论著·专题报道

纳米颗粒MntC-rePO@LS双联疫苗的构建及其对铜绿假单胞菌与金黄色葡萄球菌感染的免疫保护效果研究

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[摘要] **目的** 细菌耐药性问题日益严峻,尤其金黄色葡萄球菌(*Staphylococcus aureus*,SA)与铜绿假单胞菌(*Pseudomonas aeruginosa*,PA)共感染可协同加重病情,临床治疗困难。基于二氧四氢喋啶合酶(lumazine synthase,LS)自组装纳米颗粒,构建一种共展示SA抗原锰离子转运蛋白C(Manganese transporter C,MntC)与PA重组V抗原与外膜蛋白OprI(recombinant PcrV-OprI fusion antigen, rePO)的双联纳米疫苗MntC-rePO@LS,并假设其能在SA与PA单一及共感染模型中提供优于单体或物理混合组的免疫保护效果。**方法** 利用基因工程技术和SpyTag/SpyCatcher系统制备MntC-rePO@LS双联纳米颗粒。将6~8周龄雌性BALB/c小鼠(体质量18~20 g)按随机数字表法分为(n=5):PBS组、LS组(含8.57 μg LS)、rePO组(含9.43 μg rePO)、MntC组(含10.00 μg MntC)、物理混合组(含8.57 μg LS、10.00 μg MntC和9.43 μg rePO)及MntC-rePO@LS组(含10.86 μg rePO-ST和18.57 μg MntC@LS)。各组均以PBS缓冲液为溶剂配制相应制剂,于第0、7、14天经肌肉注射免疫。采用SDS-PAGE、分子筛层析、动态光散射和透射电镜对疫苗进行理化表征;通过红细胞溶血实验、细胞毒性实验、小鼠体质量监测、组织病理学检查、血常规与血生化分析评估其体内外安全性;于末次免疫后第7天采集血清,采用间接ELISA法检测抗MntC与抗rePO的特异性IgG抗体及其亚型水平以评价免疫原性。分别建立PA、SA及PA/SA共感染的肺炎小鼠模型,攻毒后监测体质量、感染评分,并于24 h后检测肺组织细菌载量及观察病理变化以评价保护效果。**结果** 成功构建了结构均一、粒径约65.69 nm的MntC-rePO@LS纳米颗粒。该疫苗在10~200 μg/mL浓度内无明显溶血及细胞毒性,免疫小鼠体质量正常增长,主要脏器及注射局部肌肉未见病理损伤,血常规与血生化指标均在正常范围内。免疫原性结果显示,MntC-rePO@LS组抗MntC与抗rePO特异性IgG效价在3个检测时间点(第0、7、14天)均显著高于单体组及物理混合组(P<0.05),且以IgG2b亚型为主。在PA、SA单一及共感染模型中,与各对照组相比,MntC-rePO@LS组小鼠体质量恢复更快,感染评分更低,肺组织细菌载量均显著降低(P<0.05),肺组织病理损伤明显减轻。**结论** 成功构建的MntC-rePO@LS双联纳米颗粒疫苗具有良好的安全性与免疫原性,可在SA与PA单一及共感染模型中提供显著的免疫保护,证实了其作为应对细菌耐药性及多微生物共感染候选疫苗的科学假设。

[关键词] 双联纳米疫苗;铜绿假单胞菌;金黄色葡萄球菌;免疫保护

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Bivalent nanovaccine MntC-rePO@LS conffers efective protections against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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[**Abstract**] Objective The escalating threat of antimicrobial resistance poses significant clinical challenges, particularly in cases of co-infections involving *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA), which synergistically increase disease severity and complicate therapeutic interventions. A bivalent nanovaccine, MntC-rePO@LS, was constructed by co-displaying the SA antigen Manganese transporter C (MntC) and the PA recombinant PcrV-OprI fusion antigen (rePO) based on self-assembling lumazine synthase (LS) nanoparticles. We hypothesized that this vaccine would provide superior immunoprotection against both single and co-infections with SA and PA compared to monomeric or physically mixed formulations. Methods The MntC-rePO@LS bivalent nanoparticles were prepared using genetic engineering and the SpyTag/SpyCatcher system. Female BALB/c mice (6 to 8 weeks old, weighing 18 to 20 g) were randomly divided into 6 groups: PBS, LS (8.57 μg LS), rePO (9.43 μg rePO), MntC (10 μg MntC), physical mixture (8.57 μg LS, 10 μg MntC, and 9.43 μg rePO), and MntC-rePO@LS (10.86 μg rePO-ST and 18.57 μg MntC@LS) groups, with 5 animals in each group. The mice from the above groups were immunized intramuscularly with corresponding agents dissolved in PBS buffer on days 0, 7, and 14. The vaccine was characterized by SDS-PAGE, size-exclusion chromatography, dynamic light scattering, and transmission electron microscopy (TEM). *In vitro* and *in vivo* safety of the vaccine was assessed through hemolysis assay, cytotoxicity test, body weight monitoring, histopathological examination, and hematological and serum biochemical analyses. The serum samples were collected on day 7 after the final immunization, and the titers and subtypes of anti-MntC and anti-rePO IgG were evaluated by indirect ELISA to evaluate the immunogenicity of the vaccine. Protective efficacy was assessed in mouse pneumonia models of single PA, single SA, and PA/SA co-infections by monitoring body weight and infection scores post-challenge, and by quantifying lung bacterial loads and observing histopathological changes at 24 h post-challenge. Results We successfully constructed homogeneous MntC-rePO@LS nanoparticles with a hydrodynamic diameter of approximately 65.69 nm. The vaccine exhibited no significant hemolysis or cytotoxicity at concentrations ranging from 10 to 200 μg/mL. The immunized mice demonstrated normal body weight gain, with no pathological damage observed in major organs or at the injection site, and all hematological and biochemical parameters remained within normal ranges. Immunogenicity studies revealed that the MntC-rePO@LS group induced significantly higher anti-MntC and anti-rePO specific IgG titers at all 3 time points tested (day 0, 7, and 14) compared to monomer and physical mixture groups ($P<0.05$), with a predominant IgG2b subtype response. In the models of single PA, single SA, and co-infections, the MntC-rePO@LS group exhibited faster body weight recovery, lower infection scores, significantly reduced bacterial loads in the lungs ($P<0.05$), and markedly attenuated lung histopathological damage compared to all control groups. Conclusion The successfully constructed MntC-rePO@LS bivalent nanovaccine demonstrates favorable safety and immunogenicity, providing significant immune protection against single and co-infections with SA and PA. These findings validate its potential as a candidate vaccine to address antimicrobial resistance and polymicrobial infections.

