

布鲁氏菌外膜蛋白免疫原性研究进展

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摘要: 布鲁氏菌病是由布鲁氏菌引起的世界范围内广泛流行的重要人畜共患传染病。外膜蛋白作为布鲁氏菌重要毒力因子, 不仅在宿主感染过程中起着关键作用, 还是布鲁氏菌潜在免疫原和保护性抗原, 能刺激机体产生较强免疫应答, 可作为布鲁氏菌的诊断抗原和新型疫苗靶点。本文对布鲁氏菌外膜蛋白免疫原性的研究进展进行了总结, 以期为布鲁氏菌的实验室诊断和新型疫苗研发提供参考。

关键词: 布鲁氏菌; 外膜蛋白; 免疫应答; 疫苗

Research progress in immunogenicity of outer membrane proteins of *Brucella*

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Abstract: Brucellosis, caused by the bacteria of *Brucella*, is considered one of the major zoonotic diseases worldwide. As the key virulence factors of *Brucella*, outer membrane proteins

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not only play a vital role in the host immune process against *Brucella* but also are potential immunogens and protective antigens of *Brucella*. These proteins can stimulate strong immune response of the host and be used as diagnostic antigens and targets of novel vaccines against *Brucella*. We reviewed the research progress in the immunogenicity of key outer membrane proteins of *Brucella*, aiming to provide a reference for laboratory diagnosis and new vaccine development against *Brucella*.

Keywords: *Brucella*; outer membrane proteins; immune response; vaccine

布鲁氏菌病(简称“布病”)是由布鲁氏菌引起的世界范围内广泛流行的重要人畜共患传染病。自1980年后,布病迅速在170多个国家和地区开始流行,此后每年新增病例超过50万^[1]。世界动物卫生组织(World Organization of Animal Health, WOAH)将布病列为法定报告疫病,我国将其列为二类动物疫病^[2-3]。1985年世界卫生组织(World Health Organization, WHO)布病专家委员会将布鲁氏菌属分为6个种:羊种布鲁氏菌(*Brucella melitensis*)、牛种布鲁氏菌(*B. abortus*)、猪种布鲁氏菌(*B. suis*)、绵羊附睾种布鲁氏菌(*B. ovis*)、犬种布鲁氏菌(*B. canis*)、沙林鼠种布鲁氏菌(*B. neotomae*)^[4]。此外,近些年陆续发现鲸种布鲁氏菌(*B. ceti*)、鳍种布鲁氏菌(*B. pinnipedialis*)、田鼠种布鲁氏菌(*B. microti*)、人源布鲁氏菌(*B. inopinata*)、狒狒种布鲁氏菌(*B. papionis*)等新种型^[4]。布鲁氏菌易感宿主较多,家畜中以牛、羊、猪最为易感,犬、马、骆驼等其他动物也可感染。动物患布病主要以侵害生殖系统为特征,且母畜易感性高于公畜^[5-6],其中母畜患布病主要临床症状为不孕症、流产、死胎、弱胎、乳腺炎、子宫内膜炎;公畜患布病主要临床症状为睾丸炎和附睾炎^[7]。人感染布鲁氏菌后临床症状为虚弱、发热、关节炎和骨髓炎等,严重者可引起心内膜炎或脑膜脑炎^[8]。目前,布病疫情在全球依旧呈上升趋势,严重危害畜牧业发展和人类健康^[9]。因此,加强布病的诊断与防控对畜牧业经济发展和人类健康具有重要意义。

外膜蛋白(outer membrane proteins, OMPs)位于细菌表面,作为病原相关模式分子(pathogen-associated molecular patterns, PAMPs),是大多数革兰氏阴性细菌的重要免疫原^[10-11]。布鲁氏菌外膜蛋白于20世纪80年代初通过选择性萃取技术获得^[12],根据分子质量大小将它们分为3组,依次为OMP10、OMP16和OMP19编码的分子质量分别为10.0、16.5和19.0 kDa^[13];OMP2编码的分子质量为36–38 kDa^[14];OMP22、OMP25、OMP25b、OMP25c、OMP25d、OMP31和OMP31b共同编码的分子质量为25–27 kDa、31–34 kDa^[15-16]。布鲁氏菌外膜蛋白不仅承担生物膜的重要功能(图1),还是其重要的毒力因子,与布鲁氏菌胞内生存及逃避宿主免疫密切相关^[17]。研究表明,布鲁氏菌外膜蛋白具有良好的免疫原性,可作为布病的血清学诊断抗原和新型疫苗候选靶点^[18]。本文对布鲁氏菌外膜蛋白免疫原性的相关研究进行综述,以期进一步了解布鲁氏菌外膜蛋白的免疫学特征,为布病的诊断技术和新型疫苗研发提供理论支持。

