对于军团菌的免疫遏制: 宿主能否另辟蹊径?

郭婉兰[1,2] 陈涛涛[1,2] 欧阳松应[1,2]

([1]福建师范大学生命科学学院, 福州 350117; [2]南方生物医学研究中心, 福州 350117)

摘要 嗜肺军团菌可引起严重的非典型肺炎, 其特殊的Dot/Icm IVB型分泌系统转运近330种效应蛋白(大多数作为蛋白酶发挥功能)至宿主细胞, 通过修饰细胞调节因子、抑制细胞凋亡等一系列措施调控宿主免疫应答以逃逸宿主免疫系统的监测, 完成自身的增殖与侵染。嗜肺军团菌诱发的病原相关分子模式(pathogen-associated molecular patterns, PAMPs)和效应器触发反应(effector-triggered response, ETR)为探究军团菌与宿主互作提供新思路。该文就军团菌的致病机制、对宿主免疫的应对策略以及宿主的免疫应答等方面进行讨论, 旨在探索由军团菌引起的肺部感染的相关免疫机制, 利于开发出最优的细菌性肺炎治疗方案。

关键词 嗜肺军团菌; 效应蛋白; 病原体相关分子模式; 效应蛋白触发反应

Immune Suppression of *Legionella pneumophila*: Can the Host Find a New Way?

GUO Wanlan[1,2], CHEN Taotao[1,2], OUYANG Songying[1,2]

([1]*College of Life Sciences, Fujian Normal University, Fuzhou 350117, China*;
[2]*FJNU Biomedical Research Center of South, Fuzhou 350117, China*)

Abstract *L. pneumophila* (*Legionella pneumophila*), a causative agent of a non-canonical pneumonia, injects nearly 330 virulence factors (most known of proteinases) into host cell through its unique type IVB (Dot/Icm) secretion system, modifying cell regulatory factors and inhibiting cell apoptosis to escape the host immune surveillance and promote its proliferation. PAMPs (pathogen-associated molecular patterns) and ETR (effector-triggered response) by *L. pneumophila* offers us a new insight into the interplay between host and *Legionella*. This review discussed pathogenesis, anti-host immune strategies of *Legionella*, and host immune response, to reveal the immune pathogenesis caused by *L. pneumophila*, and develop the best therapy solution for bacterial pneumonia.

Keywords *Legionella pneumophila*; effectors; pathogen-associated molecular patterns; effector-triggered response

嗜肺军团菌病是由嗜肺军团杆菌所引起的一种细菌性传染病, 临床表现为以肺部感染为主, 伴随肺外多系统损伤。90%的患者胸部X射线拍片显示肺炎征象, 主要症状有发热、咳嗽、咳痰、呼吸困难及腹泻等[1], 该菌能引起重症肺炎和非肺炎型–庞蒂亚克热(Pontiac fever)轻型。自1979年正式命名以来, 根据基因序列分型方法(sequence based typing, SBT), 军团菌属已发现58个种, 70多个血清型, 其中20多个种可以引起人类疾病, 而嗜肺军团菌引起的病例约占临床病例的90%。84%的军团菌病

收稿日期: 2021-01-05 接受日期: 2021-02-21
国家自然科学基金(批准号: 31770948)、福建省科技厅项目(批准号: 2020Y4007、2021H0004)和福建省海洋经济发展补助项目(批准号: FJHJF-L-2020-2)资助的课题
*通讯作者。Tel: 0591-22868199, E-mail: ouyangsy@fjnu.edu.cn
Received: January 5, 2021 Accepted: February 21, 2021
This work was supported by the National Natural Science Foundation of China (Grant No.31770948), the Fujian Provincial Department of Science and Technology (Grant No.2020Y4007, 2021H0004), and the Fujian Provincial Marine Economic Development Subsidy Project (Grant No.FJHJF-L-2020-2)
*Corresponding author. Tel: +86-591-22868199, E-mail: ouyangsy@fjnu.edu.cn
URL: http://www.cjcb.org/arts.asp?id=5494

由嗜肺军团菌血清1型(*Legionella pneumophla* serogroup 1, Lp01)引起, 此外, 人类易感军团菌还包括: 波兹曼军团菌(*Legionella micdadei*)、米克达德军团菌(*Legionella bozemanii*)和长滩军团菌(*Legionella longbeachae*), 其共占人类军团菌感染的2%~7%。秦天团队[2]通过序列分型方法发现血清1型(serotype 1, ST-1)是我国优势型别, 其余型别和序列群呈地区性分布特征。一项在我国的自然水环境与人工水环境内嗜肺军团菌菌株之间的差异性研究表示, 在中国的自然水环境中, 嗜肺军团菌具有高度的遗传多态性且重组频繁, 不同环境及地区的自然水环境嗜肺军团菌在分子水平上差异并不显著, 但与人工水环境中的菌株有显著差异[3]。自然环境中水温较低、营养物质较贫乏, 因此, 军团菌浓度较低且不易繁殖, 因此, 致病危险性也较低; 人工水环境中如空调系统、饮用水、热水器水、喷泉水、管道旋流池水等水温高、管道阻塞、水流停滞、含铁锈(铁离子、钙离子发挥主要作用)、生物膜等因素为军团菌提供了良好的栖息和繁殖环境, 军团菌以气溶胶的形式经呼吸道传播使人致病。表1总结了现阶段嗜肺军团菌的研究现状。本文回顾嗜肺军团菌的研究现状, 主要对军团菌的致病机制以及相关的免疫应对策略进行讨论, 旨在归纳由军团菌引起的肺部感染的相关免疫机制, 以便提出更好的细菌性肺炎治疗方案。

1 嗜肺军团菌的致病机制

肺泡巨噬细胞由单核细胞分化而来, 广泛分布于肺间质内, 能在肺泡腔游走。一般而言, 吞噬细胞通过细胞膜内陷包被细胞表面附着的病原体, 在溶酶体内将大多数入侵的病原体降解消化[14]。不同于无害病原菌, 军团菌进化躲避宿主吞噬消化作用的机制, 在吞噬细胞内寄生、繁殖, 引起大量的吞噬细胞死亡, 溶酶体酶破坏邻近的正常组织细胞, 造成组织的免疫病理损伤[15-16]。

1.1 嗜肺军团菌诱发肺泡巨噬细胞的吞噬作用

空气中的嗜肺军团菌进入肺泡和肺间质后, 军团菌相关蛋白如效应蛋白RtxA[17]、菌毛相关蛋白[18]、鞭毛[19]等参与军团菌在细胞表面的附着, 肺泡巨噬细胞表面的一型补体受体(type 1 complement receptor, CR1, 又称C3b/C3d受体或CD35)和三型补体受体(type 3 complement receptor, CR3, 也称CD18/CD11b)特异性识别军团菌[20], 伸出伪足将其包围并摄入细胞内, 形成由一层细胞膜包绕的吞噬体(*Legionella*-containing vehicles, LCVs), 军团菌多种外膜蛋白如巨噬细胞感染增强蛋白(microphage infectivity potentiator, Mip)、外膜脂蛋白等参与嗜肺军团菌的入侵[20]。

嗜肺军团菌产生特化的四型分泌系统(type IVB secretory system, T4BSS), 转运于人类宿主中侵染和增殖所需的效应蛋白。效应蛋白VipA[21]直接聚合微丝, 改变吞噬体在细胞内的运输; 效应蛋白SidK与溶酶体膜上质子泵的关键成分VatA相互作用, 抑制质子泵活性[22], 避免LCVs酸化, 是早期吞噬体中嗜肺军团菌逃避宿主溶酶体酶途径杀灭的原因之一[23]; 效应蛋白真核样锚蛋白B(eukaryotic-like ankyrin B, AnkB)等参与募集泛素化蛋白至LCVs[24], PRICE等[25]认为, 嗜肺军团菌广泛调节宿主泛素网络, 提高LCVs内的氨基酸水平, 为细菌的复制提供了碳和能量来源。近年来已发现多个嗜肺军团菌效应蛋白如MAVC[26]、Ceg23[27]等在军团菌感染过程中调控K63型泛素化、去泛素化途径, 效应蛋白Lem27是作用于K6、K11、K48双泛素位点的去泛素化酶[28]。吞噬后期, LCVs与溶酶体腔室的最终融合促进LCVs内嗜肺军团菌的生长[29]。

1.2 嗜肺军团菌的四型分泌系统

T4BSS由嗜肺军团菌基因组中一个大小约65 Kb的毒力岛基因座编码, 可分为两种: IVA型(1vh)和IVB型(Dot/Icm)[30-31], 嗜肺军团菌IVB型分泌系统是其最主要的毒力系统。目前的研究认为, 嗜肺军团菌的IVB型分泌系统是一种能够横跨细菌内膜、外膜以及宿主细胞膜的孔道结构, 能将效应蛋白直接注入宿主细胞内, 这些蛋白在其增殖和侵染的生命周期中发挥重要的功能[32]。首先, ELISABETH团队[33]通过电镜的方法获得T4BSS复合物和核心复合物的结构; 其次, DEBNATH团队[34]使用电子冷冻层析成像(electron cryotomography, ECT)在冷冻水合细菌中观察嗜肺军团菌完整的T4BSS转运装置, 包括核、外膜复合物、柄和内膜复合物, 分为上、中、下三层, 与ELISABETH等的研究结果一致。在嗜肺军团菌侵染后期, T4BSS触发宿主细胞溶解酶, 诱导宿主细胞凋亡; 此外, RAYCHAUDHURY等[35]还发现, IcmR-IcmQ蛋白复合体是嗜肺军团菌的另一个转运机制, 其中IcmQ具有成孔活性, 可以在磷脂双分子层上形

