微生物学报
Acta Microbiologica Sinica
2023, 63(2): 567–581
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20220497

Review 综 述

鼠疫减毒活疫苗研究现状与展望

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GUO Xiao, TONG Zehui, DU Zongmin. Live-attenuated *Yersinia pestis* vaccines[J]. Acta Microbiologica Sinica, 2023, 63(2): 567-581.

摘　要：鼠疫(plague)是由鼠疫耶尔森氏菌(*Yersinia pesits*)引起的烈性传染病，在人类历史上曾造成约2亿人的死亡，在我国被列为甲类传染病。由于鼠疫菌具有高度致病性、传染性，被列为最具潜力的生物战剂和生物恐怖剂。在面临鼠疫威胁时，疫苗是最为有力的武器。鼠疫疫苗研究中，减毒活疫苗是重要的研究方向，现就鼠疫减毒活疫苗的研究现状进行综述，为新疫苗的研制提供参考。

关键词：鼠疫；鼠疫耶尔森氏菌；减毒活疫苗

Live-attenuated *Yersinia pestis* vaccines

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Abstract: Plague caused by *Yersinia pestis* is a fulminating infectious disease, resulting in nearly 200 million deaths in human history. It is listed as a category A infectious disease in China. As a highly infectious pathogen, *Y. pestis* is considered one of the most potential biological weapons and biothreat agents. Prophylactic vaccination is a powerful measure for counteracting this disease. In the development of plague vaccines, live-attenuated vaccine is an important research direction.

资助项目：病原微生物生物安全国家重点实验室开放课题(SKLPBS2135)
This work was supported by the Open Project of State Key Laboratory of Pathogen and Biosecurity (SKLPBS2135).
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Received: 2022-07-01; Accepted: 2022-09-24; Published online: 2022-10-10

This review introduces the recent progress, current challenges, as well as the future prospects on the development of live-attenuated plague vaccines.

Keywords: plague; *Yersinia pestis*; live-attenuated vaccine

鼠疫是由鼠疫耶尔森氏菌(*Yersinia pesits*, 以下称为鼠疫菌)引起的严重烈性传染病，在我国被列为甲类传染病。人类历史上出现过 3 次鼠疫大流行，造成约 2 亿人死亡[1]。随着人类居住环境和卫生条件的改善、抗生素的发明和使用，鼠疫的发病率逐渐控制在较低水平。但是作为一种人兽共患病，除大洋洲、南极洲以外，鼠疫自然疫源地广泛地存在分布于各个大洲。21 世纪以来鼠疫发病率有增加趋势，2000 年世界卫生组织(World Health Organization, WHO)将鼠疫定义为重新抬头的传染病。2017 年马达加斯加暴发人间鼠疫的流行，造成约 2 400 人感染[2]。我国鼠疫自然疫源地面积辽阔[3]，近年来人间鼠疫病例时有发生。2019 年内蒙古锡林郭勒盟 2 例肺鼠疫病例、内蒙古乌兰察布市 1 名腺鼠疫病例(http://www.chinacdc.cn/jkzt/crb/jl/sy/gzdt/)，以及多国相继发现的鼠疫多耐药株都警示我们鼠疫防控任务依然十分艰巨[4-6]。

鼠疫菌在战争中曾经作为生物战剂投入战场使用[7]，迄今为止，仍然是最有威胁的生物战剂之一[8]。由于鼠疫菌易于从疫源地动物身上直接获得，也是理想的生物恐怖剂。鼠疫的宿主主要为啮齿类动物，如旱獭、家鼠、黄鼠等，以蚤类作为传播媒介进行传播。被染疫跳蚤叮咬后，宿主动物通常会发生腺鼠疫感染，被感染的动物通常会成为新的传染源，引起动物间、动物与人间、或人间的传播。腺鼠疫如未经治疗可以发展为继发性肺鼠疫或败血症鼠疫，人类可以通过飞沫途径直接罹患原发性肺鼠疫，如果未经治疗，通常在 3–5 d 内死亡，死亡率极高[2]。鼠疫作为一种再发传染病和潜在的生物武器、生物恐怖剂，是我国社会稳定、人民健康的巨大威胁。研制更为安全有效的鼠疫疫苗无疑是应对这一威胁强有力的武器。本文就鼠疫疫苗，主要是减毒活疫苗在安全性、保护效果及诱导适应性免疫方面的研究进展进行综述。

1 鼠疫菌生物学性状

自 1894 年法国生物学家亚历山大·耶尔森发现鼠疫菌以来，各国学者对鼠疫菌生化特征、毒力因子、致病机制及免疫学特性等进行了广泛的研究，这为鼠疫疫苗的研究奠定了基础。鼠疫菌是革兰阴性杆菌，无鞭毛，不形成芽孢，大约是 2 800–5 000 年前由假结核耶尔森氏菌进化而来[9]，进化过程中继承了一个质粒 pCD1 (或称 pLcr、pYV)，通过水平转移获得了 2 个外源性质粒 pMT1 (或称 pFra、pYT)和 pPCP1 (或称 pPst、pYP)，这些质粒在鼠疫菌的致病和传播过程中发挥重要作用[1-2]。pCD1 质粒约 68–75 kb，通常被称为“毒力质粒”，编码三型分泌系统(type three secretion system, T3SS)。其中 LcrV (low calcium response V)抗原是 T3SS 的重要组成成分，也是鼠疫菌的重要保护性抗原之一[10]。pMT1 质粒约 100–110 kb，它编码鼠毒素(murine toxin, Ymt)和 F1 抗原(fraction 1)，F1 抗原在抗细胞吞噬中发挥一定作用，也是鼠疫菌重要的保护性抗原之一[10]。pPCP1 质粒约 9.5 kb，主要编码纤溶酶原激活剂(plasminogen activator, Pla)。Pla 有纤溶活性，有助于细菌穿透基底膜通过组织屏障，进而增强细菌在体内传播的能力，同时其也在促进细菌细胞内存活和补体抵抗中发挥一定