1 OMP10

OMP10属于脂蛋白,是构成布鲁氏菌外膜的主要结构成分,存在于目前已知所有种型中,同时是其胞内存活的关键毒力因子,可诱导机体产生保护性细胞免疫^[19]。Tibor等发现布鲁氏菌OMP10缺失株在小鼠体内的载菌量显著降低,并且在基本培养基(minimal medium, MM)中的

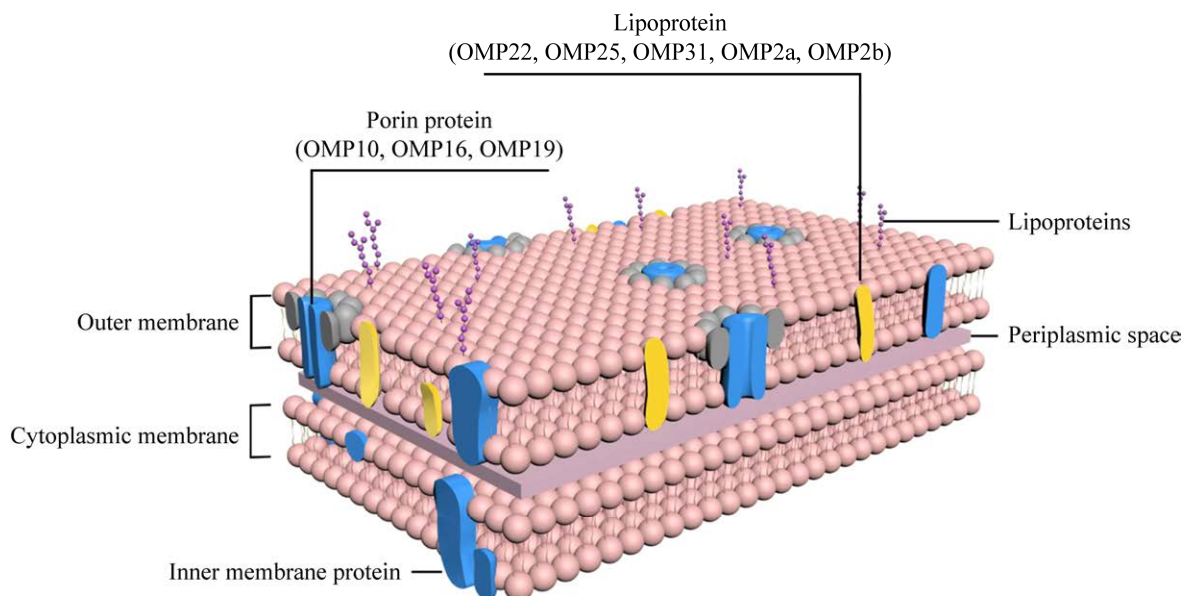


图1 布鲁氏菌外膜蛋白结构示意图

Figure 1 Structure diagram of *Brucella* outer membrane protein.

生存能力也显著减弱,但并未改变布鲁氏菌的外膜特性及细胞内的存活^[20]。使用重组的布鲁氏菌 OMP10 刺激 RAW264.7 细胞和幼稚脾细胞后,可使 RAW264.7 细胞中一氧化氮(nitric oxide, NO)、肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)和白细胞介素-1 (interleukin-1, IL-1)的水平增加及幼稚脾细胞中干扰素- γ (interferon- γ , IFN- γ)和白细胞介素-2 (interleukin-2, IL-2)的水平升高,证实重组的 OMP10 可诱导炎性细胞因子的产生^[21]。

OMP10 不与自然感染的牛发生血清学反应,但与自然感染的羊发生血清学反应。因此,可作为布病分型的血清学诊断抗原^[20]。陈瑶等采用布鲁氏菌 OMP10 和 BP26 融合蛋白作为诊断抗原,可与布鲁氏菌免疫血清发生特异性结合,证实融合表达的蛋白具有较好的免疫识别效果,不仅提高了检测的敏感性,还能区分自然感染与疫苗免疫,具有较好的应用前景^[22]。Simborio 等证实联合应用 OMP10、OMP19 和