表1 嗜肺军团菌的研究现状

Tabel 1 Current investigation of *Legionella pneumophila*

分类 Classification	分布 Distribution	环境特点 Environment feature	菌型 Germ types	检测方法[4-8] Detection method[4-8]	发病率 Morbidity	死亡率 Mortality	传播途径 Pathogen transmission	治疗(消毒)方法 Therapy (or sterilize) methods
Artificial waters	Air conditioning system Fountain Cyclone water Hot and cold water pipes	High water temperature, pipes block, stagnant water, rust (especially iron and calcium ions), and biofilm fit for *Legionella* habitation	Lp1-Lp14, mainly Lp1[9]	1 Traditional microbial culture; 2 Immunological assays: DFA detect urinary antigen; serum antibodies (gold immunochromatography assay (GICA); IgG; IgM); 3 Enzyme substrate analysis; 4 *Legionella* genome detection: polymerise chain reaction (real-time fluorescent quantitative PCR; FRET-PCR; AFLP; Enzyme segmentation type; 16S rRNA sequence; mip gene; isothermal nucleic acid amplification; 5 Genotyping: probe hybridization; DNA microarray; biosensors; 6 High-throughput sequencing, HTS; 7 Aliphatic acid typing technology; 8 Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, MALDI-TOF MS	*Legionella pneumophila* caused more than 90% *Legionella* disease. And 84% of *Legionella* infections are caused by *Legionella pneumophla* serogroup 1 (LP01)	*Legionella* pneumonia accounts about 2%-7% of all community related pneumonia, and mortality can be as high as 80% in immunosuppressed patients	1 Drinking water, cooling tower water and calorifier water contaminate *Legionella,* which is transmitted to people in the form of aerosols through respiratory system; 2 Inhalation of water contaminated by *Legionella pneumophila* sometimes causes transmission, such as nasogastric diet or head/neck surgery; 3 There is no valid evidence of human-to-human transmission	1 Drug therapy: fluoroquinolones and macrolides[10]; 2 Experimental method: eugenol and cinnamyl aldehyde[11]; peptidoglycan-associated lipoprotein, PAL; vaccine[12]; 3 Disinfection in public[13]: physical sterilization approachs: heating disinfection, ultraviolet sterilization, chlorination mixture with pasteurization, electrified sterilization; Chemical disinfection: 0.1g/mL copper or silver ionic solution, 1% formalin, 70% alcohol, 1:8 000 ammonia solution, 0.05% carbolic acid, etc
Natural water	Lakes Spring water	Lower temperature and poorer nutrients, limit the reproduction of *Legionella*	*Legionella pneumophila* predominate, followed by *Legionella* longbeach, *Legionella* fishei and *Legionella* golmannensis[3]					
Others	Soil							

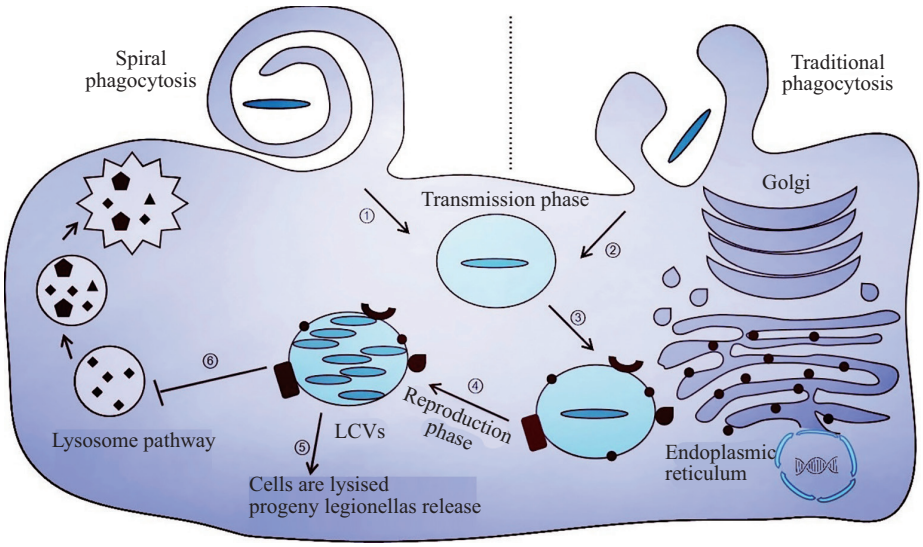
成微孔, IcmR调控IcmQ的活性。大多数Dot/Icm效应蛋白在其C末端存在被Dot/Icm分泌系统识别的转运信号, 如C末端倒数第3或第4位的氨基酸均为疏水性氨基酸[36-38]。这些信号序列富含极性氨基酸、带电性氨基酸和简单氨基酸, 如由6~8个氨基酸组成、富含谷氨酸的区域E-block结构域[36-38]等特征。

1.3 嗜肺军团菌LCVs的形成

嗜肺军团菌LCVs是其在宿主细胞内完成生长复制的场所。嗜肺军团菌入侵宿主细胞后, 通过其Dot/Icm分泌系统分泌近330个效应蛋白至宿主细胞, 干扰宿主细胞进程, 如囊泡转运、细胞凋亡等, 确保其LCVs的形成。嗜肺军团菌LCVs的形成完全依赖于其Dot/Icm分泌系统, 主要涉及宿主囊泡招募、宿主线粒体和核糖体招募及泛素化修饰。如效应蛋白SidM/DrrA[39-40]、VipD[41]、SidEs[42-45]等靶向并招募参与宿主囊泡出芽的相关蛋白Rabs、Arf和Sec22b等至LCVs表面, 招募宿主囊泡, 并与LCVs融合, 为LCVs提供膜来源; 效应蛋白SidC[46-47]、AnkB[48-49]等具有泛素连接酶活性, 锚定于LCVs膜表面, 合成并招募泛素/泛素链, 对LCVs进行泛素装饰; 效应蛋白LncP[50]和LegS2/Sp1[51]等则靶向宿主线粒体; 另外, 嗜肺军团菌的分子伴侣蛋白HtpB[52]在感染期间表达上调, 改变宿主线粒体的转运并在LCVs膜上富集。迄今, 未发现与核糖体招募相关的嗜肺军团菌蛋白因子, 但在军团菌复制期, LCVs表面核糖体数量明显增加, 且Dot/Icm系统缺陷型的军团菌则丧失招募核糖体的能力。尽管嗜肺军团菌的LCVs形成的具体分子机制尚不十分明确, 但经过大量的研究已表明, 嗜肺军团菌侵染宿主细胞、在宿主细胞内生长复制和逃避宿主免疫系统的能力完全依赖于其Dot/Icm分泌系统。

1.4 嗜肺军团菌在细胞内的生活周期

嗜肺军团菌被肺泡巨噬细胞吞噬后, 军团菌采取了一系列措施以完成其在巨噬细胞中的生命周期, 包括逃避吞噬细胞的杀伤、产生和释放各种毒素、操纵宿主囊泡运输、建立膜结合的复制液泡、引起靶细胞的损伤等过程。嗜肺军团菌在细胞内的生活周期可以分为增殖期和侵染期两个阶段(图1)[29]。吞噬体早期, 军团菌形成特殊的LCVs, LCVs不被酸化; 吞噬体后期, 细菌在LCVs中转化为耐酸的复制形式, 随后液泡与溶酶体小室融合, 形成一个酸性的、营养丰富的复制生态位[53]。军团菌在



① 细胞以螺旋吞噬的方式将军团菌摄入细胞内; ② 细胞以传统的形成伪足的方式将军团菌向内形成吞噬泡; ③ 包含军团菌的吞噬泡在细胞质中被宿主蛋白、线粒体、囊泡等修饰; ④ 修饰后的吞噬泡躲避溶酶体途径; ⑤ 军团菌在吞噬泡中复制子代; ⑥ 吞噬泡中营养耗竭后, 细胞裂解, 子代军团菌释放, 开始新一轮的侵染。

① The cells ingests *Legionella* by spiral phagocytosis; ② the cells ingests *Legionella* via the traditional phagocytic vesicles; ③ the *Legionella*-containing vesicles (LCVs) are modified by host proteins, mitochondria and vesicles in the cytoplasm; ④ the modified phagocytic vesicles avoid the lysosome pathway; ⑤ *Legionella* replicates inside vesicles; ⑥ the cells are lysised and progeny legionella are released after the depletion of nutrients in the phagocytic vesicles. *Legionella* starts a new round of infection.