作用[11]。此外部分鼠疫菌还有一个隐藏质粒pCRY (或称 pTP33)，其功能有待阐明。

2 鼠疫疫苗的分类和特点

历史上人们开发出多种疫苗应对鼠疫，部分投入过大规模临床使用。其中 Haffikine 全菌灭活疫苗、USP 全菌灭活疫苗、TJW 减毒活疫苗、MP-40 减毒活疫苗等因为不能对肺鼠疫形成有效保护，免疫持续时间短或有较强的副作用等原因而被淘汰[12]。目前仅有 EV76 减毒活疫苗及其衍生株仍然被允许在中国、蒙古国、俄罗斯及中亚地区高危人群中使用。该菌株是 *pgm* 位点缺失的减毒株，*pgm* 位点是位于鼠疫菌染色体上约为 102 kb 的序列，主要编码铁载体蛋白和高毒力岛，*pgm* 位点的缺失导致鼠疫菌经皮下或滴鼻途径攻毒对哺乳动物毒力大幅下降[13-14]。但有动物实验表明该疫苗可能导致致命性败血症[15-16]。2009 年有基于 *pgm*$^-$的鼠疫菌减毒株感染并导致实验人员死亡的个例报道，可能与该人员罹患高铁血症相关[17]。由于 EV76 减毒活疫苗可引起接种部位和/或全身不良反应[18]，所以学者们致力于开发更为安全有效的鼠疫疫苗。

在鼠疫疫苗的研究中除了减毒活疫苗外，还有亚单位疫苗、活载体疫苗、核酸疫苗、OMV 疫苗(outer membrane vesicle)等不同类别，下面逐一简述。

2.1 鼠疫亚单位疫苗

鼠疫亚单位疫苗主要基于 F1 和 LcrV 抗原辅以不同类型的佐剂进行设计和改良，在实验动物中对肺鼠疫和腺鼠疫都展现出保护作用。经过近 30 年的发展，目前国内外已经有多款亚单位疫苗进入临床试验，包括英国 PharmAthene 公司的以 rF1+rV 为组分、氢氧化铝为佐剂的亚单位疫苗[19]；美国陆军传染病医学研究所和 Dynport 疫苗公司联合研制的由 rF1-V 融合蛋白和氢氧化铝组成的亚单位疫苗[19]；美国 Frey 等研制的沙门菌鞭毛蛋白、F1、LcrV 构成的融合蛋白为组分的亚单位疫苗[20]；国内兰州生物制品研究所研制的由天然 F1 和 rV 抗原、氢氧化铝组成的亚单位疫苗等[21]。亚单位疫苗在安全性上有优势，但其制备过程复杂，部分重组抗原与天然抗原差别大，诱导细胞免疫应答水平低；自然界中存在 F1$^-$鼠疫菌，这类菌株对人依然具有致病性[22]，以及 LcrV 的多态性[23]等原因造成以 F1 和 LcrV 抗原为主要成分的亚单位疫苗不能覆盖所有鼠疫菌株，有一定局限。

2.2 活载体疫苗

活载体疫苗，基于对人体无毒或低毒的细菌、病毒作为载体，构建表达鼠疫菌 F1、LcrV、YscF、YopE 等抗原的菌株或病毒株，该类型疫苗能够同时诱导体液和细胞免疫，可以通过口服或吸入途径诱导黏膜免疫。活载体类型包括减毒假结核耶尔森氏菌[24-27]、减毒沙门氏菌[28]、复制缺陷腺病毒[29-30]等。此类疫苗表达有限的鼠疫菌抗原，在应对鼠疫致病菌多态性时保护作用仍有待商榷。

2.3 核酸疫苗

目前关于鼠疫的核酸疫苗报道较为有限，主要集中在 DNA 疫苗方面。与亚单位疫苗、活载体疫苗相似，核酸疫苗主要基于鼠疫菌有限的抗原设计，将保护性抗原编码基因 *caf1*、*lcrV*、*pla* 或 *yscF* 等直接导入宿主细胞[31-33]，借助细胞中表达系统合成抗原蛋白，诱导宿主产生特异性免疫应答。动物实验中，DNA 疫苗可以发挥保护作用。

2.4 OMV (outer membrane vesicle)疫苗

革兰氏阴性菌在生长的过程中能够形成 OMV[10]，OMV 不能复制，但具有类似细菌的免疫原性。目前鼠疫 OMV 疫苗包括减毒鼠疫菌 OMV[34]、表达 F1 抗原的假结核耶尔森氏菌