OMP28 重组蛋白可区分牛种布鲁氏菌 S19 疫苗免疫和自然感染动物,其在布病血清学诊断中具有非常高的潜力^[23]。此外, Zhu 等融合 3 个保护性抗原 OMP10、OMP28 和 L7/L12,并用重组疫苗免疫小鼠后,其抗体水平升高、外周血淋巴细胞增殖、CD4⁺和 CD8⁺ T 淋巴细胞数量及细胞因子分泌均显著高于对照组,表明重组亚单位疫苗能在小鼠体内产生特异性抗体,并发挥良好免疫原性^[19]。因此,OMP10 不仅具有作为布鲁氏菌亚单位疫苗的潜力,还可作为布鲁氏菌诊断抗原。

2 OMP16

OMP16 是肽聚糖相关脂蛋白,在所有布鲁氏菌中都能表达,可诱导机体产生较强的免疫保护^[24]。OMP16 是布鲁氏菌存活的必需基因, Zhi 等通过四环素诱导表达载体成功获得布鲁氏菌 OMP16 缺失株, OMP16 合成不足导致布鲁氏菌在生长、外膜完整性及细胞内存活均受影响,且巨噬细胞 RAW264.7 的 IL-1 β 和 IL-6 表达增强,

表明 OMP16 可维持布鲁氏菌的外膜完整性并介导宿主免疫应答^[24-26]。此外, Alizadeh 等用重组布鲁氏菌 OMP16 免疫 BALB/c 小鼠后观察到其血清中的 IgG1 和 IgG2a 抗体水平升高, 且小鼠的脾细胞培养物中 IFN- γ 和 IL-4 的水平显著增加, 表明重组布鲁氏菌 OMP16 可诱导机体产生有效的保护性免疫反应^[27]。因此, OMP16 不仅是布鲁氏菌生存所必需的外膜蛋白, 而且具有良好的免疫原性。

由于布鲁氏菌 OMP16 的脂质部分具有佐剂活性, 在无需外源性佐剂条件下也可诱导机体产生与减毒活疫苗 S19 相似的保护水平^[28]。Ibañez 等用布鲁氏菌非脂化外膜蛋白 OMP16 (unlipidated-OMP16, U-OMP16) 作为黏膜佐剂对小鼠进行免疫, 可诱导其特异性的全身 IgG 型和 Th1 型免疫应答, 并调节对牛乳蛋白的 Th2 型过敏反应^[29]。此外, U-OMP16 还能够通过 TLR4 在体外刺激树突细胞和巨噬细胞, 进而诱导 Th1 型免疫应答^[30]。U-OMP16 具有免疫自佐剂性质, 且是一种 Th1 型免疫诱导剂, 在口服疫苗研发方面具有较好的应用前景。

研究显示, 由表达布鲁氏菌核糖体蛋白 L7/L12 和 OMP16 的重组二价疫苗与 montanide gel 01 佐剂组合, 可诱导较强体液免疫和细胞免疫应答, 且与市售 S19 疫苗具有相当的高水平保护性, 所以其可用于牛的实际应用^[31]。此外, Rezaei 等证实 OMP16-IL-2 融合蛋白具有良好免疫原性, 有利于开发抗布病的口服疫苗或亚单位疫苗^[32]。因此, OMP16 不仅是布鲁氏菌必需基因, 同时具有良好的免疫原性, 可作为研发布鲁氏菌口服和亚单位疫苗的潜在候选靶点。

3 OMP19

OMP19 是布鲁氏菌外膜结构重要成分, 又是布鲁氏菌重要毒力因子之一, 与布鲁氏菌的

定殖、入侵、胞内生存等多个生理功能密切相关^[33]。布鲁氏菌 OMP19 缺失后可引起粗糙型羊种布鲁氏菌外膜结构发生改变, 进而影响其胞内存活能力^[34]。此外, Uslu 等构建了羊种布鲁氏菌 Rev.1 OMP19 缺失株, 其残留毒力可在小鼠模型中提供与 Rev.1 疫苗相似的保护性免疫, 且使用羊种布鲁氏菌 Rev.1 OMP19 缺失株免疫后, 可通过筛选布鲁氏菌 OMP19 的酶联免疫吸附试验(enzyme-linked immunosorbent assay, ELISA), 从血清学上区分自然感染和免疫接种动物^[35]。Pasquevich 等用弗氏不完全佐剂与 U-OMP19 或 U-OMP16 联合免疫小鼠, 其诱导产生的免疫保护水平与布鲁氏菌减毒活疫苗 S19 相似, 表明 U-OMP19 和 U-OMP16 将是针对人和动物布病的亚单位疫苗的重要候选者^[36]。