图1 嗜肺军团菌在宿主细胞中的生活周期(根据参考文献[29]修改)

Fig.1 The intracellular life cycle of *L. pneumophila* in host cells (modified from reference [29])

LCVs中大量增殖, 即增殖期(reproduction phase); 在增殖后期, LCVs内氨基酸被耗尽, 子代军团菌转化为毒性形式, 逃离被消耗的宿主, 并在另一个吞噬细胞中开始新一轮的侵染与增殖, 这个过程被称为侵染期(transmission phase)[29]。

2 军团菌应对宿主免疫的策略

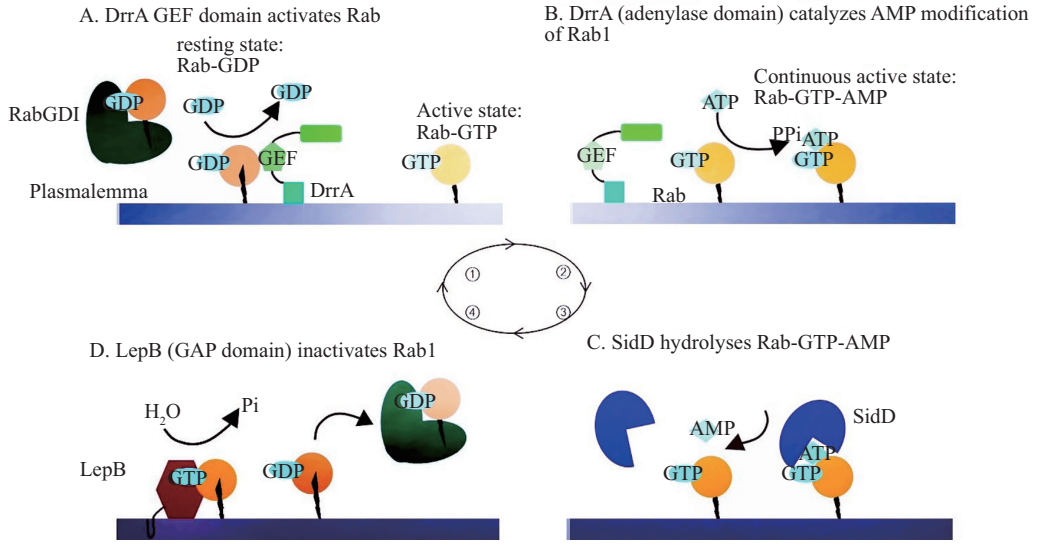
吞噬是消除病原体的重要过程: 吞噬细胞质膜上受体与抗原的结合、网格蛋白介导的假足的延伸和靶标周围质膜的封闭、浆膜分裂新生的液泡或吞噬体。通过分子马达, 吞噬泡将颗粒物质输送到细胞中, 与来自内吞或内溶酶体途径的异质小泡融合, 内容物酸化并暴露于水解酶, 从而促进内容物的有效消化。吞噬过程需要细胞膜的大量重塑, 如膜融合和膜裂变, 最后剩余未消化的液泡内容物被排除[54-55]。Rab GTPase蛋白在囊泡出芽、转运、黏附、锚定、融合的各阶段发挥重要作用[56], 调节Rab蛋白的功能是军团菌应对宿主免疫的一种生存策略。军团菌调控Rab GTPase家族的成员活性、调节LCVs膜的蛋白质组成, 抑制细菌吞噬体进入溶酶体途径[57], 宿主细胞中形成非溶酶体的复制空泡[58]。

2.1 军团菌修饰细胞调节因子Rab的策略

在军团菌感染期间, 广泛精确调控宿主蛋白(如Rab GTPase)的活动对军团菌LCVs的膜重构以及宿主囊泡运输至关重要, 也是军团菌干扰宿主免疫功能和进行LCVs细胞内迁移的方式之一。鸟嘌呤核苷酸交换因子结构域(guanosine nucleotide exchange factor, GEF)改变Rab与GTP或GDP的结合状态, 活性形式Rab-GTP与效应蛋白相互作用, 引发信号级联, 调控细胞内囊泡发生、靶向运输、靶膜的泊位和融合等生物过程[59]。多种嗜肺军团菌的效应蛋白参与对Rab GTPase的俘获、修饰和去修饰[41,60], 如SiM/DrrA催化腺苷化修饰、AnkX催化磷酸胆碱化修饰及SidEs家族催化新型泛素化修饰、SetA(subversion of eukaryotic vesicle trafficking A)催化糖基化修饰等。

2.1.1 效应蛋白SidM/DrrA修饰Rab1 嗜肺军团菌效应蛋白DrrA由3个结构域组成: 磷脂酰肌醇-4-磷酸(phosphatidylinositol 4-phosphate, PI4P)结构域(C-端)、GEF和腺苷酸转移酶(adenylyltransferase, Atase)结构域(N-端)。其中, C-端结构域将DrrA固定在LCVs膜上, 为GEF、ATase发挥酶活提供稳定的支架作用, 还有证据表明, C-端结构域直接影响吞噬体蛋白质的定位和功能[61]; GEF结构域招募细胞质中游离的Rab1; ATase催化Rab1的腺苷化并将Rab1固定在LCVs膜上。SidM/DrrA募集Rab1, 并催化Rab1b的腺苷化修饰, 将Rab1b截留在LCVs上[62], Rab1b招募宿主内质网来源的囊泡修饰LCVs, 阻止初级溶酶体与LCVs融合机制的发生。军团菌干扰宿主囊泡的运输, 还包括其他多种军团菌效应蛋白共同参与, 并最终形成活性开闭的膜循环(图2)。鸟嘌呤分离抑制剂移位因子[guanine nucleotide dissociation nhibitor (GDI) displacement factor, GDF]将Rab从GDI复合物中释放, DrrA募集游离的Rab1-GDP, DrrA GEF结构域催化Rab1从GDP的结合态向GTP结合态的发生, 被激活的Rab1-GTP具有招募细胞内其他宿主蛋白的活性(图2A)。DrrA的腺苷化结构域(ATase)催化Rab1的腺苷化修饰(AMP-Rab1)。GTPase激活蛋白(GTPase activating proteins, GAPs)催化Rab1从GTP结合态向GDP结合态的转变, 腺苷化Rab1抑制GAPs的催化活性, 同时也抑制Rab1与GDI作用(图2B)。AMP-Rab1的去腺苷化是GAPs解除Rab1持续激活的状态的前提, 军团菌另一个效应蛋白SidD催化AMP-Rab1去腺苷化修饰[63](图2C)。MISHRA等[64-65]报道了嗜肺军团菌效应蛋白LepB在Rab1复合物中的过渡态模拟结构, LepB的GAPs结构域能灭活Rab1, 将其从LCVs膜上释放, 这可能与增殖后期军团菌诱发的细胞裂解等活动相关(图2D)。军团菌通过DrrA、SidD等一套非常完整的操纵宿主Rab1活性的效应蛋白, 对Rab1b在LCVs上的定位和激活进行精确的时间控制, 保证军团菌捕获足够的宿主来源的核糖体、线粒体等进行LCVs修饰。腺苷化修饰Rab1是一种有效的策略, 可以在空间和时间上调节Rab1在膜上的功能, 既能在复制期满足军团菌在LCVs内复制的需求, 保障复制生态位的形成, 同时在侵染期时有利于保障军团菌的细胞逃逸。

2.1.2 效应蛋白AnkX修饰Rab 军团菌中还有另外一套机制用于调控Rab的活性, 嗜肺军团菌效应蛋白AnkX(PDB:4BER)含有FIC结构域(filamentation induced by cyclic adenosine monophosphate moiety)和13个真核样锚蛋白重复结构域(eukaryotic-like ankyrin repeat domains, Anks), 所有结构域相互之间建立多个交互, 产生一个紧密结合的结构[67]。AnkX调控巨噬细胞内微管依赖的囊泡运输、晚期LCVs与



① Rab被DrrA GEF结构域激活, 从Rab-GDP结合态转换成Rab-GTP结合态, 从细胞质固定至膜上; ② DrrA的腺苷化酶结构域催化Rab-GTP的AMP修饰, 形成持续的活跃态; ③ SidD催化Rab-GTP-AMP的AMP水解; ④ LepB GAP结构域催化Rab-GTP从活跃态转换成静息态。

① The Rab is activated by DrrA GEF domain, Rab-GDP is transformed to Rab-GTP binding state, and then recruited on membrane; ② DrrA (adenylase domain) catalyzes AMP modification of Rab-GTP, containing a continuous active state; ③ SidD catalyzes Rab-GTP-AMP hydrolysis; ④ LepB (GAP domain) catalyzes the conversion of Rab-GTP from an active state to a resting state.