[**Key words**] bivalent nanovaccine; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; immunoprotection

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全球每年有700~770万人死于细菌感染,其中495万与耐药病原体相关,127万直接归因于抗生素耐药感染[1]。相较于单一微生物感染,多微生物共感染的临床处理更为复杂,尤其以金黄色葡萄球菌(*Staphylococcus aureus*, SA)与铜绿假单胞菌(*Pseudomonas aeruginosa*,PA)共感染最为突出,二者可协同增强毒力因子表达、促进生物膜形成,并削弱宿主免疫清除能力,从而加剧病情进展并增加患者病死率[2-3]。目前临床上常联合使用抗SA与抗PA抗生素治疗,然而并未显著改善肺功能或降低急性加重风险,导致治疗失败率上升[4],这一现象在医院获得性肺炎、慢性伤口及囊性纤维化患者中尤为常见[5-7]。

疫苗接种是防控细菌感染的有效策略[8]。在SA疫苗研究中,锰转运蛋白C(manganese transporter C, MntC)作为锰ABC转运体高亲和力锰摄取系统的关键组分,具有高度保守性,并在细菌致病过程中发挥核心作用,因而被视为极具潜力的疫苗抗原[9]。对于PA,其Ⅲ型分泌系统关键蛋白V抗原(pseudomonas clearance regulator,PcrV)负责介导毒素向宿主细胞的转运[10],而外膜脂蛋白I(outer membrane lipoprotein I, OprI)不仅可激活TLR2/4免疫通路,还能与其他外膜蛋白协同参与细菌的营养物质运输和膜结构稳定性维持[11],二者均为公认的免疫靶标。本课题组前期构建的重组PcrV-OprI融合抗原rePO(recombinant PcrV-OprI fusion antigen,rePO)在PA感染动物模型中已证实具有良好的免疫保护效力[12-13]。然而,上述抗原仍存在免疫原性有限的问题,所诱导的保护性应答的强度与持久性仍有进一步提升的空间。

纳米颗粒疫苗作为新型抗原递送平台,能够模拟天然病原体空间构象,在提升抗原稳定性与树突状细胞递呈效率的同时,可诱导强效且持久的免疫应答,因而备受关注[14-16]。二氧四氢喋啶合酶(lumazine synthase, LS)可自组装形成六十聚体二十面体结构,其表面能够实现多种异源抗原的高密度、精准排布,已成为开发多价疫苗的理想平台[17-18]。有研究通过构建同时展示SARS-CoV-2、SARS-CoV-1和MERS-CoV刺突蛋白的纳米颗粒,在小鼠模型中成功诱导广谱中和抗体,并对包括变异株在内的SARS-CoV-2感染提供有效保护[19]。此外,另一项研究基于LS融合纳米颗粒,利用SpyCatcher/SnoopCatcher系统将基孔肯雅病毒E2蛋白与寨卡病毒EDIII抗原共同展示,在小鼠中同步激发针对两种病原体的高水平IgG与中和抗体[20]。

基于此,本研究以LS纳米颗粒为载体平台,通过基因工程技术在其N端与C端分别连接SpyCatcher与MntC,并利用SpyTag/SpyCatcher系统介导的共价连接将rePO抗原定向展示于颗粒表面,构建双联纳米疫苗MntC-rePO@LS,旨在同时靶向SA与PA感染,为应对其单一及共感染提供新型免疫干预策略。

1 材料与方法

1.1 材料

1.1.1 实验动物 6~8周龄SPF级雌性BALB/c小鼠,体质量18~20 g,购自北京维通利华实验动物技术有限公司。本研究涉及的动物实验均已经陆军军医大学实验动物福利伦理审查委员会批准(AMUWEC20223336)。实验动物在SPF级环境下饲养,温度维持在20~26 ℃,日温差≤4 ℃,相对湿度为40%~70%,通风次数≥8次/h,光照周期为12 h明/12 h暗。动物饲养于独立通气笼具中,可自由摄食和饮水,每笼动物数量符合国家相关标准。所有可能引起疼痛的操作均在麻醉镇痛下进行,麻醉剂为阿佛丁。实验过程中密切观察,若出现感染、异常死亡、自残等预期外不良事件,应立即上报实验动物伦理委员会,并由专业人员指导采取干预措施或终止实验,详细记录不良事件发生的时间、表现及处理过程。建立人道终点,当动物出现以下情况之一时实施安乐死:①持续3 d以上体质量下降超过20%;②无法自主进食饮水、严重脱水或濒死状态。动物监测频率为实验前期每日1次,实验后期每日2次。

1.1.2 菌株与细胞 PA XN-1菌株、SA USA300菌株和DC2.4细胞由国家免疫生物制品工程技术研究中心保存。BL21(DE3)感受态细胞购自重庆铠创生物公司。pET-21a(+)-SpyCatcher-Lumazine Synthase-MntC由南京金斯瑞生物科技有限公司合成,转化至*E. coil* BL21(DE3)大肠杆菌感受态细胞中,制得pET-21a(+)_SpyCatcher-Lumazine Synthase-MntC/*E. coil* BL21(DE3)工程菌。pET-28a(+)_rePcrV OprI-SpyTag/*E. coli* BL21(DE3)工程菌由本课题组前期研究构建[13]。

1.1.3 主要试剂与仪器 胰蛋白胨、酵母粉购自OXOID;琼脂粉、氨苄青霉素钠、卡那霉素、异丙基-β-D硫代半乳糖苷(IPTG)购自生工生物;金属亲和层析介质购自中科森辉;Tris购自索莱宝;甘氨酸、SDS粉末购自BBI;DMEM培养基、胰酶细胞消化液购自GIBCO;胎牛血清购自普诺赛,青霉素-链霉素溶液(100×)、4%多聚甲醛通用型组织固定液购自白鲨。LB培养基由胰蛋白胨、酵母粉和氯化钠配制,LB琼脂培养基由胰蛋白胨、酵母粉、氯化钠和琼脂粉配制。常温高速离心机购自上海卢湘仪实验室仪器有限公司,Avant Pure 150纯化仪购自美国General Electric公司。