工程菌 OMV[35]、表达鼠疫菌 F1、LcrV 抗原的多形拟杆菌工程菌 OMV[36]几种，在动物实验中均展现出对腺鼠疫及肺鼠疫的保护性。

3 鼠疫减毒活疫苗研究进展

鼠疫疫苗研究中鼠疫减毒活疫苗由于包含抗原种类全面，可以同时诱导体液免疫和细胞免疫，是重要的研究方向，近年来鼠疫减毒活疫苗的研制得到了进一步的发展。现代鼠疫减毒活疫苗主要通过基因工程获得减毒株，其亲本株可以是强毒株或 *pgm* 位点缺失的减毒株。近 10 年鼠疫减毒活疫苗研究情况见表 1。基因改造的靶标

表 1 候选鼠疫减毒活疫苗及其保护效果

Table 1 Candidate live-attenuated vaccines and their protective efficacies against plague

Vaccine candidates	Animal strains	Challenge route	LD_{50} (CFU)	Immunization	Protective efficacy
Naturally attenuated strain					
Y. pestis Microtus 201[37]	Human adults	Skin Scracth	>1.5×10^7[38]	Chinese-origin rhesus macaques were immunized with 1.4×10^{10} CFU of *Y. pestis Microtus* 201 by s.c. route	83.3% protection against s.c. challenge with 1.79×10^9 CFU of *Y. pestis* 141 strain[37]
Y. pestis I-2948/3 (pPCP1-, pCD1-, pCRY-), *Y. pestis* I-3749(pCD1-), *Y. pestis И*-3480 (pPCP1-, pCD1-)[39]	White mice		nd	Mice received subcutaneous injection of mutant strains in a dose of 10^6 CFU	Activate cellular immunity
Attenuated strains defective in T3SS components					
CO92Δ*yscN*[40-41]	Female Swiss Webster	s.c.	>3.2×10^7	BALB/c mice immunized by s.c. route with two dose (1×10^7 CFU/dose) at 0 d and 21 d	Complete protection against s.c. infection with 316×LD_{50} *Y. pestis* CO92 and complete protection against i.n. infection with 7×LD_{50} *Y. pestis* CO92
C12Δ*yscN*(C12:F1-negative capsule minus strain)[41]	Female BALB/c	s.c.	>2×10^7	BALB/c mice were immunized with two dose (1×10^7 CFU/dose) at 0 d and 21 d by s.c. route	40% protection against s.c. infection with 316×LD_{50} *Y. pestis* CO92 and no protection against i.n. infection with 7×LD_{50} *Y. pestis* CO92
Y. pestis Microtus 201Δ*yscB*[42]	BALB/c	s.c.	>10^6	s.c. immunization with 1.64×10^4 CFU of mutant strain	87.5% protection against s.c. challenge with 1.24×10^6 CFU of virulent *Y. pestis* 141 strain and complete protection against i.n. challenge with 1.24×10^6 CFU of virulent *Y. pestis* 141 strain
LPS synthesis mutants (some of them with transcriptional regulator or invasion factor deletion)					
χ10030(pCD1Ap)(KIM6+Δ*lpxP32*Ω P_{lpxL} *lpxL*ΔP_{crp21}::TT*araC* P_{BAD}*crp*)[43]	Female Swiss Webster	s.c.	>10^8	Female Swiss Webster mice immunized with 1.4×10^7 CFU of mutant strain by s.c. route	Provide complete protection against s.c. challenge with 3.57×10^7 CFU of virulent *Y. pestis* KIM6+ (pCD1Ap) strain at 35 d post immunization and 70% protection against i.n. challenge with 1.24×10^4 CFU[43]. But no protection in BALB/c mice[41]
YPS19(pCD1Ap) (Δ*lpxP32*::P_{lpxL}*lpxL pgm*-pPCP-)[44]	Swiss Webster	s.c.	1×10^8 90% survival of mice	Swiss Webster mice immunized i.m. with 2×10^7 CFU of the mutant strain on day 0 and day 21	70% protection against i.n. challenge with 50×LD_{50} KIM6+ (pCD1Ap)
	Hfe$^{-/-}$ C57BL/6	i.m.	>1×10^7		
YPS20(pCD1Ap) (Δ*lpxP32*::P_{lpxL}*lpx*LΔlacI23::P_{lpp}*lpxE pgm*$^-$ pPCP$^-$)[44]	Swiss Webster	s.c.	>2×10^7	Swiss Webster mice immunized i.m. with 5×10^7 CFU of the mutant strain	20% protection against i.n. or s.c. challenge with 50×LD_{50} KIM6+ (pCD1Ap)

(待续)

(续表 1)