图2 嗜肺军团菌调控Rab1循环(根据参考文献[66]修改)

Fig.2 Rab1 cycles regulated by *L. pneumophila* (modified from reference [66])

溶酶体融合[68]。AnkX FIC结构域以CDP-胆碱(CDP-choline, C5P)作为磷酸胆碱基团供体, 将磷酸胆碱转移到Rab1b、Rab35, 形成C5P-Rab[69]。军团菌效应蛋白Lem3催化AnkX的去磷酸胆碱修饰[70]。此外, 军团菌效应蛋白AnkX与LidA存在另一种调控方式, C5P-Rab与GEF、GDI的相互作用被抑制, 但对C5P-Rab与GAPs或LidA的相互作用影响不大[71], 复合物LidA:C5P-Rab-GDP与Rab-GTP具有几乎相同的活性[72]。磷酸胆碱修饰将Rab锁定在Rab-GDP的状态, 细胞中与活跃态Rab-GTP相互作用后发挥功能的蛋白均被抑制, LidA结合Rab-GDP满足军团菌重新获得Rab GTPase活性的需求。效应蛋白AnkX介导的Rab1磷酸胆碱化修饰不能代替Rab1的腺苷化修饰, AnkX对Rab1的磷酸胆碱修饰既不是维持LCVs上Rab1的必要条件, 也不是充分条件, 这表明, 腺苷化修饰和磷酸胆碱修饰在功能上具有不同的生物学活性。

2.1.3 SetA催化的糖基化修饰 效应蛋白SetA定位于哺乳动物细胞晚期溶酶体中, 影响酵母的小泡运输。SetA具有糖基转移酶活性, 交联质谱分析发现Rab1是SetA的靶点之一, SetA优先修饰Rab1-GDP, 而不是Rab1-GTP, 将葡萄糖共价连接到Rab1第75位苏氨酸Thr75上, Rab1糖基化修饰抑制了其GTPase活性[73], 但不影响其接受GTP的能力。SetA的活性增加了Rab1-GTP复合物, SetA与其他效应蛋白(如SidM)协同发挥作用, 确保在嗜肺乳杆菌感染期间内, 活性Rab1与LCVs相关联[74]。TAK1(TGF-β activated kinase 1)与宿主先天免疫有关, Rab1 Thr 75被TAK1磷酸化, 细菌与宿主竞争控制Rab1的活性[74-75]。此外, SetA在多个位点糖基化转录因子EB(transcription factor EB, TFEB), 真核细胞中氨基酸的稳态由TFEB调节, TFEB易位进入细胞核激活自噬和溶酶体生物发生相关的基因, TFEB易位取决于SetA的糖基转移酶活性[76]。

2.1.4 嗜肺军团菌新型泛素化机制 泛素化在蛋白质周转、细胞周期、囊泡运输、信号传递、DNA修复、先天免疫和适应性免疫的细胞过程中必不可少, 不同泛素链的长度和连接类型具有不同的功能, 如蛋白降解、酶活性改变、细胞定位、蛋白相互作用等。嗜肺军团菌SidE家族(即SidEs), 包括SidE、SdeA、SdeB和SdeC, 形成一种不依赖于E2或E3连接酶的新型泛素化机制。SdeA由ADP-核糖转移酶域(mono-ADP-ribosyltransferase, mART)和磷酸二酯酶域(phoshopdiesterase, PDE)组成, 以NAD^+为能量来源, 驱动多步生化反应。mART对泛素的第42位精氨酸(R42)进行ADP-核糖基化(ADP-ribosylated ubiquitin, ADPR-Ub), 激活泛素。PDE在

多种宿主蛋白如Rab GTPases、管状内质网重排蛋白Rtn4(reticulon 4)[45,77]等的丝氨酸残基催化泛素/泛素链连接[78]。效应蛋白SidJ位于SidEs基因座中, 是一种CaM依赖的蛋白质聚谷氨酰胺酶, 对SdeA的活性谷氨酸残基E860进行谷氨酸修饰, 抑制了SdeA介导的泛素精氨酸R42的ADP-核糖基化[79-80]。在细菌感染过程中, SidJ能够抑制SidE家族的细胞毒性。此外, 效应蛋白SidC、SdcA和LegA14属于新型的泛素连接酶, 具有新型的催化三元体Cys-His-Asp催化泛素链的形成[46,81]; 效应蛋白RavD具有去泛素化酶活性, 特异性地识别并水解线性泛素链, 抑制宿主的免疫反应[41]; 效应蛋白LubX含有2个U-box结构域, 介导宿主因子Cdc2样激酶1和效应蛋白SidH泛素化, 导致其被蛋白酶体降解[82]。

2.2 军团菌与细胞凋亡

当细胞受到病原体入侵时, 细胞启动细胞凋亡清除细胞内病原体, 是一种有效的细胞防御感染机制。嗜肺军团菌在诱导和抑制宿主细胞死亡之间保持平衡。嗜肺军团菌感染早期, 宿主细胞的过早凋亡不利于军团菌在胞内的增殖与侵染。嗜肺军团菌感染早期, 巨噬细胞的caspase-3被激活参与囊泡转运而非细胞凋亡。细胞表面受体识别病原体后触发信号级联, 胞质内磷酸化激酶IKKs(IκB kinase)激活核转录因子NF-κB(nuclear transcription factor kappa B)家族成员, NF-κB亚基易位进入细胞核, 调控促炎细胞因子和凋亡抑制剂的转录[83-84]。嗜肺军团菌通过效应蛋白激活NF-κB信号或通过其他抗凋亡途径, 抑制细胞凋亡。如LegK1具有真核样丝氨酸/苏氨酸激酶活性, 磷酸化IKKα第32位丝氨酸Ser32、IKKβ第36位丝氨酸Ser36及NF-κB家族抑制因子p100, 激活经典NF-κB信号通路[85]; MavC利用其谷氨酰胺转移酶活性催化泛素的第40位谷氨酰胺的谷氨酰胺转移反应, 转移至泛素结合酶UBE2N的第94位赖氨酸, 抑制宿主NF-κB信号通路激活[86]。此外, LnaB[87]、SdhA和LubX等均可能参与激活宿主细胞抗凋亡NF-κB通路[88]。Lpg1137为丝氨酸蛋白酶, 水解突触融合蛋白Syntaxin 17, 抑制细胞自噬和依赖于Bax-Drp1的细胞凋亡[69]。RavZ识别并结合自噬体膜上的LC3, 将LC3从自噬体膜抽离, 抑制细胞自噬[89]。SidF抑制宿主细胞凋亡分子BCL-RAMBO或BNIP3相关的信号通路控制宿主细胞的死亡[90], 但也有研究表明, 在没有capsase-11依赖的凋亡作用的情况下, 军团菌诱导的巨噬细胞死亡延迟并不依赖于SidF[91]。VipD是磷脂酶A2, 位于线粒体膜水解磷脂酰乙醇胺(phosphatidylethanolamine, PE)和胆碱磷酸(phosphocholine, PC), 引起线粒体膜的不稳定性, 激活caspase-3[92]。SdhA调控宿主细胞I型干扰素的产生, 抑制宿主细胞凋亡, 维持嗜肺军团菌LCVs的完整性[93-94]。嗜肺军团菌分泌效应蛋白调控宿主抗凋亡基因的表达, 暂时性抑制细胞凋亡或延长细胞的存活时间, 为自身获取增殖的时间和空间。

嗜肺军团菌在感染后期则激活宿主细胞死亡信号, 诱导宿主细胞坏死或焦亡, 裂解细胞, 以便其从宿主细胞中逃逸及开始下一轮的侵染。目前, 对嗜肺军团菌的逃逸机制并不清楚, 一般的机制模型认为: 在完成复制后, 嗜肺军团菌可诱导具有成孔能力的毒力因子表达, 诱发宿主细胞的坏死或焦亡, 在其LCVs膜及宿主细胞膜上形成孔洞裂解细胞, 完成嗜肺军团菌的释放[95-96]。

3 宿主细胞的免疫应答

先天免疫系统是宿主抵御病原体攻击的第一道防线, 并向适应性免疫系统发出潜在的感染信号。宿主细胞的先天免疫系统对于成功消除病原体至关重要[97], 而军团菌Dot/Icm系统在诱导多种先天性免疫反应途径(包括NF-κB途径、MAPK途径和宿主应激反应[98])中具有重要作用[99-100]。

3.1 病原体触发反应

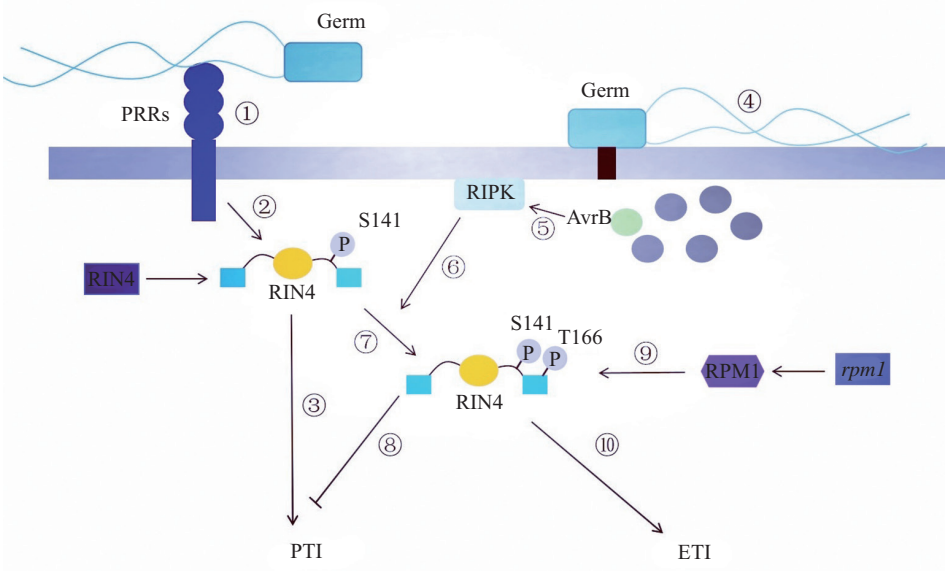
细胞识别病原相关分子的模式被称为病原相关分子模式(pathogen-associated molecular patterns, PAMPs)或损伤相关的分子模式(damage-associated molecular patterns, DAMPs), 并将宿主受体称为模式识别受体(pattern recognition receptors, PRRs), 如Toll样受体(Toll-like receptors, TLRs)和核苷酸结合寡聚域样受体[nucleotide-binding oligomerization domain (NOD)-like receptors, NLRs][101-103]。已在人类细胞中发现10个TLRs, 部分TLRs配体和衔接蛋白, 其中衔接蛋白髓样分化初级反应蛋白88(myeloid differentiation primary response protein 88, MyD88)是TLR/IL1-R超家族的关键信号传递介质, 促进转录因子NF-κB的核易位, 调节免疫或发育靶基因的表达[104-105]。CELESE团队[106]研究发现, 与野生型相比, 嗜肺军团菌二型分泌系统(type II secretion system, T2SS)突变体对丝裂原活化蛋白激酶(mitogen-activated protein