1.2 方法

1.2.1 MntC@LS和rePO-ST的表达与纯化 分别将工程菌pET-21a(+)_MntC@LS/*E. coli* BL21(DE3)和pET-28a(+)_rePO-ST/*E. coli* BL21(DE3)划线于含氨苄青毒素(100 μg/mL)LB琼脂平板,挑取单菌落于20 mL含氨苄青毒素(100 μg/mL)的LB培养基中,在37 ℃下以220 r/min振荡培养过夜,转接至2 L含Amp的LB培养基,同条件培养至*OD*(600)为0.6~0.8,16 ℃降温2 h,加入IPTG至终浓度0.2 mmol/L,16 ℃、160 r/min诱导20 h。离心收集菌体重悬于PBS缓冲液中超声破菌,离心取上清与Ni填料4 ℃结合30 min,用梯度浓度含咪唑的PBS缓冲液洗脱,收集目标组分经G25脱盐柱置换至PBS缓冲液,即得MntC@LS和rePO-ST蛋白。

1.2.2 MntC-rePO@LS的制备与理化性质研究 将MntC@LS与rePO-ST按不同摩尔比(1.00∶0.50、1.00∶0.75、1.00∶1.00、1.00∶1.25、1.00∶1.50)混合,利用SpyTag/SpyCatcher蛋白连接系统在4 ℃下孵育过夜进行共价偶联。通过SDS-PAGE凝胶电泳、Image J灰度分析、Superose 6 Increase 10/300GL分子筛层析、动态光散射和透射电镜对MntC-rePO@LS的理化性质进行检测。

1.2.3 安全性研究 在红细胞溶血实验中,取空白小鼠1只,经阿佛丁(0.02 mL/g)麻醉后采集眼眶血,PBS洗涤后制备为5%红细胞悬浮液,与梯度浓度(10~200 μg/mL)的MntC-rePO@LS于37 ℃共孵育2 h,随后以8 000 r/min离心10 min,取上清测定吸光度[*OD*(545)]值。CCK-8法检测细胞毒性,将DC2.4细胞以1×10^{5}个/孔接种于96孔板,37 ℃、5%CO_2培养过夜后加入梯度浓度(0~200 μg/mL)的MntC-rePO@LS,继续培养24 h,加入10 μL CCK-8反应2 h后,测定*OD*(450)。首次免疫后每间隔48 h监测小鼠体质量,持续28 d。末次免疫后第7天,取免疫MntC-rePO@LS和空白小鼠中各1只,麻醉后解剖,采集心、肝、脾、肺、肾组织及局部肌肉组织,4%多聚甲醛固定、石蜡包埋切片后进行HE染色并观察组织形态。另从上述2组小鼠中各取1只,麻醉后采集眼球血,采用血常规分析仪检测全血中白细胞计数(white blood cell count,WBC)、血红蛋白(hemoglobin,HGB)、血小板计数(platelet count,PLT)、中性粒细胞百分比(neutrophil percentage,Neu)及红细胞压积(hematocrit,HCT);另取血液以8 000 r/min离心10 min分离血清,使用生化分析仪测定血清中肝功能:丙氨酸氨基转移酶(alanine aminotransferase,ALT)、白蛋白(albumin,ALB)、天冬氨酸氨基转移酶(aspartate aminotransferase,AST),肾功能:肌酐(creatinine,CREA)及心肌酶:肌酸激酶(creatine kinase,CK)指标。

1.2.4 疫苗制备与小鼠免疫 选取6~8周龄SPF级雌性BALB/c小鼠,按随机数字表法分为(*n*=5):PBS组、LS组(含8.57 μg LS)、rePO组(含9.43 μg rePO)、MntC组(含10 μg MntC)、物理混合组(MntC,rePO,LS mix组,含8.57 μg LS、10 μg MntC和9.43 μg rePO)及MntC-rePO@LS组(含10.86 μg rePO-ST和18.57 μg MntC@LS)。各组均以PBS缓冲液为溶剂配制相应制剂,于第0、7、14天经肌肉注射途径免疫,每只接种体积为200 μL,其中PBS组注射等体积PBS缓冲液。所有制剂现配现用,且不同批次的疫苗配制、免疫剂量及免疫过程中所遵循的标准操作流程均保持一致。

1.2.5 抗体应答研究 于每次免疫后第7天经尾静脉采血,37 ℃孵育2 h后,以8 000 r/min离心10 min取血清,采用间接ELISA法检测血清中抗rePO和抗MntC的特异性IgG抗体效价。以6 μg/mL抗原(rePO和MntC)100 μL/孔包被酶标板,4 ℃过夜。PBST洗涤后,每孔加入250 μL 1%BSA-PBST,37 ℃封闭2 h。洗涤后加入稀释至合适浓度的血清200 μL/孔,进行梯度稀释,37 ℃孵育1 h。洗涤后加入HRP标记山羊抗鼠IgG二抗(1∶7 500)100 μL/孔,37 ℃反应45 min。洗涤后加入TMB底物显色液100 μL/孔,37 ℃避光显色10 min,加入终止液50 μL/孔,测定*A*(450)。

1.2.6 保护效果研究 于末次免疫后第7天将各免疫组小鼠分别建立PA、SA及PA/SA共感染的肺炎模型。每组小鼠经阿佛丁麻醉后,通过气管插管分别接种20 μL XN-1(1.33×10^6 CFU/只)、40 μL USA300(2.00×10^8 CFU/只)和30 μL混合菌液(由10 μL XN-1 4.76×10^5 CFU/只和20 μL USA300 1.72×10^7 CFU/只混合)。攻毒后每12 h记录体质量变化及感染状态评分,持续监测168 h。攻毒24 h后每组取5只小鼠,经阿佛丁麻醉后摘眼球采血,随后实施安乐死,75%酒精体表消毒后取肺组织,加入1 mL PBS匀浆后取20 μL进行10倍梯度稀释,分别取10 μL滴种于含氨苄青霉素(50 μg/mL)的LB平板、MHA平板和MSA平板,37 ℃培养过夜后计数菌落。同时于攻毒24 h后每组各取1只小鼠肺组织,4%多聚甲醛固定24 h,经石蜡包埋、切片及HE染色后,于光镜下组织病理变化。