Vaccine candidates	Animal strains	Challenge route	LD_{50} (CFU)	Immunization	Protective efficacy
Invasion/adhesion/outer membrane protein mutants					
Y. pestis CO92pPst^{-}*pgm*$^{-}$[41]	Female BALB/c	s.c.	>1×10^{8}	BALB/c mice or CD-1 mice were immunized with 10^{7} CFU of mutant strain by s.c. route	Complete protection for BALB/c mice against subcutaneous challenge with 235×LD_{50} *Y. pestis* CO92 and complete protection aerosol challenge with 8×LD_{50} *Y. pestis* CO92; Complete protection for CD-1 mice against subcutaneous challenge with 416×LD_{50} *Y. pestis* CO92 and 60% protection against aerosol challenge with 26×LD_{50} *Y. pestis* CO92
Δ*nlpD Y. pestis* 231 Δ*nlpD Y. microtus* I-3455 and Δ*nlpD Y. microtus* I-2359[45]	BALB/c Guinea pigs	i.n. s.c.	>10^{7} >10^{10}	s.c. immunization with different dosages of each mutant strain	Immunization with the Δ*nlpD* mutants provided potent immunity against plague in mice but failed to do so in guinea pigs
Y. pestis CO92Δ*lpp*Δ*msbB* Δ*ail*[46-47]	Female Swiss Webster	i.n.	>3.4×10^{6}	Female Swiss Webster mice or female Brown Norway rats were immunized by the i.m. route with two doses (2×10^{6} CFU) of mutant strain at 0 d and 21 d	On day 120, 80% protection for Swiss Webster mice against intranasal challenge with 24×LD_{50} *Y. pestis* CO92 *luc2*. On day 91 100% protection for Brown Norway rats against intranasal challenge with 31×LD_{50} *Y. pestis* CO92 *luc2*
Y. pestis CO92Δ*lpp*Δ*msbB:ail2*[46-47]	Female Swiss Webster		nd	Female Swiss Webster mice were immunized by the i.m. route with two doses (2×10^{6} CFU) of mutant strain at 0 d and 21 d	On day 120, 80% protection against intranasal challenge with 24×LD_{50} *Y. pestis* CO92 *luc2*
Y. pestis CO92Δ*lpp*Δ*msbB* Δ*pla*[47]	Female Swiss Webster	i.n.	>5×10^{6}	Swiss Webster mice or Brown Norway rats were immunized by the i.m. route with two doses (2×10^{6} CFU) of mutant strain at 0 d and 21 d	On day 120, 100% protection for Swiss Webster mice against intranasal challenge with 24×LD_{50} *Y. pestis* CO92 *luc2*. On day 91 100% protection for Brown Norway rats against intranasal challenge with 31×LD_{50} *Y. pestis* CO92 *luc2*
EV76-B-SHUΔ*pla*[48]	Female BALB/c	s.c.	1×10^{8} 50% survival of mice	Mice were immunized by s.c. route with three doses (1×10^{6} CFU) of mutant strain at 0 d, 21 d and 42 d	Complete protection against i.t. challenge with 61×LD_{50} *Y. pestis* 201 and complete protection against s.c. challenge with *Y. pestis* 201
		i.t.	> 10^{6}	Mice were immunized by i.t. route with three doses (1×10^{6} CFU) of mutant strain at 0 d, 21 d and 42 d	
Others					
Y. pestis KIM1001Δ*brnQ*[49]	BALB/c C57BL/6	s.c.	10^{7}	Survival mice after s.c. infection with 1 000 CFU of *Y. Pestis* KIM1001 Δ*brnQ*	93.75% protection against s.c. challenge with 1 000 CFU (20LD_{50}) of *Y. pestis* KIM1001
Y. pestis CO92Δ*lpp*Δ*cyoABCDE*[50]	Female Swiss Webster	i.n.	5.5×10^{3} CFU 90% survival of mice	Survival mice re-challenge	On day 35, 50% protection against intranasal challenge with 10×LD_{50} *Y. pestis* CO92
CO92Δ*vasK*Δ*hcp6*[50]	Female Swiss Webster	i.n.	4.5×10^{3} CFU 60% survival of mice	Survival mice re-challenge	On day 21, 80% protection against intranasal challenge with 8×LD_{50} *Y. pestis* CO92
CO92Δ*ypo2720-2733*Δ*hcp3*[50]	Female Swiss Webster	i.n.	4.5×10^{3} CFU 60% survival of mice	Survival mice re-challenge	On day 21, 100% protection against intranasal challenge with 8×LD_{50} *Y. pestis* CO92

CFU, colony forming units; LD_{50}, 50% lethal dose; s.c., subcutaneous; i.n., intranasal; i.m., intramuscular; i.t., aerosolized intratracheal inoculation; nd, not detected.

主要聚焦在 T3SS 相关毒力因子基因、脂多糖(lipopolysaccharide, LPS)修饰基因、侵袭黏附相关膜蛋白基因、应激反应功能相关基因等。这些基因位于鼠疫菌染色体或不同质粒上，基因的联合敲除进一步降低了减毒株毒力恢复的可能。目前暂未报道有鼠疫减毒活疫苗进入临床试验。

3.1 自然减毒候选疫苗株

田鼠型鼠疫菌对大型哺乳动物无毒[38,51]，可以作为候选疫苗株。国内 Zhang 等在中国猕猴上评价了田鼠型鼠疫菌 201 株的免疫效果，该菌株通过皮下途径高度减毒，可诱导与 EV76 相似的 F1 特异性抗体滴度，诱导 Th1 型细胞因子(IFN-γ、IL-2 和 TNF-α)和 Th2 型细胞因子(IL-4、IL-5 和 IL-6)以及趋化因子 MCP-1 和 IL-8 的分泌。免疫接种 201 株后，使用鼠疫菌 141 强毒株皮下攻毒，在中国猕猴中发挥了与 EV76 相似的保护效果[37]。但在随后的毒力比较中发现鼠疫菌 201 株在恒河猴中残存毒力较 EV76 更高[52]。

Balakhonov 等对含有不同质粒的鼠疫菌进行了毒力和病理学评价，发现 *Y. pestis* subspecies altaica I-2948/3 (pPCP1-、pCD1-、pCRY-)、I-3749 (pCD1-)、И-3480 (pPCP1-、pCD1-)株在小鼠中无毒，且能够刺激机体细胞免疫[39]。