kinase, MAPK)和NF-κB通路的刺激更强, T2SS系统抑制人巨噬细胞中的TLR2/MyD88信号通路, 从而支持了T2SS抑制先天免疫反应触发的假说[106]。军团菌气溶胶感染豚鼠肺部的研究表明, 受感豚鼠产生特异性抗军团菌的抗体, 特异性抗体增强细胞的吞噬作用[107], 但对细菌在细胞内的增殖没有明显的抑制作用, 因此, 军团菌引起的体液免疫可能不是有效的宿主防御机制[108-109]。

3.2 效应器触发反应

效应蛋白不仅在军团菌LCVs的生物发生中起重要作用, 而且可触发宿主免疫反应[102]。MARY团队[101]发现, 缺乏Dot/Icm系统的嗜肺军团菌能被TLRs识别, 介导NF-κB依赖性转录反应, 而表达功能性Dot/Icm系统的军团菌诱导独特的转录靶标, 这种依赖于Dot/Icm的免疫反应被称为效应器触发反应(effector-triggered response, ETR)。研究嗜肺军团菌分泌蛋白(major secretory protein, MSP)在豚鼠中诱导体液免疫、细胞免疫的能力, MSP免疫后的豚鼠对嗜肺军团菌气雾剂表现出明显的细胞免疫和体液免疫应答, 且抑制嗜肺军团菌在肺部的增殖[110]。MSP、OmpS、Hsp60对豚鼠和人类表现出明显的迟发型超敏反应(delayed-type hypersensitivity, DTH)[111]。

我们实验室在探索军团菌致病机制的过程中发现, 嗜肺军团菌效应蛋白Lem23(lpg2406)的三维结构与丁香假单胞菌效应蛋白AvrB(avirulence B)高度相似(未发表的数据)。AvrB在拟南芥中触发的植物基础免疫已被广泛研究, 其抑制植物病原体相关分子模式触发的免疫(PAMPs-triggered immunity, PTI)同时又能诱导效应因子触发的免疫(effector-triggered immunity, ETI)(图3)。在此, 我们推测, 军团菌效应蛋白Lem23在宿主细胞中起与AvrB具有类似的功能, 抑制宿主细胞的PTI, 诱导宿主细胞的迟发型超敏反应(图4), 但其具体的分子机制及在宿主细胞中是否存在抗军团菌感染的免疫反应所需的分子基础有待进一步的探究。探究病原体拮抗宿主细胞的模式识别受体介导的防御机制, 对临床治疗军团菌病具有重要作用, 将有助于呼吸道感染的治疗和预防策略的制定。

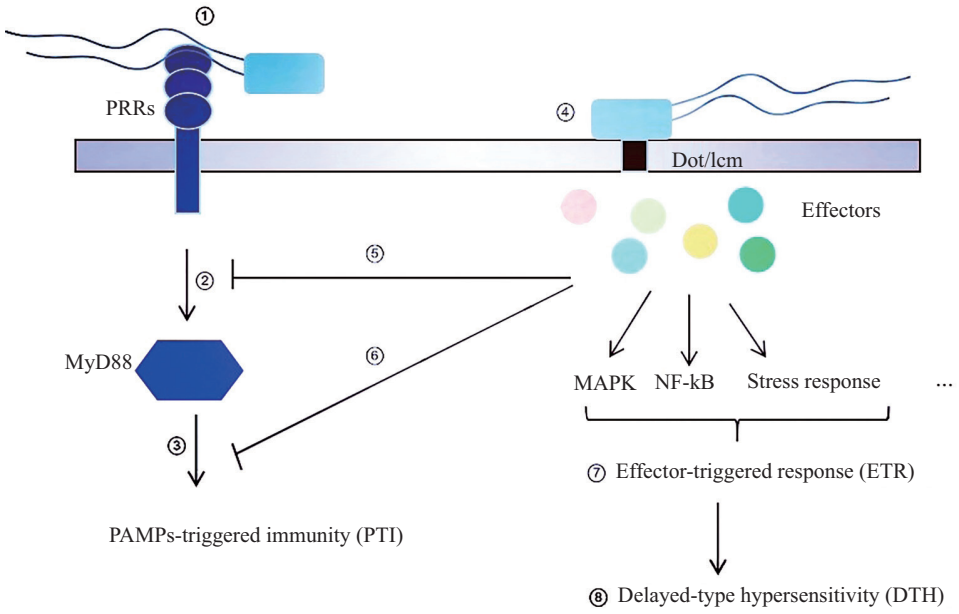


① 细胞表面PRRs识别细菌表面抗原; ② PRRs将抗原信号传递至胞内RIN4; ③ 细胞启动PTI杀灭病原菌; ④ 丁香假单胞菌通过分泌系统向细胞内传送效应蛋白, 其中包括AvrB; ⑤ AvrB激活宿主蛋白RIPK; ⑥ RIPK对RIN4进行磷酸化修饰; ⑦ RIN4在多个位点被磷酸化修饰, 其中包括S141、T166等; ⑧ 过度磷酸化修饰的RIN4抑制植物PTI活动; ⑨ RPM1识别过度磷酸化的RIN4; ⑩ AvrB引起的RIN4过度磷酸化最终触发植物ETI活动, 受感细胞局部死亡。

① PRRs (pattern recognition receptors) on cell surface detect antigens of bacteria; ② the antigen signal is transmitted by PRRs to intracellular RIN4; ③ PTI is activated to kill pathogenic bacteria; ④ *Pseudomonas sycamore* delivers effectors into cells through secretory system, including AvrB; ⑤ AvrB activates host protein RIPK; ⑥ phosphorylation of RIN4 catalyzed by RIPK; ⑦ RIN4 is phosphorylated at multiple sites, including S141, T166 and etc; ⑧ RIN4 that is hyperphosphorylated inhibites plants PTI; ⑨ RPM1 recognizes RIN4 that is hyperphosphorylated; ⑩ RIN4 hyperphosphorylated caused by AvrB triggers plant ETI and eventually break out local death in affected cells.

图3 RIN4对AvrB的免疫应答

Fig.3 Immune responses of RIN4 to AvrB



① 细胞表面PRRs识别细菌表面抗原; ② PRRs将抗原信号传递至胞内蛋白, 如MyD88; ③ 细胞启动PTI杀灭病原菌; ④ 军团菌通过分泌系统(如Dot/Icm)向细胞内传送效应蛋白; ⑤、⑥ 效应蛋白抑制PTI; ⑦ 效应蛋白触发ETR; ⑧ 受感细胞产生迟发型超敏反应, 细胞局部死亡。

① PRRs (pattern recognition receptors) on cell surface detect antigens of bacteria; ② the antigen signal is transmitted by PRRs to intracellular protein, such as MyD88; ③ PTI is activated to kill pathogenic bacteria; ④ *Legionella pneumophila* delivers effectors into cells through secretory system (Dot/Icm); ⑤,⑥ effectors inhibites immune response PTI; ⑦ effectors triggers ETR; ⑧ eventually break out local death in affected cells.