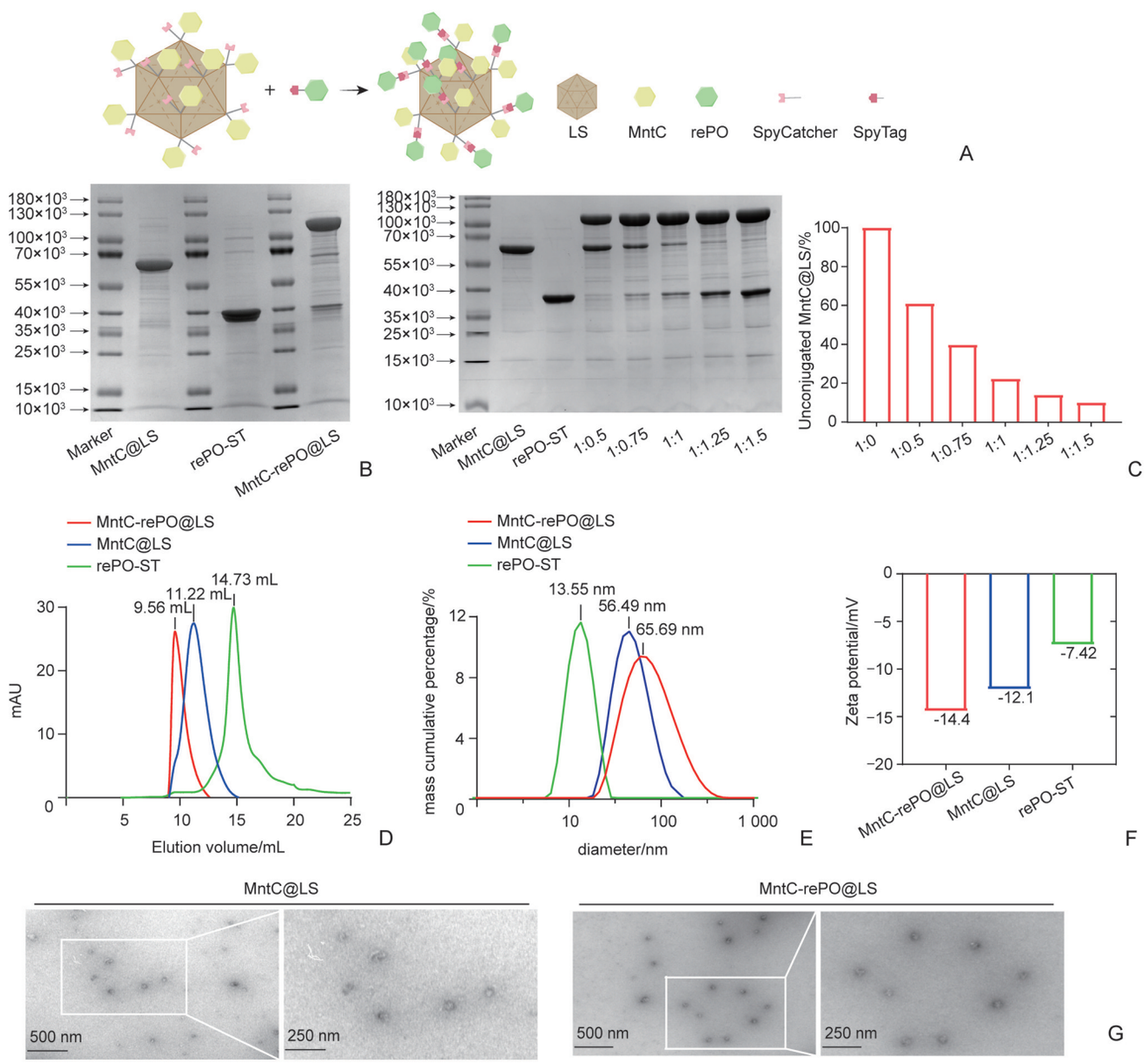
1.3 统计学分析

采用GraphPad Prism 10.0软件进行统计学分析,实验结果以$\bar{x}\pm s$表示,两组数据间采用非配对*t*检验,多组数据间采用ANOVA因素方差分析,$P<0.05$为差异具有统计学意义。

2　结果

2.1　双联纳米颗粒疫苗MntC-rePO@LS的成功构建与表征

本研究构建了双联纳米颗粒疫苗MntC-rePO@LS,其构建策略如图1A所示。为实现该构建方案,分别培养pET-21a(+)-SpyCatcher-Lumazine Synthase-MntC/*E. coli* BL21(DE3)和pET-28a(+)_rePcrV OprI-SpyTag/*E. coli* BL21(DE3)工程菌并诱导表达,以获得表面展示MntC和SpyCatcher标签的LS纳米颗粒蛋白(MntC@LS)和偶联SpyTag标签的rePO蛋白(rePO-ST)。表达产物经纯化后成功得到MntC@LS和rePO-ST(图1B)。将二者按不同摩尔比(1∶0.5~1∶1.5)混合后,SDS-PAGE检测显示均形成了MntC-rePO@LS复合物(图1C)。经Image J灰度分析,在1∶1摩尔比条件下结合效率最高,约为83%。分子筛层析显示,rePO-ST、



A: Schematic diagram of the MntC-rePO@LS components; B: SDS-PAGE analysis of purified MntC@LS and rePO-ST; C: SDS-PAGE analysis of the products after incubation of MntC@LS with rePO-ST at varying molar ratios, and the corresponding percentage of unconjugated MntC@LS; D: Characterization of MntC@LS, rePO-ST, and MntC-rePO@LS by Superose 6 size-exclusion chromatography (SEC); E: Size analysis by dynamic light scattering (DLS) of MntC@LS, rePO-ST, and MntC-rePO@LS; F: Zeta potential analysis by DLS of MntC@LS, rePO-ST, and MntC-rePO@LS; G: Images of MntC@LS and MntC-rePO@LS nanoparticles by transmission electron microscopy (TEM)