U-OMP19 也是一种自佐剂蛋白, 口服和静脉注射该蛋白均能引起保护性反应, 表明 U-OMP19 可作为黏膜免疫系统及全身免疫系统的自身佐剂^[37]。此外, 引起的全身保护性反应可抵御牛种、羊种和猪种布鲁氏菌的感染^[37]。Nikam 等通过融合伤寒沙门氏菌 *sseB* 和 *ompL* 基因, 构建了融合蛋白 r-BL, 将 r-BL 与黏膜佐剂 U-OMP19 蛋白联合免疫小鼠后, 导致小鼠粪便样品和肠洗液中黏膜抗体滴度显著增加, 表明 U-OMP19 有助于保护靶分子免受水解消化, 同时刺激抗原特异性免疫应答, 并增加抗原半衰期^[38], 研究报道 U-OMP19 是目前研制新型佐剂疫苗中的一个新概念^[39]。OMP19 的蛋白质部分具有自身诱导保护性应答的能力, 可解决制剂中副作用、毒性、成本等问题^[37]。因此, OMP19 不仅是布鲁氏菌重要的毒力因子, 而且是良好的亚单位疫苗和新型佐剂疫苗的候选靶点。

4 OMP22

OMP22 也称 OMP3b, 具有较高保守性, 是

布鲁氏菌免疫优势抗原^[40]。研究显示, OMP22 的表达依赖布鲁氏菌的 BvrS/BvrR 双组分调节系统, 在 *BvrS/BvrR* 缺失株中 OMP22 的表达受到抑制^[41-42]。Caro-Hernández 等证实绵羊附睾种布鲁氏菌 OMP22 缺失株在小鼠模型中减毒^[43]。此外, 布鲁氏菌 OMP22 缺失株对 HeLa 和 J774.A1 细胞的侵袭能力降低, 并且胞内增殖明显减弱, 表明 OMP22 可能与布鲁氏菌毒力有关^[44]。

Dehghani 等采用 ELISA 检测 OMP22、OMP25 和 OMP31 重组蛋白与 37 例患者和 27 例健康人的血清反应性, 结果显示重组蛋白对布病诊断具有良好敏感性和特异性, 因此该重组蛋白可作为布病诊断靶标^[45]。Kim 等^[40]构建了表达牛种布鲁氏菌 BCSP31、OMP3b 和 SOD 重组蛋白的鼠伤寒沙门氏菌减毒活疫苗, 该疫苗在小鼠模型中可以诱导 Th1 型免疫应答。此外, 小鼠脾细胞培养上清中 IFN- γ 和 TNF- α 的水平显著升高, 并且口服和腹内注射候选疫苗, 均能有效抵御牛种布鲁氏菌的侵袭, 并诱导强烈的黏膜和细胞介导的免疫应答^[40]。然而, 针对布鲁氏菌 OMP22 的研究较少, 其重组蛋白的免疫效果仍需要进一步研究。

5 OMP25

OMP25 是布鲁氏菌重要的外膜结构蛋白, 其基因组在不同菌株间高度保守, 通过共价键与布鲁氏菌的肽聚糖层结合。布鲁氏菌侵入树突状细胞(dendritic cell, DC)后, 外膜蛋白 OMP25 可通过抑制 TNF- α 的产生和分泌, 进一步抑制 DC 的成熟。此外, OMP25 还可抑制 IL-6 和 IFN- β 的分泌, 以促进其胞内存活, 从而逃避宿主免疫系统的杀伤^[46-47]。Edmonds 等用牛种、羊种和猪种布鲁氏菌的 OMP25 缺失株免疫小鼠后, 其脾脏载菌量下降, 且羊种和猪种布鲁氏菌的

