及阳离子修饰聚氨酯海绵均能够吸附OMVs(图3)。该海绵为咪唑阳离子接枝聚氨酯,已被证明是可吸附清除绿脓杆菌LPS的抗菌敷料,在绿脓杆菌及其LPS致小鼠皮肤伤口感染模型中表现出抗炎、促进伤口愈合的显著治疗效果^[85]。

然而,抗菌材料的使用也可能刺激细菌的包膜应激反应,生成更多的OMVs。因此,在最大化提高OMVs吸附清除效率的前提下,尽可能减少对细菌OMVs生成

图3 绿脓杆菌OMVs吸附于不同聚合物材料表面的扫描电子显微镜图。(a) PET膜; (b) 阳离子聚合物膜; (c), (d) 吸附OMVs前后的咪唑阳离子接枝聚氨酯海绵。其中抗菌海绵是具有LPS吸附清除功能的咪唑阳离子接枝聚氨酯Foam 3(含6 mmol的咪唑离子二醇)^[85], 与OMVs的作用时间为4 h(未发表数据)

Figure 3 Scanning electron microscope images of *P. aeruginosa* OMVs after contacting with different polymers. (a) PET film; (b) cationic polymer film; (c), (d) imidazolium-type polyurethane foams before and after adsorption of OMVs for 4 h (unpublished). The antibacterial foam (containing 6 mmol imidazolium ionic diol, named Foam 3) has been demonstrated to have the endotoxin adsorption and removal for wound healing^[85]

的刺激, 成为进一步设计阳离子聚合物抗菌材料的要求和目标。

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6 总结

脓毒症是细菌感染后宿主免疫失控造成的全身性炎症及多器官功能障碍。革兰氏阴性菌是脓毒症最常见的病原体, 细菌侵入后, 宿主的应激环境使OMVs的合成显著增多, 通过细菌-细菌或细菌-宿主相互作用发挥对机体的致病作用。

细菌感染发生后, OMVs先后与多种宿主细胞相互作用, 参与脓毒症的病理进程。首先, OMVs黏附于上皮细胞, 破坏上皮屏障, 随后激活免疫细胞。一旦侵入血管, OMVs可进一步活化血管内皮细胞、血小板, 形成炎症-凝血正反馈环路, 最终引起全身炎症反应及器官功能障碍。

OMVs的致病机理推动了脓毒症防治策略的发展。以OMVs为疫苗平台设计的多价重组疫苗, 在预防感染性疾病及脓毒症中展现了保护作用。以缓解OMVs介导的炎症反应为目标, 可开发出更具有针对性的药物。此外, 设计出新型的抗菌材料可有效清除OMVs、杀灭微生物, 防止局部感染进展为脓毒症等严重疾病。

总之, 脓毒症的发生是OMVs作用于宿主细胞后通过多种致病机制综合作用的结果, 目前靶向OMVs的防治策略在动物模型中获得了成功, 但将其应用于临床仍需克服多种障碍。随着疫苗开发、药物研发、抗菌材料等的不断进展, 未来定能开发出针对OMVs的脓毒症治疗新手段。

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Summary for “细菌外膜囊泡在脓毒症中的作用机制及应对策略”

Pathogenic roles and coping strategies of bacterial outer membrane vesicles in sepsis

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Sepsis is a life-threatening disease that characterized by systemic inflammatory response and multiple organ dysfunction and mostly caused by Gram-negative bacterial infections. Outer membrane vesicles (OMVs) are multifunctional spherical structures (10–300 nm) released from outer membrane of Gram-negative bacteria. Usually, OMVs carry a variety of virulence factors and pathogen-associated molecular patterns (PAMPs), such as toxins, digestive enzymes, lipopolysaccharide (LPS), peptidoglycan, bacterial DNA, etc. Roles of OMVs in bacterial survival include nutrient acquisition, bacterial biofilm formation, antibiotic resistance delivery, and killing of competing microbes.

In the pathogenesis of sepsis, OMVs have versatile effects on host cells (such as epithelial cells, immune cells, endothelial cells and platelets, etc.), causing the immune response out of control and the positive feedback loop of inflammation and coagulation formed, which ultimately leading to the systemic inflammatory response and multiple organ dysfunction. Pathogenic effects of OMVs are versatile, including adhesion of host cells, delivery of virulence factors and PAMPs, promotion of inflammatory and immune responses, and facilitation of bacteria survival in the host.

Moreover, the main mechanism of bacterial OMVs invading host cells and causing sepsis is related to the recognition of their carried PAMPs with pattern recognition receptors (PRRs) of host cells, including Toll-like receptors (TLRs) on the surface of cell membrane, NOD-like Receptors (NLRs) and cysteinyl aspartate specific proteinases (Caspases) in the cytoplasm. Here, we will briefly introduce the structure composition, biosynthesis and functions of OMVs, particularly focuses on the pathogenic roles of OMVs in the occurrence of sepsis.

In addition, the coping strategies of bacterial OMVs in sepsis have been recently investigated since OMVs can carry and transmit a variety of virulence factors. Firstly, OMVs vaccines can prevent the bacterial infection and reduce the OMVs production from the source. Secondly, therapeutic molecules, such as antimicrobial peptides and polymyxin B, can target LPS directly, the common toxic component of bacterial out membranes, and other drugs using TLR4 and Caspase-4/5/11 as targets, also have therapeutic potential to block the inflammatory pathways activated by LPS. Recently, the new drug screening system was established based on bacterial OMVs directly. The treatment effects of the existing drugs for sepsis, including blocking the release of inflammatory factors induced by OMVs, and alleviating the clinical symptoms of sepsis, were reevaluated.

Cationic antimicrobial polymers could adsorb the anionic groups of bacterial membrane through the electrostatic interaction, followed by inserting the phospholipid bilayer to destroy the integrity of bacterial membrane and ultimately kill bacteria. Based on the outer membrane structure characteristics of bacterial OMVs (the outmost LPS with more negative charges, toxic protein components, and the inner phospholipid layer with hydrophobicity), the development of cationic antibacterial materials with the integrated function of adsorption and removal of OMVs is feasible to prevent sepsis and other bacterial infection related diseases. Recently, our team designed and synthesized a series of cationic antibacterial polymers, and observed the adsorption capacity of different polymer surfaces to the OMVs of *Pseudomonas aeruginosa* by electron microscopy. The results confirmed that both the cationic polymer membrane and the cation-modified polyurethane foam could adsorb the OMVs. However, the antimicrobial polymers have the possibility to stimulate the release of bacterial OMVs. Therefore, the simultaneous effects of cationic polymers with antimicrobial activities need to be investigated on the release and removal of OMVs in future studies.

This review briefly introduces the structural composition, biosynthesis and functions of OMVs, particularly focuses on the pathogenic roles of OMVs in the occurrence of sepsis, and the corresponding coping strategies for the clinical prevention and treatment of sepsis.

sepsis, outer membrane vesicles, lipopolysaccharide, Gram-negative bacteria, cationic antimicrobial polymers

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