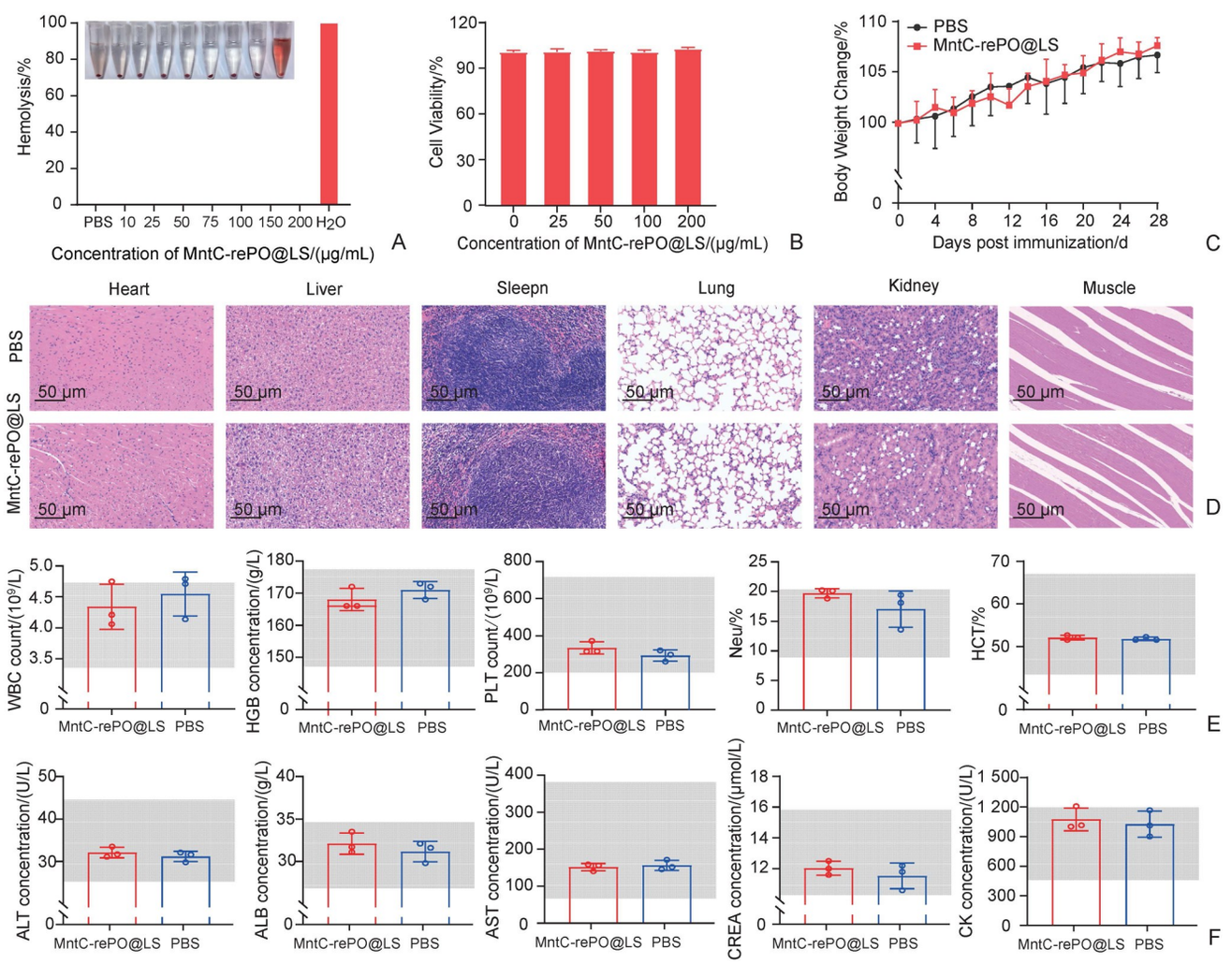
图1　双联纳米颗粒疫苗MntC-rePO@LS的构建与表征

Figure 1　Construction and characterization of the bivalent nanovaccine MntC-rePO@LS

MntC@LS和MntC-rePO@LS的峰形均对称,出峰体积分别为14.73、11.22和9.56 mL(图1D)。与MntC@LS蛋白相比,MntC-rePO@LS蛋白的出峰体积前移1.66 mL,提示其分子量增加。动态光散射结果显示三者流体直径分别为13.55、56.49、与65.69 nm(图1E),Zeta电位分别为-7.42、-12.1、-14.4(图1F)。透射电镜进一步发现MntC@LS与MntC-rePO@LS均呈现均匀球形结构(图1G)。以上结果表明,本研究成功制备了结构均一的双联纳米颗粒疫苗MntC-rePO@LS。

2.2 双联纳米颗粒疫苗MntC-rePO@LS具有良好的体内外安全性

为了评价MntC-rePO@LS在体内外的安全性,本研究采用细胞溶血实验、细胞毒性实验、小鼠体质量监测、组织病理学检查、小鼠血常规及血生化分析。细胞溶血和细胞毒性结果显示,MntC-rePO@LS在10~200 μg/mL浓度内均未引起明显溶血反应(图2A),且在0~200 μg/mL浓度内对DC2.4细胞活力均无显著影响(图2B)。体质量监测显示,MntC-rePO@LS免疫28 d内小鼠体质量正常增加,与PBS组无显著差异(图2C)。组织切片结果显示,与PBS组类似,MntC-rePO@LS组小鼠心脏、肝脏、脾脏、肺脏、肾脏均未发现异常病理改变,注射部位肌肉组织亦未出现明显炎症或损伤(图2D)。血常规和血生化检测结果表明,MntC-rePO@LS免疫组小鼠的WBC、HGB、PLT、Neu、HCT、ALT、ALB、AST、CREA及CK水平均在正常范围内(图2E、F)。上述结果表明,MntC-rePO@LS蛋白在体内外具备良好的安全性。