OMP25 缺失株所产生的免疫保护作用与布鲁氏菌 Rev.1 疫苗相似^[48]。因此, 布鲁氏菌 OMP25 缺失株可作为潜在的疫苗候选物。

Yousefi 等使用重组蛋白 OMP25-BLS 与热休克蛋白 60 (heat shock proteins 60, HSP60)联合免疫小鼠, 所诱导的 IFN- γ 和 TNF- α 的抗体滴度高于单独免疫, 并且可提高 IgG2a 与 IgG1 的比值; 此外, 与其他各组相比, 重组蛋白 OMP25-BLS 所诱导的总抗体滴度更高, 且与 HSP60 联合免疫可提高细胞免疫水平^[49]。Sun 等用重组山羊痘病毒(recombinant goat pox virus, rGTPV)表达布鲁氏菌外膜蛋白 OMP25 免疫小鼠, 证明 rGTPV 可引起抗布鲁氏菌特异性免疫球蛋白 IgG 应答; 接种 rGTPV 的小鼠在肾脏和肝脏中未表现病理学改变, 证实新型 rGTPV 能够有效驱动布鲁氏菌 OMP25 的表达并激活免疫反应, 有望在活载体疫苗及相关研究中得到应用^[50]。因此, OMP25 不仅与布鲁氏菌胞内生存和免疫逃避密切相关, 也是亚单位疫苗的候选抗原。

6 OMP31

OMP31 是布鲁氏菌的表面孔道蛋白, 不同种属间高度保守, 在维持布鲁氏菌外膜结构稳定性与毒力方面发挥重要作用。OMP31 缺失株导致布鲁氏菌毒力降低, 改变布鲁氏菌的外膜特性, 导致细菌在小鼠巨噬细胞 J774.A1 和 HeLa 细胞中的内化、存活和细胞内复制显著降低^[51]。

研究显示, 用重组布鲁氏菌 OMP31 免疫小鼠可诱导机体产生显著水平的 IFN- γ 、IL-12、IL-10 和 IL-4 及其他细胞因子, 表明重组布鲁氏菌 OMP31 能诱导机体产生 Th1 为主的细胞免疫应答^[52]。此外, 用重组布鲁氏菌 OMP31 免疫的小鼠血清中 IgG 抗体显著增加, 在 IgG 亚型中, IgG2a 的水平明显高于 IgG1, 表明重组布鲁氏菌

OMP31 能诱导潜在保护性抗体^[53]。使用核糖体蛋白 L7/L12、OMP22、OMP25 和 OMP31 蛋白作为单亚单位疫苗(single subunit vaccine, SSV)或联合亚单位疫苗(combined subunit vaccine, CSV)皮下免疫 BALB/c 小鼠,结果表明免疫小鼠中 IFN- γ 和 IL-2 大量产生,且 IgG2a 的滴度均高于 IgG1,提示 SSV 和 CSV 在体内诱导了典型的 Th1 细胞主导的免疫应答^[54]。此外,CSV 诱导保护水平显著高于 SSV 诱导的保护水平,且与用 RB51 疫苗免疫组相比无显著差异。因此,这些抗原可能是开发布鲁氏菌亚单位疫苗的潜在候选物^[54]。研究显示,布鲁氏菌嵌合重组 OMP25-OMP31 抗原可诱导机体产生更高水平的 IFN- γ 和 TNF- α 细胞因子,且比单独注射重组的 OMP25 和 OMP31 产生更高滴度的 IgG。因此,OMP31 可能是作为联合疫苗来增强免疫的候选者^[55]。

7 OMP2

OMP2 蛋白包含两个同源性较高的基因组 OMP2a 和 OMP2b^[17]。OMP2a 所表达出的孔道蛋白具有较好的亲水性和透水性,有利于布鲁氏菌获得充足养分以供其生长、分裂和繁殖^[56]。OMP2b 具有良好的免疫原性和抗原保护作用,可引起机体产生较强的 Th1 型免疫反应^[57]。用重组布鲁氏菌 OMP2b 刺激 RAW264.7 细胞 24 h 后诱导产生 TNF- α 和 IL-6。此外,在重组布鲁氏菌 OMP2b 的体外刺激中,小鼠幼稚脾细胞产生高水平 IFN- γ 和低水平 IL-4。在体内试验中,重组布鲁氏菌 OMP2b 免疫小鼠也产生高滴度的抗原特异性 IgM 和 IgG 抗体,表明重组的 OMP2b 有较高的免疫原性,可作为布鲁氏菌属血清学诊断的候选抗原^[58]。