3.2 T3SS 相关基因突变候选减毒疫苗株

pCD1 质粒编码的 T3SS 对鼠疫菌的毒力至关重要，是由几十个蛋白组成的横跨细菌内外膜的类注射器样结构。鼠疫菌可以通过 T3SS 向宿主细胞中转运效应蛋白，破坏细胞骨架、抑制核转录因子(nuclear factor kappa-B, NF-κB)和有丝分裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)等信号通路，进而抑制宿主免疫响应激活、促进鼠疫菌在巨噬细胞内的生存和增殖[18,53]。针对 T3SS 的关键结构和调控蛋白进行突变失活可以显著降低鼠疫菌的毒力。

国内 Zhang 等敲除了田鼠型鼠疫菌 201 株中的 *yscB* 基因，构建了 201Δ*yscB* 株。*yscB* 基因编码鼠疫菌 YscB (Yop secretion protein B)蛋白，是 YopN 的伴侣分子，对 YopN 负调控 T3SS 分泌使之在接触宿主细胞或低钙环境下触发效应蛋白分泌的功能是必须的。201Δ*yscB* 株高度减毒，对 BALB/c 小鼠皮下攻毒的 LD_{50} 上升约 4×10^5 倍。经单剂皮下免疫 BALB/c 后 6 周，2.2×10^5 倍 LD_{50} 鼠疫菌 141 强毒株皮下攻毒，保护率为 87.5%；283 倍 LD_{50} 鼠疫菌 141 强毒株滴鼻攻毒，保护率为 100%，与相同剂量 EV76 疫苗株免疫组无显著差异，而且可以刺激小鼠产生比 EV76 免疫组更高滴度的抗 F1 抗体[42]。

YscN (Yop secretion protein N)编码 ATP 转移酶，负责为 T3SS 转运效应蛋白提供能量[40]。美国陆军传染病医学研究所对 CO92Δ*yscN*、C12Δ*yscN*、CO92*pgm*$^-$pPCP1$^-$三株菌在 BALB/c 小鼠安全性进行了评价，其中 C12Δ*yscN* 为敲除 *yscN* 基因且不表达 F1 的 CO92 株。3 菌株在 1×10^7 CFU 皮下免疫后，小鼠均存活。C12Δ*yscN* 单次皮下免疫的 BALB/c 小鼠经 329 倍 LD_{50} 鼠疫菌 CO92 株皮下攻毒生存率只有 30%，经 22 倍 LD_{50} 气溶胶攻毒时无存活。C12Δ*yscN* 菌株不表达 F1 抗原，同时 LcrV 抗原表达下调，对强毒株攻毒只能提供有限的保护作用。CO92Δ*yscN* 株、CO92*pgm*$^-$pPCP1$^-$株单次皮下免疫 BALB/c 和 CD-1 小鼠，经 200 倍以上 LD_{50} 的 CO92 株皮下攻毒 100%存活。CO92Δ*yscN* 株、CO92*pgm*$^-$pPCP1$^-$株单次皮下免疫后的 BALB/c 小鼠在 8 倍 LD_{50} 鼠疫菌 CO92 株气溶胶攻毒时能够 100%存活，但是在 26 倍 LD_{50} 鼠疫菌 CO92 株气溶胶攻毒时

CO92Δ*yscN* 株皮下免疫 CD-1 小鼠只有 20%存活，CO92*pgm*$^{-}$pPCP1^{-}株皮下免疫 CD-1 小鼠有 60%存活。表明不同动物品系中疫苗保护率可能存在显著差异，需要引起重视。随后使用 CO92Δ*yscN* 株对 BALB/c 小鼠进行 2 剂免疫，或使用 CO92Δ*yscN* 株和 C12Δ*yscN* 株先后免疫的免疫方案时，对 7 倍 LD_{50} 气雾剂攻毒保护率约为 90%–100%[41]，与单剂免疫没有显著差异。

3.3 基于脂多糖结构改造的候选减毒活疫苗株

LPS 是革兰氏阴性菌中重要的细胞壁结构，由 O 抗原、核心多糖、脂质 A 构成。脂质 A 的酰基化程度不同其生物学活性有显著差异。LpxL、LpxM 和 LpxP 是革兰氏阴性细菌 LPS 生物合成过程后期的酰基转移酶，是向四酰化前体脂质 IV_A 中添加次级酰基链所必需的[54]。鼠疫菌相较于其他革兰阴性菌在合成 LPS 的过程中有其自身的特点，在不同温度下合成不同乙酰化修饰类型的脂质 A。鼠疫菌具有活性的 *lpxP* 和 *lpxM* (又称 *msbB*)基因，但 *lpxL* 基因发生突变失活，26 ℃下可形成六酰化脂质 A。在 37 ℃时，*lpxP* 基因表达受抑制，主要合成四酰化脂质 A，其激活 Toll 样受体 4 (Toll like receptor 4，TLR4)的活性较差，从而有助于逃逸固有免疫系统识别[55-56]。多种鼠疫减毒活疫苗基于这个特点进行设计。