图4 哺乳动物效应器触发免疫

Fig.4 Effectors-triggered immune of mammalian cells

4 总结与展望

嗜肺军团菌作为引起军团菌病的病原菌, 我国已将军团菌病列为重点防治的一种新发传染病, 而当前军团菌的绝大多数效应蛋白的生物学功能尚未完全阐明。首先, 深入研究军团菌调控宿主细胞的免疫应答机制, 对于理解细菌性感染疾病的发生、提出致命性下呼吸道感染的治疗方案和预防策略至关重要。其次, 模式识别受体不仅触发宿主先天应答, 而且调节特异性的适应性免疫应答。识别病原体诱发的活动将有利于宿主区分高毒力或低致病潜力的病原体, 为精准治疗传染病提供新策略。再次, 对嗜肺军团菌治病机制的阐明, 可为胞内致病菌结核分枝杆菌、沙寒沙门菌等与宿主先天性免疫系统的互作机制给予重要启示。我们课题组综合运用X-射线晶体学方法、Cryo-EM、生物化学与分子生物学、细胞生物学等技术致力于阐明病原体–宿主相互作用等生命过程中关键科学问题, 以致病性嗜肺军团菌为模型, 系统地筛选并发现与病原菌入侵以及胞内存活相关的病原菌致病因子和宿主因子, 在分子水平上研究病原菌蛋白与宿主蛋白之间的相互作用, 如效应蛋白Ceg23在军团菌感染过程中调控K63型多泛素修饰的蛋白分子的去泛素化过程[27]; 效应蛋白Lem27通过抑制细菌的E3泛素连接酶SidC和SdcA的活性共同调控Rab10的泛素化[28]; 效应蛋白MavC(Lpg2147)通过转谷氨酰胺酶活性将Ub的谷氨酰胺残基转移到靶点E2泛素结合酶UBE2N(ubiquitin conjugating enzyme E2 N), 完成对宿主UBE2N的非典型泛素化过程, 从而抑制NF-κB信号通路的激活[112]; 效应蛋白MvcA(Lpg2148)可以特异性逆转由MavC催化的泛素化过程[113], 从而在感染期间对宿主信号进行精确的时空调控; 效应蛋白SidJ协作钙调蛋白以及AMP腺苷单磷酸化作用对SdeA进行谷氨酸化修饰的全新酶学机理[79]。病原菌与宿主互作中的新的作用机制, 泛素和类泛素化蛋白等修饰及其他新型修饰如何调控病原菌进入宿主细胞并在胞内存活及相关信号通路的分子机制, 深入揭示了病原菌发生作用的分子机制, 为新型药物和疫苗的设计提供了理论基础。

参考文献 (References)

[1] 吴建辉, 张灿辉, 彭志文, 等. 6型嗜肺军团菌肺炎并发肺脓

肿一例并文献复习[J]. 中国呼吸与危重监护杂志(WU J H, ZHANG C H, PENG Z W, et al. Severe cavitary pneumonia caused by *Legionella pneumophila* serotype 6: a case report and literature review [J]. Chin J Respir Crit Care Med Chin), 2021, 20(2): 106-13.

[2] 任红宇, 周海健, 李马超, 等. 206株中国环境分离嗜肺军团菌1型菌株基因序列分型研究[J]. 疾病监测(REN H Y, ZHOU H J, LI M C, et al. Sequence based typing of *Legionella pneumophila* serotype 1 isolated from natural environment in China [J]. Dis Surveillance Chin), 2015, 30(2): 97-102.

[3] 郭景玉. 中国城市自然水环境中军团菌分布情况调查及嗜肺军团菌的遗传多态性研究[D]. 北京: 中国人民解放军军事医学科学院, 2015.

[4] 张秋, 李杰, 王猛, 等. 嗜肺军团菌快速检测胶体金试纸的研制[J]. 检验医学(ZHANG Q, LI J, WANG M, et al. Development of colloidal gold immunochromatography assay for the rapid detection of *Legionella pneumophila* [J]. Lab Med Chin), 2020, 35(7): 726-33.

[5] PASCALE M R, MAZZOTTA M, SALARIS S, et al. Evaluation of MALDI-TOF mass spectrometry in diagnostic and environmental surveillance of *Legionella* species: a comparison with culture and Mip-gene sequencing technique [J]. Front Microbiol, 2020, 11: 589369.

[6] 鲁曦, 李王平, 李春梦, 等. 嗜肺军团菌致病机制及快速检测最新研究进[J]. 中华肺部疾病杂志(LU X, LI W P, LI C M, et al. Current progress on oathogensis and rapid detection of *Legionella pneumophila* [J], Chin J Lung Dis Chin), 2018, 11(1): 101-4.

[7] 林韵, 肖晓臻, 肖贤声, 等. 嗜肺军团菌分子检测最新进展[J]. 生物技术通讯(LIN Y, XIAO Q Z, XIAO X S, et al. Recent progress of molecular methods for *Legionella pneumophila* detection [J]. Lett Biotech Chin), 2017, 28(5): 704-8.

[8] 高艳, 李啸, 段杉, 等. 酶底物法与过滤培养法检测非饮用水中嗜肺军团菌的比较[J]. 净水技术(GAO Y, LI X, DUAN S, et al. Comparison of enzyme substrate and filter culture methods for determination of *Legionella pneumophila* [J]. Water Purif Technol Chin), 2020, 39(8): 8-12.

[9] 孔德川, 肖文佳, 陈明亮, 等. 2011—2018年上海市公共场所人工水环境中军团菌污染情况调查及其病原型别鉴定[J]. 中华预防医学杂志(KONG D C, XIAO W J, CHEN M L, et al. Incestigation and identification of *Legionella* contamination in artificial water environment of public places in Shanghai from 2011 to 2018 [J]. Chin J Preven Med), 2021, 55(1): 72-7.

[10] 薛洪源, 葛向华, 蔡长春, 等. 社区获得性嗜肺军团菌肺炎治疗的抗菌药物使用分析[J]. 中国感染与化疗杂志(XIE H Y, GE X H, CAI C C, et al. Analysis of the antibacterial use in community-acquired pneumonia due to *Legionella pneumophila* [J]. Chin J Infect Chemother), 2012, 12(5): 348-51.

[11] 马江伟. 丁香酚及肉桂醛对嗜肺军团菌的抗菌作用及机制的研究[D]. 沈阳: 中国医科大学, 2018.

[12] KIM S J, SIN J I, KIM M J. $CD8^+$ T Cells directed against a peptide epitope derived from peptidoglycan-associated lipoprotein of *Legionella pneumophila* confer disease protection [J]. Front Immunol, 2020, 11: 604413.

[13] 边国林, 王爱红. 公共场所军团菌污染现状研究进展[J]. 环境与职业医学(BIAN G L, WANG A H. Research progress on *Legionella* contamination in public places [J]. J Environ Occup Med Chin), 2014, 31(12): 981-4.

[14] FLANNAGAN R S, COSíO G, GRINSTEIN S. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies [J]. Nature Rev Microbiol, 2009, 7(5): 355-66.

[15] CIANCIOTTO N P. Pathogenicity of *Legionella pneumophila* [J]. Int J Med Microbiol, 2001, 291(5): 331-43.

[16] MIYAKE M. Intracellular survival and replication of *Legionella pneumophila* within host cells [J]. Yakugaku Zasshi, 2008, 128(12): 1763-70.

[17] CIRILLO S L G, LUM J, CIRILLO J D. Identification of novel loci involved in entry by *Legionella pneumophila* [J]. Microbiology, 2000, 146(6): 1345-59.

[18] STROM M S, LORY S. Structure-function and biogenesis of the type IV pili [J]. Annual Rev Microbiol, 1993, 47(1): 565-96.

[19] DIETRICH C, HEUNER K, BRAND B C, et al. Flagellum of *Legionella pneumophila* positively affects the early phase of infection of eukaryotic host cells [J]. Infect Immun, 2001, 69(4): 2116-22.

[20] PAYNE N R, HORWITZ M A. Phagocytosis of *Legionella pneumophila* is mediated by human monocyte complement receptors [J]. J Exp Med, 1987, 166(5): 1377-89.

[21] FRANCO I, SHOHDY N, SHUMAN H. The *Legionella pneumophila* effector VipA is an actin nucleator that alters host cell organelle trafficking [J]. PLoS Pathog, 2012, 8(2): e1002546.

[22] XU L, SHEN X, BRYAN A, et al. Inhibition of host vacuolar H^+-ATPase activity by a *Legionella pneumophila* effector [J]. PLoS Pathog, 2010, 6(3): e1000822.

[23] STURGILL-KOSZYCKI S, SWANSON M S. *Legionella pneumophila* replication vacuoles mature into acidic, endocytic organelles [J]. J Exp Med, 2000, 192(9): 1261-72.

[24] PRICE C T D, AL-QUADAN T, SANTIC M, et al. Exploitation of conserved eukaryotic host cell farnesylation machinery by an F-box effector of *Legionella pneumophila* [J]. J Exp Med, 2010, 207(8): 1713-26.

[25] PRICE C T D, AL-QUADAN T, SANTIC M, et al. Host proteasomal degradation generates amino acids essential for intracellular bacterial growth [J]. Science, 2011, 334(6062): 1553-7.

[26] PUVAR K, IYER S, FU J, et al. *Legionella* effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination [J]. Nat Commun, 2020, 11(1): 2365.

[27] MA K, ZHEN X, ZHOU B, et al. The bacterial deubiquitinase Ceg23 regulates the association of Lys-63-linked polyubiquitin molecules on the *Legionella* phagosome [J]. J Biol Chem, 2020, 295(6): 011758.

[28] LIU S, LUO J, ZHEN X, et al. Interplay between bacterial deubiquitinase and ubiquitin E3 ligase regulates ubiquitin dynamics on *Legionella* phagosomes [J]. eLife, 2020, 9: e58114.

[29] AND M S S, HAMMER B K. *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages [J]. Annu Rev Microbiol, 2000, 54(1): 567-13.

[30] BRASSINGA A K C, HILTZ M F, SISSON G R, et al. A 65-kilobase pathogenicity island is unique to Philadelphia-1 strains of *Legionella pneumophila* [J]. J Bacteriol, 2003, 185(15): 4630-37.