Pathak 等克隆、表达和纯化了重组的 OMP2a,并采用间接酶联免疫吸附试验(indirect

enzyme-linked immunosorbent assay, iELISA)和 Western blotting 试验对 185 份血清样本进行检测,结果显示灵敏度为 93.75%,特异性为 95.83%,表明重组 OMP2a 可作为布病血清学诊断的候选抗原^[59]。此外,Vatankhah 等使用 iELISA 系统评估了重组 OMP2b 作为一种新的血清学诊断抗原,发现重组 OMP2b 可区分布鲁氏菌感染动物与非感染动物,并将该方法与虎红平板凝集试验和血清试管凝集试验进行比较,其灵敏度、特异性和准确性分别为 88.5%、100% 和 90.8%,表明重组 OMP2b 对布病的血清学诊断具有较高的灵敏度和特异性,可作为布病的诊断抗原^[60]。Sha 等利用生物信息学方法分析了 OMP2b、P39 和 BLS 的理化性质、结构特征和优势表位,发现布鲁氏菌 OMP2b 具有 3 个线性 B 细胞优势表位、5 个 CD8⁺ T 细胞优势表位和 3 个 CD4⁺ T 细胞优势表位,3 种布鲁氏菌疫苗候选蛋白抗原表位的预测可为构建理想的布鲁氏菌多价抗原表位疫苗提供理论依据^[61]。

8 其他外膜蛋白

BP26 又称 CP28 或 OMP28,是分子质量为 28 kDa 的布鲁氏菌细胞周质蛋白,最初被鉴定为布鲁氏菌感染动物中的主要免疫反应性蛋白^[62],在牛、绵羊、山羊和人中 BP26 具有较好的免疫原性^[63]。Li 等构建了羊种布鲁氏菌 M5-90 BP26 缺失株,其毒力降低并具有良好的免疫保护性,且该缺失株在人胎盘滋养层 8 细胞(human placenta trophoblastic 8 cells, HPT-8 cell)和 BALB/c 小鼠中的存活率降低;同时,M5-90 BP26 缺失株引起抗布鲁氏菌特异性 IgG 应答,这暗示 BP26 抗原是区分自然感染和疫苗免疫的候选抗原^[64]。因此,羊种布鲁氏菌 BP26 被认为是一种潜在抗布病的新型疫苗候选物。此外,S19 BP26 缺失株没有改变菌株的生物学特性及亲本

菌株的免疫保护性^[65]。因此, BP26 被认为是目前最合适的布鲁氏菌疫苗标志物之一。

Thavaselvam 等基于重组布鲁氏菌 BP26 建立了一种高灵敏度和特异性的 iELISA 方法, 从收集的血清优化微量滴定板和斑点印迹形式的 iELISA 显示, 重组 BP26 仅与已证实的阳性样品反应, 而与阴性样品没有反应, 证实该重组蛋白具有较强的免疫原性^[66]。此外, Wu 等采用 iELISA 方法评估了布鲁氏菌外膜蛋白 BP26 与 OMP31 和 OMP2b 作为联合诊断抗原的检测效果, 结果显示其灵敏度和特异性分别为 85.5% 和 93.2%, 该抗原组合不仅提高了检测布病的有效性, 而且可作为人布病血清学检测的诊断抗原^[67]。

SP41 是分子质量为 41 kDa 的布鲁氏菌表面蛋白, 易被宿主免疫系统识别, 具有作为布鲁氏菌免疫原性优势抗原的能力。研究发现, 布鲁氏菌 SP41 缺失株比亲本菌株具有更低的黏附性和侵袭性, 表明 SP41 可介导布鲁氏菌与宿主细胞的黏附^[68], 是布鲁氏菌的重要的毒力因子。此外, SP41 是目前唯一明确的与布鲁氏菌黏附和侵袭宿主细胞有关的蛋白。Al-Mariri 等用 pCIp39 和 pCIsp 41 DNA 疫苗免疫 BALB/c 小鼠, 可引发 T 细胞的增殖及产生高水平的 IFN- γ , 表明该二价苗可诱导机体产生 Th1 型细胞免疫应答^[69]。因此, SP41 可能成为开发布鲁氏菌 DNA 疫苗及预防布病的新靶点。表 1 对上述提到的布鲁氏菌外膜蛋白进行分类及作用总结, 以便理解。