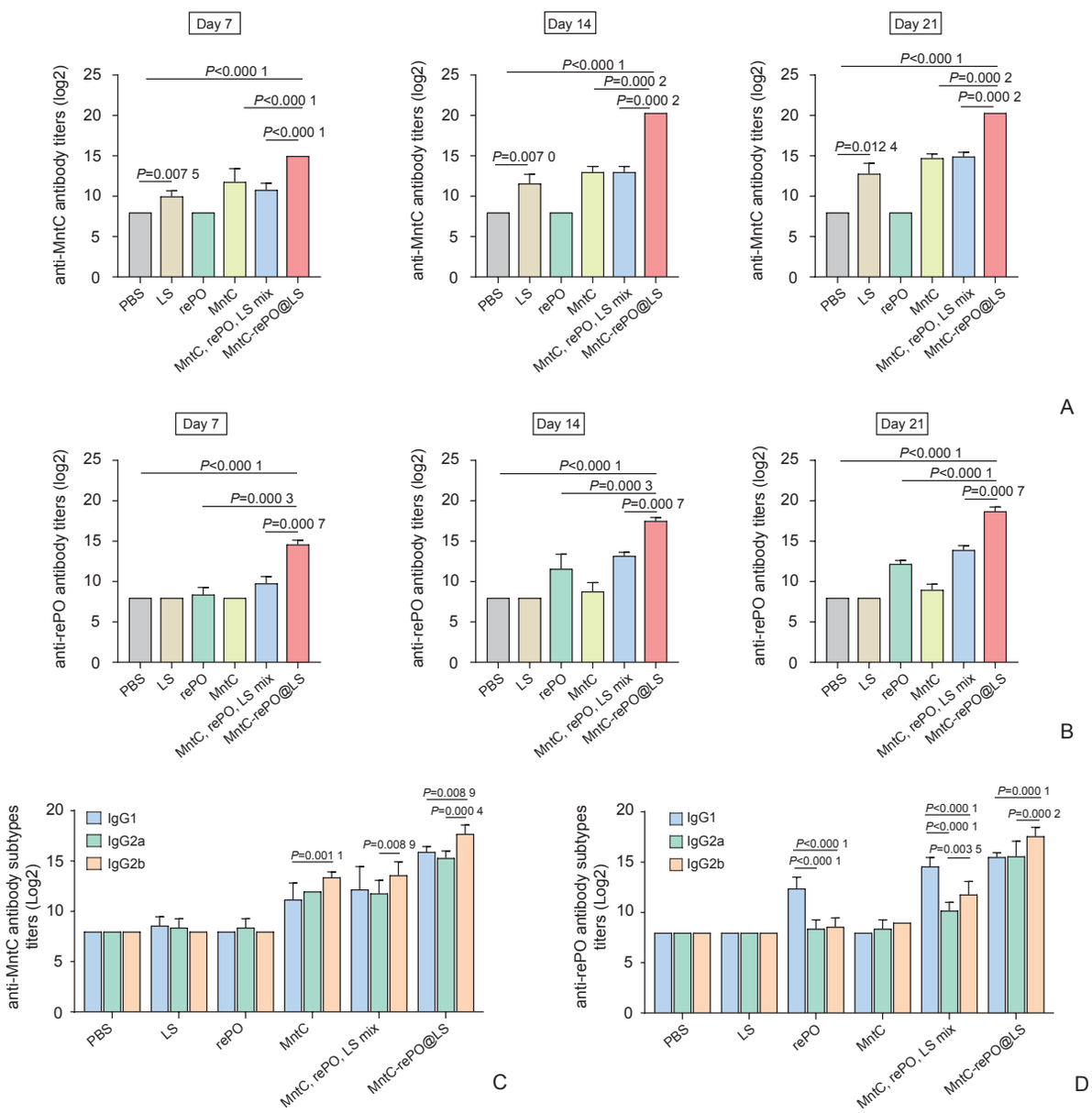
A: Hemolysis assay of MntC-rePO@LS at different concentrations; B: Cytotoxicity assay of MntC-rePO@LS at different concentrations; C: Body weight changes in mice during the immunization period (n=5); D: HE-stained sections of major organs (heart, liver, spleen, lungs, kidneys) and local muscle tissue from mice on day 7 after the final immunization; E: Analysis of hematological parameters (WBC, HGB, PLT, Neu, HCT) in mice; F: Analysis of serum biochemical parameters (ALT, ALB, AST, CREA, CK) in mice. Shaded areas indicate the reference intervals defined by healthy controls

图2 双联纳米颗粒疫苗MntC-rePO@LS体内外安全性研究(n=3,$\bar{x}\pm s$)

Figure 2 *In vitro* and *in vivo* safety evaluation of the bivalent nanovaccine MntC-rePO@LS (n=3,$\bar{x}\pm s$)

2.3　双联纳米颗粒疫苗MntC-rePO@LS有效增强抗原特异性免疫应答

为了评价MntC-rePO@LS的免疫原性,采用间接ELISA法检测免疫后小鼠血清中抗MntC与抗rePO的特异性总IgG及其亚型水平。结果显示,在3个检测点(第7、14、21天),MntC-rePO@LS组的抗MntC和抗rePO IgG总效价显著高于单体组和物理混合组(P<0.05,图3A、B)。抗MntC IgG总效价在第14天即达峰值,显著早于单体组和物理混合组(图3A)。此外,与PBS组相比,LS组小鼠血清中在3个检测点(第7、14、21天)均可检测到抗MntC IgG抗体(P<0.05,图3A)。抗体亚型分析显示,MntC-rePO@LS组诱导的抗MntC与抗rePO IgG2b水平均显著高于IgG1和IgG2a(P<0.05,图3C、D),提示疫苗主要引发Th1型免疫应答。而单体rePO和物理混合组的抗rePO IgG2b水平均显著低于IgG1(P<0.05,图3D)。综上,MntC-rePO@LS诱导的特异性抗体总效价显著高于单体组和物理混合组,且抗体亚型以IgG2b为主的特异性抗体。



A: Anti-MntC-specific total IgG titers; B: Anti-rePO-specific total IgG titers; C: Anti-MntC-specific antibody isotypes; D: Anti-rePO-specific antibody isotypes