Feodorova 等把鼠疫 EV NIIEG 株中 *lpxM* 基因敲除，使其在 26 ℃下合成毒性较小的五酰化 LPS，而在相同的培养条件下，亲本菌株产生毒性更大的六酰化脂质 A[57]。研究者进一步对 EVΔ*lpxM* 株在远交小鼠、BALB/c 小鼠和豚鼠中进行了安全性评估。2×10^2–2.5×10^4 CFU 不同剂量 EVΔ*lpxM* 株皮下途径感染 BALB/c 小鼠后，小鼠均存活，而不同剂量组亲本株 EV NIIEG 皮下感染后均有小鼠死亡；在远交小鼠和豚鼠的实验中 EVΔ*lpxM* 株和亲本株残存毒力无明显差异。使用 EVΔ*lpxM* 株单剂皮下免疫后，在 BALB/c 小鼠、远交小鼠、豚鼠中对鼠疫菌 231 强毒株皮下攻毒中均较亲本株保护率高[57-58]。

Wang 等在鼠疫菌 KIM10 (pCD1Ap)株(*pgm*$^{-}$、pPCP1^{-}、导入外源性 pCD1 质粒)中针对酰基转移酶进行了改造，构建了 YPS19 (pCD1Ap)(Δ*lpxP32*::P_{lpxL}*lpxL*)使其在 37 ℃时主要合成六酰基脂质 A，同时构建了 YPS20 (pCD1Ap)(Δ*lpxP32*::P_{lpxL}*lpxL*Δ*lacI*23::P_{lpp}*lpxE*)使其在 37 ℃主要合成 1-去磷酸化脂质 A (低毒性脂质 A)。用这 2 株菌大剂量(>1×10^7 CFU)皮下和肌肉攻毒后，Swiss Webster 小鼠存活率较亲本株均显著提高。YPS19 (pCD1Ap)株在高铁血症模型小鼠中 1×10^7 CFU 皮下攻毒后生存率为 100%，而同样条件下 KIM10 (pCD1Ap)生存率为 80%。虽然 YPS20 (pCD1Ap)株经皮下或肌肉免疫后刺激机体产生了特异性抗体，但却不能有效保护小鼠抵抗滴鼻攻毒。YPS19 (pCD1Ap)免疫对 50 倍 LD_{50} 的 KIM6+ (pCD1Ap)鼠疫菌皮下和滴鼻攻毒能够起到与其亲本株相近的保护作用。该研究还评估了经皮下和肌肉免疫的差异，认为在肌肉免疫后能够可诱导平衡的 Th1 和 Th2 反应，而皮下免疫刺激偏向 Th2 反应[44]。

3.4 侵袭黏附等相关膜蛋白突变候选减毒活疫苗株

许多鼠疫外膜蛋白是重要的毒力因子，在致病过程中发挥不同的作用。Ail 黏附蛋白编码基因位于染色体上，具有细胞外环 2 (L2)结构，它与鼠疫菌补体抵抗、细菌黏附、抑制炎性反应等相关[59]；Braun 脂蛋白(Braun lipoprotein, Lpp)在肠杆菌科中广泛存在，其编码基因位于染色体上，该蛋白可以通过 TLR-2 促进炎症细胞因子

的产生[60-61]。Pla (plasminogen activator)纤溶酶原激活剂是由 pPCP1 质粒编码的外膜蛋白，有助于细菌穿透基底膜通过组织屏障进而增强细菌在体内扩散，同时也在促进细菌细胞内存活和补体抗性发挥一定作用[11]；NlpD (novel lipoprotein D)编码一种新型脂蛋白，其缺失株在皮下和滴鼻攻毒中毒力显著下降[62]。

Tiner 等在鼠疫菌 CO92 强毒株基础上构建了 Δ*lpp*Δ*msbB*Δ*ail* (LMA)、Δ*lpp*Δ*msbB*::*ailL2*、Δ*lpp*Δ*msbB*Δ*pla* (LMP) 3 株减毒株。3 株多重敲除株 LD_{50} 较其亲本株毒力下降约 5×10^5 倍，经过 2 剂肌肉免疫小鼠和大鼠，都激发了长期体液免疫和细胞免疫。初免后 120 d 使用 24 倍 LD_{50} 鼠疫菌 CO92 *luc*2 株(CO92 株携带萤光素酶基因)滴鼻攻毒，LMP 株能够 100%保护小鼠，LMA 和 Δ*lpp*Δ*msbB*::*ailL2* 保护率为 80%[47,63]。

国内 Feng 等在鼠疫 EV76-B-SHU 疫苗株基础上，敲除了 *pla* 基因，构建了 EV76-B-SHUΔ*pla* 株。10^8 CFU EV76-B-SHUΔ*pla* 株在皮下攻毒途径中小鼠生存率为 50%，而 EV76-B-SHU 疫苗株生存率为 0；10^6 CFU EV76-B-SHUΔ*pla* 株肺递送攻毒途径中小鼠生存率为 100%，其亲本株生存率为 50%。小鼠经过肺递送或皮下进行 3 次免疫后保护率相同，使用 61 倍 LD_{50} 鼠疫菌 201 株进行肺递送途径攻毒，小鼠 100%存活[48]。

鼠疫菌脂蛋白 NlpD 是腺鼠疫和肺鼠疫疾病发展中的重要毒力因子[62]。Dentovskaya 等在鼠疫菌 231 株、I-3455 株、I-2359 株中敲除了 *nlpD* 基因，敲除株均对小鼠和豚鼠无毒。用 3 株不同的 *nlpD* 突变株免疫动物后用鼠疫菌 231 强毒株皮下攻毒，231Δ*nlpD* 株、I-3455Δ*nlpD* 株较 EV-NIIEG 株展现了更好的保护性，而在豚鼠中，231Δ*nlpD* 株、I-3455Δ*nlpD* 株、I-2359Δ*nlpD* 株较 EV-NIIEG 株保护性差[45]，这种在不同动物模型中疫苗的保护效果差异不容忽视。