表 1 布鲁氏菌外膜蛋白分类及作用

Table 1 The classification and function of *Brucella* outer membrane protein

分类	名称	作用	参考文献
Classification	Name	Function	Reference
脂蛋白 Lipoprotein	OMP10	毒力因子、诊断抗原、激活细胞/体液免疫	[19,22]
		Virulence factor, diagnostic antigen, activation of cellular/humoral immunity	
	OMP16	必需基因、佐剂活性、激活细胞/体液免疫	[24,27-28]
		Essential gene, adjuvant activity, activation of cellular/humoral immunity	
	OMP19	毒力因子、诊断抗原、佐剂活性、激活细胞/体液免疫	[35,39]
		Virulence factor, diagnostic antigen, adjuvant activity, activation of cellular/humoral immunity	
孔道蛋白 Porin protein	OMP22	毒力因子、诊断抗原	[44-45]
		Virulence factor, diagnostic antigen	
	OMP25	毒力因子、激活/抑制细胞免疫	[46-47]
		Virulence factor, activation/inhibition of cellular immunity	
	OMP31	毒力因子、诊断抗原、激活细胞/体液免疫	[51-53]
		Virulence factor, diagnostic antigen, activation of cellular/humoral immunity	
	OMP2a	诊断抗原	[59]
		Diagnostic antigen	
	OMP2b	诊断抗原、激活细胞/体液免疫	[58,60]
		Diagnostic antigen, activation of cellular/humoral immunity	
其他外膜蛋白 Other outer membrane proteins	BP26	毒力因子、诊断抗原、激活细胞/体液免疫	[64,66]
		Virulence factor, diagnostic antigen, activation of cellular/humoral immunity	
	SP41	毒力因子、激活细胞免疫	[68-69]
		Virulence factor, activation of cellular immunity	

9 展望

布病是由布鲁氏菌引起的人畜共患传染病,目前,世界范围内仍无获得批准的预防人类布病疫苗,而动物布病预防主要靠疫苗免疫为主^[70]。现阶段我国预防动物布病常用的疫苗是牛种布鲁氏菌 A19 疫苗、猪种布鲁氏菌 S2 疫苗和羊种布鲁氏菌 M5 疫苗,尽管这些疫苗在预防和控制布病方面发挥了重要作用,但仍然存在诸多问题亟待解决,如:(1) 具有残余毒力;(2) 可引起怀孕动物流产;(3) 无法区分自然感染与疫苗免疫的问题。因此,有必要寻找更安全、有效、敏感、特异的新型疫苗对布病进行诊断及早期预防。近年来,利用与布鲁氏菌毒力及免疫原性密切相关的外膜蛋白进行减毒活疫苗、亚单位疫苗、重组蛋白疫苗、载体疫苗等的研制成为热点。目前,开发布鲁氏菌新型疫苗的最佳方法是基于毒力基因缺失设计的减毒活疫苗,它们不仅可以促进 T 细胞、促炎细胞因子和抗体的产生,而且与传统减毒活疫苗相比其安全性更高、保护效果更好^[71]。因此,基于毒力基因缺失的减毒活疫苗被认为是一种很有前景的布鲁氏菌疫苗。

针对目前布鲁氏菌疫苗所存在的上述问题,作者在前期构建了一系列的布鲁氏菌基因缺失株,包括外膜蛋白、转录调控因子和 GTP 酶等。OMP16 条件缺失株毒力降低且免疫原性增强,且针对 OMP16 构建了 iELISA 的方法,为布病新型疫苗研发提供候选靶标及布病检测提供一种高灵敏度的技术方法^[24-25,72-73]。布鲁氏菌转录调控因子 ArsR6 调控外膜蛋白 OMP25d 的表达并参与布鲁氏菌毒力^[16,74]。转录调控因子 GntR 在布鲁氏菌感染前期促进 TNF- α 的分泌,但 GntR 通过何种途径影响 TNF- α 的分泌有待后续的研究^[75]。后续作者将继续探究这些基因对布

鲁氏菌毒力及免疫原性的影响,并优化实验方案,通过联合缺失等技术方法,进一步降低现有疫苗毒力,增强免疫原性,从而为新型生物安全疫苗研发奠定基础。

外膜蛋白是布鲁氏菌重要的毒力因子,具有较强的免疫原性,可作为布鲁氏菌减毒活疫苗、亚单位疫苗和口服疫苗等新型疫苗候选靶点,同时也可作为血清学诊断的抗原。此外,由于单个布鲁氏菌外膜蛋白免疫保护有限,多组分联合亚单位疫苗已成为研制布鲁氏菌新型疫苗的热点,但仍处于实验室阶段。相信在不久的将来新型疫苗会被研发出来并取代传统疫苗来预防和控制布病。

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