[31] LLOSA M, ROY C, DEHIO C. Bacterial type IV secretion systems in human disease [J]. Mol Microbiol, 2009, 73(2): 141-

51.
[32] ENSMINGER A W. *Legionella pneumophila*, armed to the hilt: justifying the largest arsenal of effectors in the bacterial world [J]. Curr Opin Microbiol, 2016, 29: 74-80.
[33] GROHMANN E, CHRISTIE P J, WAKSMAN G, et al. Type IV secretion in gram-negative and gram-positive bacteria [J]. Mol Microbiol, 2018, 107(4): 455-71.
[34] GHOSAL D, CHANG Y W, JEONG K C, et al. *In situ* structure of the *Legionella* Dot/Icm type IV secretion system by electron cryotomography [J]. EMBO Rep, 2017, 18(5): 726-32.
[35] RAYCHAUDHURY S, FARELLI J D, MONTMINY T P, et al. Structure and function of interacting IcmR-IcmQ domains from a type IVb secretion system in *Legionella pneumophila* [J]. Structure, 2009, 17(4): 590-601.
[36] NAGAI H, CAMBRONNE E D, KAGAN J C, et al. A C-terminal translocation signal required for Dot/Icm-dependent delivery of the *Legionella* RalF protein to host cells [J]. Proc Natl Acad Sci USA, 2005, 102(3): 826-31.
[37] KUBORI T, HYAKUTAKE A, NAGAI H. *Legionella* translocates an E3 ubiquitin ligase that has multiple U-boxes with distinct functions [J]. Mol Microbiol, 2010, 67(6): 1307-19.
[38] HUANG L, BOYD D, AMYOT W M, et al. The E block motif is associated with *Legionella pneumophila* translocated substrates [J]. Cell Microbiol, 2010, 13(2): 227-45.
[39] MüLLER M P, PETERS H, BLüMER J, et al. The *Legionella* effector protein DrrA AMPylates the membrane traffic regulator Rab1b [J]. Science, 2010, 329(5994): 946-9.
[40] DEL CAMPO C M, MISHRA A K, WANG Y H, et al. Structural basis for PI(4)P-specific membrane recruitment of the *Legionella pneumophila* effector DrrA/SidM [J]. Structure, 2014, 22(3): 397-08.
[41] KU B, LEE K-H, PARK W S, et al. VipD of *Legionella pneumophila* targets activated Rab5 and Rab22 to interfere with endosomal trafficking in macrophages [J]. PLoS Pathog, 2012, 8(12): e1003082.
[42] BHOGARAJU S, KALAYIL S, LIU Y, et al. Phosphoribosylation of ubiquitin promotes serine ubiquitination and impairs conventional ubiquitination [J]. Cell, 2016, 167(6): 1636-49.
[43] QIU J, SHEEDLO M J, YU K, et al. Ubiquitination independent of E1 and E2 enzymes by bacterial effectors [J]. Nature, 2016, 533(7601): 120-4.
[44] AKTURK A, WASILKO D J, WU X, et al. Mechanism of phosphoribosyl-ubiquitination mediated by a single *Legionella* effector [J]. Nature, 2018, 557(7707): 729-33.
[45] KALAYIL S, BHOGARAJU S, BONN F, et al. Insights into catalysis and function of phosphoribosyl-linked serine ubiquitination [J]. Nature, 2018, 557(7707): 734-8.
[46] WASILKO D J, HUANG Q, MAO Y. Insights into the ubiquitin transfer cascade catalyzed by the *Legionella* effector SidC [J]. eLife, 2018, 7: e36154.
[47] HSU F, LUO X, QIU J, et al. The *Legionella* effector SidC defines a unique family of ubiquitin ligases important for bacterial phagosomal remodeling [J]. Proc Natl Acad Sci USA, 2014, 111(29): 10538-43.
[48] BRUCKERT W M, ABU KWAIK Y. Lysine11-linked polyubiquitination of the AnkB F-box effector of *Legionella pneumophila* [J]. Infect Immun, 2016, 84(1): 99-107.
[49] WONG K, PERPICH J D, KOZLOV G, et al. Structural mimicry by a bacterial F box effector hijacks the host ubiquitin-proteasome system [J]. Structure, 2017, 25(2): 376-83.
[50] DOLEZAL P, AILI M, TONG J, et al. *Legionella pneumophila* secretes a mitochondrial carrier protein during infection [J]. PLoS Pathog, 2012, 8(1): e1002459.
[51] ABU KHWEEK A, KANNEGANTI A, GUTTRIDGE D D, et al. The sphingosine-1-phosphate Lyase (LegS2) contributes to the restriction of *Legionella pneumophila* in murine macrophages [J]. PLoS One, 2016, 11(1): e0146410.
[52] CHONG A, LIMA C A, ALLAN D S, et al. The purified and recombinant *Legionella pneumophila* chaperonin alters mitochondrial trafficking and microfilament organization [J]. Infect Immun, 2009, 77(11): 4724-39.
[53] TILNEY L G, HARB O S, CONNELLY P S, et al. How the parasitic bacterium *Legionella pneumophila* modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to the ER membrane [J]. J Cell Sci, 2001, 114(24): 4637-50.
[54] GUERRIER S, PLATTNER H, RICHARDSON E, et al. An evolutionary balance: conservation vs innovation in ciliate membrane trafficking [J]. Traffic, 2017, 18(1): 18-28.
[55] HUYNH K K, KAY J G, STOW J L, et al. Fusion, fission, and secretion during phagocytosis [J]. Physiol, 2007, 22: 366-72.
[56] HUTAGALUNG A H, NOVICK P J. Role of Rab GTPases in membrane traffic and cell physiology [J]. PhysiolRev, 2011, 91(1): 119-49.
[57] RUSSELL M R G, NICKERSON D P, ODORIZZI G. Molecular mechanisms of late endosome morphology, identity and sorting [J]. Curr Opin Cell Biol, 2006, 18(4): 422-8.
[58] FINSEL I, RAGAZ C, HOFFMANN C, et al. The *Legionella* effector RidL inhibits retrograde trafficking to promote intracellular replication [J]. Cell Host Microb, 2013, 14(1): 38-50.
[59] 管睿, 崔英. Rab家族及其在囊泡运输中的作用[J]. 国际遗传学杂志(GUAN R, CUI Y. The RaB family and their role in vesicular trafficking [J]. Section Genet Foreign Med Sci Chin), 2005(2): 91-3.
[60] 肖婷, 赵桂华, 尹昆. 嗜肺军团菌调控宿主囊泡转运的效应因子及其分子机制[J]. 微生物学通报(XIAO T, ZHAO G H, YI A. *Legionella pneumophila* effectors and mechanisms in regulating host vesicular trafficking [J]. Microbiol Chin), 2016, 43(11): 2488-94.
[61] FRATTI R A, BACKER J M, GRUENBERG J, et al. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest [J]. J Cell Biol, 2001, 154(3): 631-44.
[62] MÜLLER M P, PETERS H, BLÜMER J, et al. The *Legionella* effector protein DrrA AMPylates the membrane traffic regulator Rab1b [J]. Science, 2010, 329(5994): 946-9.
[63] TAN Y, LUO Z Q. *Legionella pneumophila* SidD is a deAMPylase that modifies Rab1 [J]. Nature, 2011, 475(7357): 506-9.
[64] MIHAI GAZDAG E, STRELLER A, HANEBURGER I, et al. Mechanism of Rab1b deactivation by the *Legionella pneumophila* GAP LepB [J]. EMBO Rep, 2013, 14(2): 199-205.
[65] MISHRA A K, DEL CAMPO C M, COLLINS R E, et al. The *Legionella pneumophila* GTPase activating protein LepB accelerates Rab1 deactivation by a non-canonical hydrolytic mechanism

[J]. J Biol Chem, 2013, 288(33): 24000-11.

[66] HARDIMAN C A, ROY C R. AMPylation is critical for Rab1 localization to vacuoles containing *Legionella pneumophila* [J]. mBio, 2014, 5(1): e01035-13.

[67] LI J, MAHAJAN A, TSAI M D. Ankyrin repeat: a unique motif mediating protein-protein interactions [J]. Biochem, 2006, 45(51): 15168-78.

[68] PAN X, LüHRMANN A, SATOH A, et al. Ankyrin repeat proteins comprise a diverse family of bacterial type IV effectors [J]. Science, 2008, 320(5883): 1651-4.

[69] MUKHERJEE S, LIU X, ARASAKI K, et al. Modulation of Rab GTPase function by a protein phosphocholine transferase [J]. Nature, 2011, 477(7362): 103-6.

[70] HELLER K, OCHTROP P, ALBERS M F, et al. Covalent protein labeling by enzymatic phosphocholination [J]. Angew Chem Int Ed Engl, 2015, 54(35): 10327-30.

[71] GOODY P R, HELLER K, OESTERLIN L K, et al. Reversible phosphocholination of Rab proteins by *Legionella pneumophila* effector proteins [J]. EMBO J, 2012, 31(7): 1774-84.