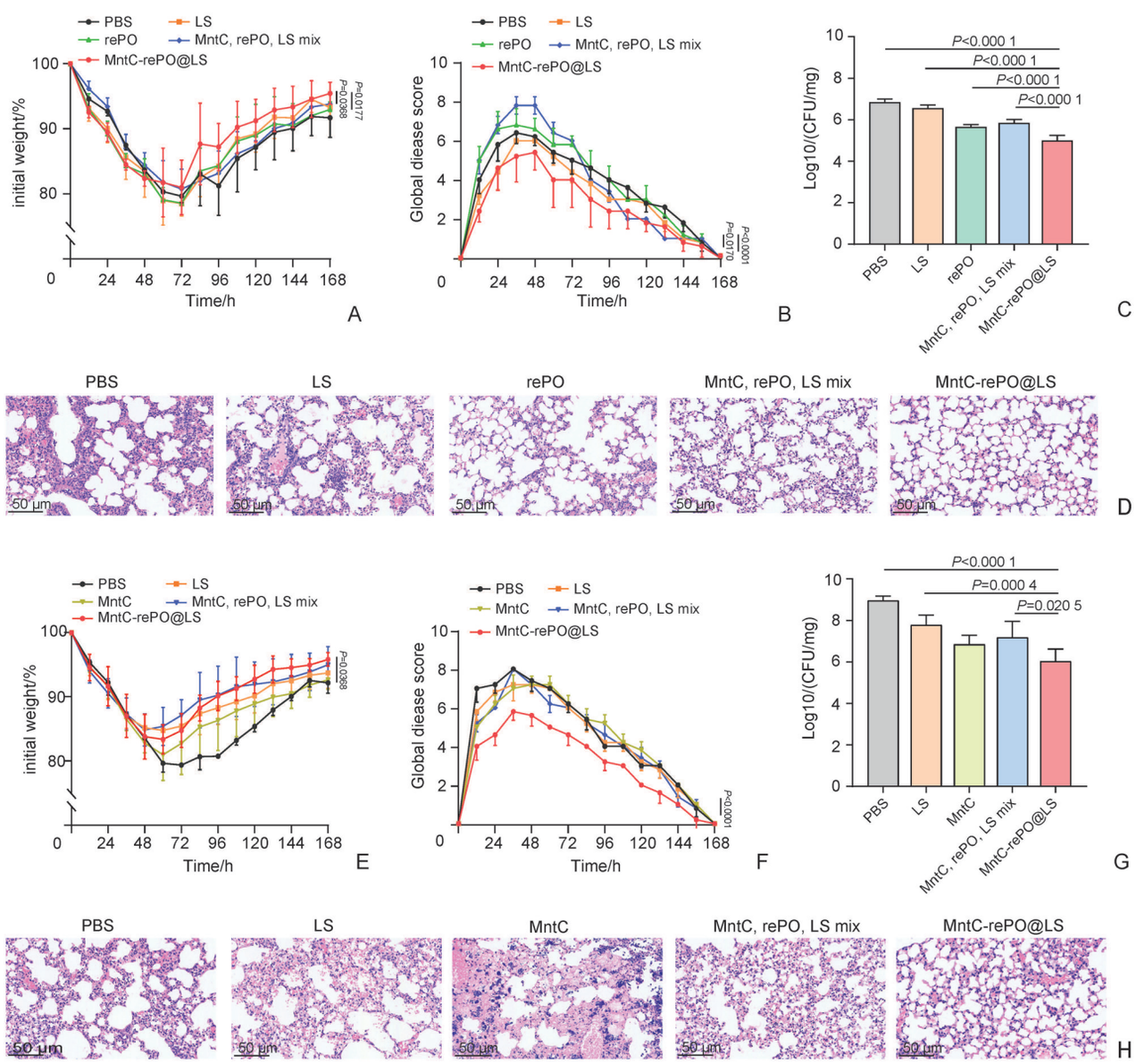
图3　双联纳米颗粒疫苗MntC-rePO@LS免疫应答研究(n=5,$\bar{x}\pm s$)

Figure 3　Immunological response induced by the bivalent nanovaccine MntC-rePO@LS(n=5,$\bar{x}\pm s$)

2.4　双联纳米颗粒疫苗MntC-rePO@LS对SA与PA感染具有显著保护效果

为了评价MntC-rePO@LS免疫对PA肺部感染的保护效果，小鼠末次免疫后第7天经气管插管接种亚致死剂量的XN-1，并监测体质量变化、感染状态评分及肺部细菌载量。结果显示，XN-1感染后84 h，MntC-rePO@LS组体质量恢复优于LS组和物理混合组（$P<0.05$，图4A）。小鼠感染状态评分结果显示，MntC-rePO@LS组感染症状最轻、恢复最快，感染后48 h评分显著低于rePO组和物理混合组（$P<0.05$，图4B）。肺部细菌定植结果进一步表明MntC-rePO@LS组每毫克肺组织细菌数显著低于PBS组、LS组、rePO单体组及物理混合组（$P<0.05$，图4C）。肺组织病理切片显示，MntC-rePO@LS组小鼠肺组织基本保持正常结构，而PBS组和LS组出现明显炎症细胞浸润与肺泡结构破坏等病变（图4D）。

同时，利用肺炎模型评价MntC-rePO@LS免疫对SA的保护效果。结果显示，USA300感染后72 h，



A: Body weight changes of mice post-challenge with XN-1; B: Infection scores of the mice post-challenge with XN-1; C: Lung bacterial loads after XN-1 infection; D: HE-stained lung sections at 24 h post XN-1 infection; E: Body weight changes in mice post-challenge with USA300; F: Infection scores of mice post-challenge with USA300; G: Lung bacterial loads after USA300 infection; H: HE-stained lung sections at 24 h post-USA300 infection

图4　双联纳米颗粒疫苗MntC-rePO@LS单独感染保护效果评价（$n=5$，$\bar{x}\pm s$）

Figure 4　Evaluation of the protective efficacy of the bivalent nanovaccine MntC-rePO@LS against single infection（$n=5$，$\bar{x}\pm s$）

MntC-rePO@LS组体质量恢复程度显著优于PBS组(P<0.05,图4E)。感染后36 h评分显著低于LS组、MntC组和物理混合组(P<0.05,图4F)。MntC-rePO@LS组每毫克肺组织细菌数显著低于各对照组(P<0.05,图4G)。组织学分析显示,MntC-rePO@LS组肺组织病变明显减轻(图4H)。

此外,在PA和SA共感染模型中,感染48 h后各组小鼠体质量开始逐步恢复,于感染后60 h,MntC-rePO@LS组体质量恢复显著优于LS组、rePO组、MntC组及物理混合组(P<0.05,图5A)。共感染48 h后,感染状态评分显著低于上述各组(P<0.05,图5B)。与PBS组、rePO组、MntC组及物理混合组相比,该组针对两种病原的肺部载量均显著降低(P<0.05,图5C、D)。病理切片进一步证实,与rePO组、MntC组和物理混合组相比,MntC-rePO@LS可有效减轻共感染引起的肺组织损伤(P<0.05,图5E)。