3.5 调控因子突变株候选疫苗株

细菌中存在一种由(p)ppGpp 分子介导的高度保守的严谨响应调节系统，可以对营养匮乏等外界环境做出响应，调节细菌中多种基因包括毒力因子的表达。鼠疫菌中 *relA*、*spoT* 基因是 ppGpp 合成的主要基因。Sun 等在 KIM5+基础上构建了 χ10004 (pCD1) (敲除了 *relA*、*spoT* 基因，导入 pCD1 质粒)，其在皮下攻毒途径中毒力下降约 10^5 倍。Swiss Webster 小鼠单剂皮下免疫后，能够 100%抵御 1×10^5 CFU 鼠疫 KIM5+株皮下攻毒，对 2×10^4 CFU KIM5+株滴鼻攻毒有 60%保护率[64]。

CRP (cAMP receptor protein)是全局调控因子，控制大量基因的表达，包括毒力因子 *pla* 和鼠疫菌素 *pst* 基因，同时 CRP 也是 T3SS 表达调控所必需的[18]。Sun 等在鼠疫菌 KIM6+株上构建了敲除 *crp* 的 χ10010 (Δ*crp18*)株、χ10017 (pCD1Ap) (ΔP_{crp21}::TT *araC* P_{BAD} *crp*)株，其中 χ10017 株的 *crp* 表达受浓度依赖性阿拉伯糖诱导，而宿主组织中不含阿拉伯糖，导致细菌在侵入宿主后毒力显著下降。比较 χ10017 株与 χ10010 株的毒力和免疫原性，发现这 2 种菌株皮下注射感染均高度减毒，χ10010 的 LD_{50} 高于 χ10017 株。然而 χ10010 只产生了强烈的 Th2 偏向免疫反应，单次皮下免疫后，对 10^6 倍 LD_{50} 鼠疫菌 KIM5+强毒株皮下攻毒提供了 80%的保护，而对 100 倍 LD_{50} 鼠疫菌 KIM5+强毒株滴鼻攻毒没有保护作用。相比之下，χ10017 株产生了更平衡的 Th1/Th2 反应，单次皮下免疫后，能够抵御 10^4 倍 LD_{50} KIM5+株皮下攻毒，对 100 倍 LD_{50}KIM5+株滴鼻攻毒保护率为 70%[65]。

3.6 其他减毒候选疫苗

六型分泌系统(type six secretion system, T6SS)是一种多功能的分泌系统，参与多种生理

过程，包括细菌竞争、宿主感染和应激反应等。鼠疫菌中存在 6 个 T6SS 基因簇[66]，其中部分基因可能为潜在的毒力因子。Andersson 等在鉴定鼠疫菌新型毒力因子的过程中，发现了几个潜在的毒力因子，包括 *vasK* (T6SS 的重要组成成分)、*cyoABCDE* (编码细胞色素 O 氧化酶操纵子)、*hcp* [T6SS 的效应蛋白溶血素共调节蛋白(hemolysin-coregulated protein, Hcp)]、*ypo*2715-2733 (编码毒素 60，有 RNA 酶毒性)。该研究团队基于鼠疫菌 CO92 株构建了多个多重敲除株，其中 Δ*lpp*Δ*cyoABCDE* 、 Δ*vasK*Δ*hcp6* 、 Δ*ypo2720-2733*Δ*hcp3* 三株经滴鼻免疫后，在 21 d 使用 10 倍 LD_{50} 鼠疫菌 CO92 株滴鼻攻毒，小鼠存活率在 55%–100%[50]。

3.7 鼠疫菌新发现的毒力相关基因

近几年，随着对鼠疫菌致病机制的认识不断深入，陆续有新的毒力因子或相关调控基因等被发现或证实，相关基因如表 2 所述。

表 2 鼠疫菌中新发现的毒力相关基因

Table 2 Newly discovered virulence-related genes in *Yersinia pestis* in recent 10 years

Genes	Functions	Reference
mgtA	Mg^{2+} transporter, part of the complex PhoPQ regulon	[67]
yp_3416/yp_3418	E3 ubiquitin ligase that can be translocated by T3SS of *Y. pestis*	[68-69]
surA	Encodes a protein related to outer membrane proteins biogenesis	[70]
YPO3903	Involved in adherence to lung epithelial cells	[71]
pagP	encodes a lipid A modifying enzyme, the inactivation of this gene in *Y. pestis* associate with the escape from innate immune recognition	[72]
bipA	encodes a bacterial GTPase that has been shown to regulate expression of virulence factors	[73]
sORF_yp1	encodes SEP_yp1 that is necessary for the virulence of *Y. pestis*	[74]
sORF_yp2	encodes SEP_yp2 that is necessary for the virulence of *Y. pestis*	[74]

其中 *mgtB*、*surA*、*sORF_yp1*、*sORF_yp2* 基因敲除株相较于其亲本株在皮下攻毒中毒力下降显著；*bipA*、*yp_3416/yp_3418* 基因敲除株相较于其亲本株滴鼻攻毒中毒力都有不同程度的降低。这些新的毒力因子或相关调控基因是潜在的鼠疫菌减毒活疫苗构建的靶点。