[72] CHENG W, YIN K, LU D, et al. Structural insights into a unique *Legionella pneumophila* effector LidA recognizing both GDP and GTP bound Rab1 in their active state [J]. PLoS Pathog, 2012, 8(3): e1002528.

[73] WANG Z, MCCLOSKEY A, CHENG S, et al. Regulation of the small GTPase Rab1 function by a bacterial glucosyltransferase [J]. Cell Discov, 2018, 4(1): 53.

[74] BECK W H J, KIM D, DAS J, et al. Glucosylation by the *Legionella* effector seta promotes the nuclear localization of the transcription factor TFEB [J]. iScience, 2020, 23(7): 101300.

[75] LEVIN R S, HERTZ N T, BURLINGAME A L, et al. Innate immunity kinase TAK1 phosphorylates Rab1 on a hotspot for post-translational modifications by host and pathogen [J]. Proc Natl Acad Sci USA, 2016, 113(33): E4776-83.

[76] BELYI Y, JANK T, AKTORIES K. Effector glycosyltransferases in *Legionella* [J]. Front Microbiol, 2011, 2:76.

[77] KOTEWICZ K M, RAMABHADRAN V, SJOBLOM N, et al. A single *Legionella* effector catalyzes a multistep ubiquitination pathway to rearrange tubular endoplasmic reticulum for replication [J]. Cell Host Microb, 2017, 21(2): 169-81.

[78] DONG Y M Y, XIE Y, ZHANG Y. Structural basis of ubiquitin modification by the *Legionella* effector SdeA [J]. Nature, 2018,.

[79] GAN N, ZHEN X, LIU Y, et al. Regulation of phosphoribosyl ubiquitination by a calmodulin-dependent glutamylase [J]. Nature, 2019, 572(7769): 387-91.

[80] SULPIZIO A, MINELLI M E, WAN M, et al. Protein polyglutamylation catalyzed by the bacterial calmodulin-dependent pseudokinase SidJ [J]. eLife, 2019, 8: e51162.

[81] YI-HAN L, MARíA L, EVANS T R, et al. RavN is a member of a previously unrecognized group of *Legionella pneumophila* E3 ubiquitin ligases [J]. Plos Pathog, 2018, 14(2): e1006897.

[82] VALLEAU D, QUAILE A T, CUI H, et al. Discovery of ubiquitin deamidases in the pathogenic arsenal of *Legionella pneumophila* [J]. Cell Rep, 2018, 23(2): 568-83.

[83] KARIN M, LIN A. NF-kappaB at the crossroads of life and death [J]. Nat Immunol, 2002, 3(3): 221-7.

[84] HAYDEN M S, GHOSH S. Shared principles in NF-kappaB signaling [J]. Cell, 2008, 132(3): 344-62.

[85] GE J, XU H, LI T, et al. A *Legionella* type IV effector activates the NF-kappaB pathway by phosphorylating the IkappaB family of inhibitors [J]. Proc Natl Acad Sci USA, 2009, 106(33): 13725-30.

[86] GAN N, NAKAYASU E S, HOLLENBECK P J, et al. *Legionella pneumophila* inhibits immune signalling via MavC-mediated transglutaminase-induced ubiquitination of UBE2N [J]. Nature microbiol, 2019, 4(1): 134-43.

[87] LOSICK V P, HAENSSLER E, MOY M Y, et al. LnaB: a *Legionella pneumophila* activator of NF-kappaB [J]. Cell Microbiol, 2010, 12(8): 1083-97.

[88] HAENSSLER E, ISBERG R. Control of host cell phosphorylation by *Legionella pneumophila* [J]. Front Microbiol, 2011, 2: 64.

[89] AIMIN YANG S P, YAO-WEN WU. Elucidation of the anti-autophagy mechanism of the *Legionella* effector RavZ using semisynthetic LC3 proteins [J]. eLife, 2017, 6: e23905.

[90] BANGA S, GAO P, SHEN X, et al. *Legionella pneumophila* inhibits macrophage apoptosis by targeting pro-death members of the Bcl2 protein family [J]. Proc Natl Acad Sci USA, 2007, 104(12): 5121-6.

[91] SPEIR M, VOGRIN A, SEIDI A, et al. Strain 130b evades macrophage cell death independent of the effector SidF in the absence of flagellin [J]. Front Cell Infect Microbiol, 2017, 7: 35.

[92] ZHU W, HAMMAD L A, HSU F, et al. Induction of caspase 3 activation by multiple *Legionella pneumophila* Dot/Icm substrates [J]. Cell Microbiol, 2013, 15(11): 1783-95.

[93] CREASEY E A, ISBERG R R. The protein SdhA maintains the integrity of the *Legionella*-containing vacuole [J]. Proc Natl Acad Sci USA, 2012, 109(9): 3481-6.

[94] CLARE, HARDING, CHARLOTTE, et al. The Dot/Icm effector SdhA is necessary for virulence of *Legionella pneumophila* in *Galleria mellonella* and A/J mice [J]. Infect Immun, 2013, 81(7): 2598-605.

[95] KWAIK M M A Y A. How does *Legionella pneumophila* exit the host cell [J]? Trends Microbiol, 2002, 10(6): 258-60.

[96] MEI L, QIU X, JIANG C, et al. Host delipidation mediated by bacterial effectors [J]. Trends Microbiol, 2020, 29(3): 238-50.

[97] EDDENS T, KOLLS J K. Host defenses against bacterial lower respiratory tract infection [J]. Curr Opin Immunol, 2012, 24(4): 424-30.

[98] LOSICK V P, ISBERG R R. NF-kappaB translocation prevents host cell death after low-dose challenge by *Legionella pneumophila* [J]. J Exp Med, 2006, 203(9): 2177-89.

[99] PALUSINSKA-SZYSZ M, JANCZAREK M. Innate immunity to *Legionella* and toll-like receptors-review [J]. Folia microbiol, 2010, 55(5): 508-14.

[100] NAUJOKS J, LIPPMANN J, SUTTORP N, et al. Innate sensing and cell-autonomous resistance pathways in *Legionella pneumophila* infection [J]. Int J Med Microbiol, 2018, 308(1): 161-7.

[101] FONTANA M F, BANGA S, BARRY K C, et al. Secreted bacterial effectors that inhibit host protein synthesis are critical for induction of the innate immune response to virulent *Legionella pneumophila* [J]. PLoS Pathog, 2011, 7(2): e1001289.

[102] VANCE R E, ISBERG R R, PORTNOY D A. Patterns of pathogenesis: discrimination of pathogenic and nonpathogenic microbes by the innate immune system [J]. Cell Host Microb,

2009, 6(1): 10-21.

[103] CHISHOLM S T, COAKER G, DAY B, et al. Host-microbe interactions: shaping the evolution of the plant immune response [J]. Cell, 2006, 124(4): 803-14.

[104] PARK B, PARK G, KIM J, et al. Innate immunity against *Legionella pneumophila* during pulmonary infections in mice [J]. Arch Pharm Res, 2017, 40(2): 131-45.

[105] ARCHER K A, ALEXOPOULOU L, FLAVELL R A, et al. Multiple MyD88-dependent responses contribute to pulmonary clearance of *Legionella pneumophila* [J]. Cell Microbiol, 2009, 11(1): 21-36.

[106] MALLAMA C A, MCCOY-SIMANDLE K, CIANCIOTTO N P. The type II secretion system of *Legionella pneumophila* dampens the MyD88 and Toll-like receptor 2 signaling pathway in infected human macrophages [J]. Infect Immun, 2017, 85(4): e00897-916.

[107] BREIMAN R F, HORWITZ M A. Guinea pigs sublethally infected with aerosolized *Legionella pneumophila* develop humoral and cell-mediated immune responses and are protected against lethal aerosol challenge. A model for studying host defense against lung infections caused by intracellular pathogens [J]. J Exp Med, 1987, 165(3): 799-811.

[108] HORWITZ M A, SILVERSTEIN S C. Interaction of the Legionnaires' disease bacterium (*Legionella pneumophila*) with human phagocytes. II. Antibody promotes binding of *L. pneumophila* to monocytes but does not inhibit intracellular multiplication [J]. J Exp Med, 1981, 153(2): 398-406.

[109] ALIM A. Immunopathogenesis of Legionnaires' disease [J]. Mikrobiyol bul, 2004, 38(3): 295-303.

[110] BLANDER S J, HORWITZ M A. Vaccination with the major secretory protein of *Legionella pneumophila* induces cell-mediated and protective immunity in a guinea pig model of Legionnaires' disease [J]. J Exp Med, 1989, 169(3): 691-705.

[111] WEERATNA R, STAMLER D A, EDELSTEIN P H, et al. Human and guinea pig immune responses to *Legionella pneumophila* protein antigens OmpS and Hsp60 [J]. Infect Immun, 1994, 62(8): 3454-62.

[112] GUAN H, FU J, YU T, et al. Molecular basis of ubiquitination catalyzed by the bacterial transglutaminase MavC [J]. Adv Sci, 2020, 7(12): 2000871.

[113] GAN N, GUAN H, HUANG Y, et al. *Legionella pneumophila* regulates the activity of UBE2N by deamidase-mediated deubiquitination [J]. EMBO J, 2020, 39(4): e102806.