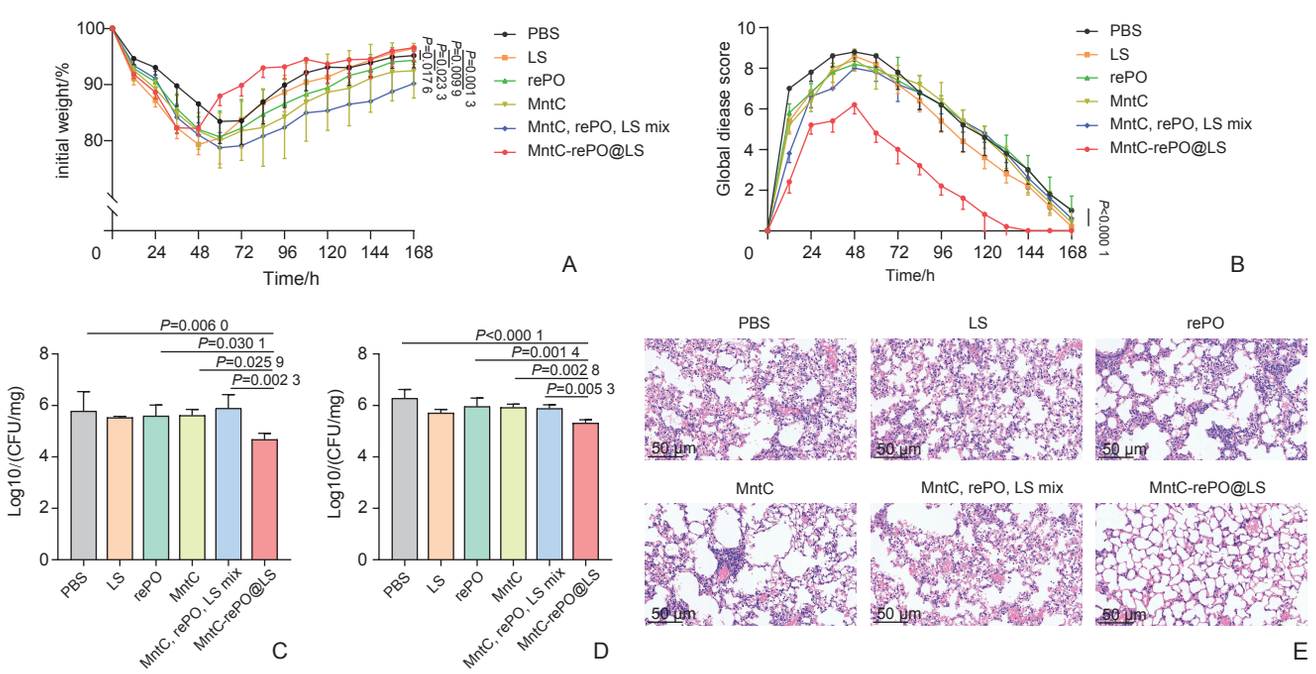
3　讨论

3.1　LS纳米颗粒可增强抗原特异性抗体应答

本研究发现,MntC-rePO@LS免疫组可诱导抗MntC与抗rePO的特异性IgG效价快速升高且其抗体效价峰值显著高于单体组和物理混合组,此效应可能与其形成的纳米颗粒理化特性密切相关。研究表明,尺寸在20~200 nm内的纳米颗粒更易被抗原呈递细胞摄取并靶向淋巴器官[21];而颗粒表面正电荷可通过静电作用增强其与细胞膜的相互作用,促进抗原内化[22-23]。此外,基于蛋白质自组装的纳米颗粒能够实现抗原的高密度、有序排列,这种多价展示可有效交叉连接B细胞受体,从而强力激活B细胞并促进生发中心反应,可能是早期高效体液免疫应答的关键机制之一[24]。但具体的机制还需在后续的研究中进一步明确。

3.2　LS纳米颗粒与MntC存在潜在交叉反应

本研究发现,LS纳米颗粒可诱导抗MntC IgG抗体,但并未转化为针对SA感染的有效保护效力。该交叉反应可能因为LS蛋白与MntC存在共同表位,并且其诱导的免疫应答无保护效果。研究表明,铁蛋白等天然自组装纳米颗粒能精确模拟病原体表面抗原的天然构象与排布,从而被免疫系统识别并引发强效交叉应答[24]。抗体的有效性还依赖于B细胞的状态与抗体识别靶标的表位[25]。因此,我们推测LS引发的交叉反应所针对的表位可能并非MntC的关键保护性表位,提示在后续研究中需鉴定该表位信息,且在疫苗优化设计中予以删除。



A: Body weight changes in mice following co-infections with XN-1 and USA300; B: Infection scores of mice following co-infections with XN-1 and USA300; C: Lung bacterial loads of XN-1 following co-infections with XN-1 and USA300; D: Lung bacterial loads of USA300 following co-infectiond with XN-1 and USA300; E: HE-stained lung sections at 24 h following co-infections with XN-1 and USA300

图5　双联纳米颗粒疫苗MntC-rePO@LS共感染保护效果评价(n=5,$\bar{x}\pm s$)

Figure 5　Evaluation of the protective efficacy of the bivalent nanovaccine MntC-rePO@LS against co-infections(n=5,$\bar{x}\pm s$)

3.3 LS纳米颗粒共递送策略可逆转repPO的免疫应答类型

本研究发现,通过LS纳米颗粒递送repPO后,针对repPO的特异性IgG2b水平显著高于IgG1与IgG2a。然而,repPO单体组IgG1水平却高于IgG2b。该亚型转换现象可能与repPO抗原形成的纳米颗粒的理化性质密切相关。研究表明,具有正电荷特性的纳米载体(如几丁质衍生的壳聚糖纳米复合物)可通过调节树突状细胞分化状态促进Th1型免疫应答,强调了表面电荷性质对免疫极化的重要调控作用[5]。此外,小尺寸纳米颗粒(<500 nm)更易通过淋巴管运输至淋巴结,从而被其中富集的抗原呈递细胞有效捕获,为高效启动T细胞免疫提供了有利条件[21]。但具体的抗体亚型转换的机制还需在后续研究中进一步明确。

细菌耐药性问题日益严峻,开发安全有效的多价疫苗具有重要的临床价值。本研究构建的MntC-repPO@LS双联纳米疫苗在PA和SA单感染及共感染模型中均展现出良好的保护效果,显示出应对临床多微生物共感染的潜在应用价值。然而,现阶段的研究主要聚焦于抗体应答及保护效果评价,细胞免疫机制尚待阐明。此外,本研究中的双联纳米颗粒疫苗仅采用了单一抗原配比与免疫剂量,未来需通过系统的梯度实验,进一步揭示抗原配比、免疫剂量与免疫效果之间的量效关系,从而为疫苗优化提供依据。

综上所述,本研究利用LS自组装形成的60聚体纳米颗粒作为支架,成功构建了共展示SA抗原MntC与PA抗原repPO的双联纳米疫苗MntC-repPO@LS。该疫苗结构均一、安全性良好,在PA和SA感染模型中均能诱导高效体液免疫应答并减轻肺组织损伤,为抗耐药菌双联纳米疫苗的研发提供了基础。

利益冲突 本文所有作者声明不存在利益冲突

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