本研究组在 EV76 疫苗株的基础上敲除了 *sORF_yp1* 或 *sORF_yp2* 基因，发现敲除株的毒力较 EV76 疫苗株有进一步下降，而保护作用与 EV76 株相当(相关结果暂未发表)，这一结果提示 *sORF_yp1* 和 *sORF_yp2* 为靶点基因在鼠疫菌减毒活疫苗研究中具有一定价值。

4 展望

虽然基于不同技术路径发展了多种鼠疫候选疫苗，但是距离成熟的鼠疫疫苗问世还有很长的路要走。2018 年，WHO 鼠疫疫苗研讨会提出鼠疫疫苗的应用包括预防性应用和应急性应用 2 种情景。预防性应用旨在保护流行地区的人群或从事鼠疫研究或检测的卫生工作者，而应急性应用旨在保护身处疫区的人群并阻断传播链[75]。不同类型疫苗由于其设计特点适用于不同使用情景，其中鼠疫减毒活疫苗相较于其他类型疫苗生产成本低，能同时诱导体液免疫和细胞免疫，在单剂免疫后 10 d 左右即提供保护[75]，其保护作用可以持续 10–12 个月[18]，在应急性应用中可以发挥重要作用。

本研究组在对 EV76 株进行研究时，注意到除 F1 抗原外，还有大量其他抗原参与诱导适应性免疫。有理由相信减毒活疫苗能够更好地

应对鼠疫菌的多态性问题。由于F1$^-$鼠疫菌在自然界中广泛存在[76]，且对人依然具有致病性[22]，需要关注鼠疫减毒活疫苗对 F1$^-$菌株的保护作用。我们实验中发现经 EV76 株皮下免疫后小鼠可以完全抵御 F1$^-$菌株皮下攻毒，但对 F1$^-$菌株滴鼻攻毒的保护作用则极为有限(相关结果未发表)。其可能原因包括免疫途径、菌株自身特点等，需要进一步地探讨。

鼠疫减毒活疫苗也有其自身需要解决的问题。首先，鼠疫减毒活疫苗有残存毒力和毒力恢复的可能性。通常引入多重突变可以更好地减毒同时降低毒力恢复的可能性，但也导致了疫苗株免疫原性的下降，这可能由于细菌无法在宿主器官中生存足够长的时间以刺激保护性免疫反应。安全性与保护率的平衡是重要的问题，其中安全性评价需要摆在首位。不同动物模型、不同攻毒方式，鼠疫菌减毒株毒力表现可以相差很大。在评价鼠疫菌减毒株安全性时，全面且合理的指标选择是必要的，俄罗斯学者Feodorova等设计了鼠疫减毒活疫苗评价体系[77]可以作为借鉴，但其动物实验量大，需要进一步地优化。其次，特异性LcrV抗体是鼠疫菌重要的保护性抗体，但是在包括 EV76、CO92*pgm*$^-$pPst$^-$免疫后的动物(人)血清中几乎检测不到特异性LcrV抗体[41,78-79]，本研究组在基于鼠疫菌201株构建的减毒活疫苗对小鼠进行免疫后也几乎检测不到特异性LcrV抗体(相关结果暂未发表)。这些基于不同亲本株的鼠疫减毒活疫苗，均不能有效刺激宿主产生 LcrV 抗体，提示这可能是鼠疫减毒活疫苗的共性问题。

由于鼠疫减毒活疫苗不能有效刺激宿主产生特异性LcrV抗体，与亚单位疫苗、病毒载体疫苗、核酸疫苗等的联合免疫或作为序贯免疫中的一环可能能够弥补这方面的不足。有团队使用鼠疫腺病毒载体疫苗和减毒活疫苗进行序贯免疫或联合免疫，相较于传统的免疫方案，序贯免疫能够刺激机体产生更为均衡的免疫应答，有更高的保护率，且对F1$^-$鼠疫菌有很好的保护作用[80]。这也是鼠疫减毒活疫苗研究的新方向。

目前我国将鼠疫 EV76 减毒活疫苗作为储备疫苗，它对腺鼠疫及肺鼠疫都有较好的保护效果。但是前文已经提到 EV76 疫苗可能在高铁血症病人中恢复毒性，且接种 EV76 疫苗时可引起接种部位和/或全身不良反应。我们认为鼠疫减毒活疫苗相对于 EV76 减毒活疫苗至少有以下某方面优势时才能够取代或部分取代之，包括：(1) 更低的残存毒力，尤其是在铁过载条件下的残存毒力；(2) 更高的保护率，特别是对经呼吸道感染和对F1$^-$鼠疫菌的保护率。未来取代 EV76 的鼠疫疫苗也可能不仅是减毒活疫苗株，而是多种疫苗技术路线获得的候选疫苗的组合应用，以达到提高疫苗的安全性和对各种型别鼠疫菌株的有效覆盖。

鼠疫有着广泛的自然疫源地，短期内难以消除，在面对未来可能出现的生物战和生物恐怖威胁时，鼠疫疫苗是最为有力的武器。鼠疫减毒疫苗作为重要的研究方向，有其独特的优势，可以与其他技术方向的疫苗优势互补，因此研发鼠疫减毒活疫苗有着重要的现实意义。

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