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动物细小病毒新型疫苗研究进展

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摘 要: 细小病毒是动物病毒中最小最简单的一类单链线状DNA病毒, 其在自然界的宿主范围广, 感染后呈长期隐性感染, 对畜牧养殖业有重要影响。目前, 对细小病毒的防治仍以接种减毒疫苗和灭活疫苗为主, 但它们仍存在一些不足, 如: 灭活疫苗成本高; 减毒疫苗存在返强的可能性。因此, 研制更安全、高效、制备方便的新型疫苗是未来研究的趋势。本文介绍了新型疫苗的发展概况以及近年来细小病毒新型疫苗的研究进展, 以期为细小病毒新型疫苗的研究及应用提供参考。

关键词: 细小病毒; 新型疫苗; DNA疫苗; 亚单位疫苗; 重组活病毒载体疫苗

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Progress in Development of Novel Vaccines Against Parvoviruses

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Abstract: Parvoviruses are the smallest and simplest single-stranded linear DNA viruses among animal viruses. They have a wide host range in nature, causing a long-lasting and inapparent infection and having a big impact on livestock farming. The current commercial vaccines, including inactivated vaccines and attenuated live vaccines, exhibit some defects. The former is costly, and the latter may have a risk of reversion to virulence. Thus, novel vaccines that are safer, more efficient and easier to prepare should be developed in future research. Herein, an overview of novel vaccines and the recent progress in parvovirus vaccine are provided, which would be helpful to further research and application of vaccines against parvoviruses.

Key words: parvovirus; novel vaccine; DNA vaccine; subunit vaccine; recombinant vector vaccine

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细小病毒是目前动物病毒中最小最简单的一类单链线状DNA病毒, 无囊膜, 直径18~26 nm, 病毒粒子(virus particle, VP)具有二十面体对称性。细小病毒科(Parvoviridae)多数成员只引起轻微感染或不引起严重的疾病, 所以并没有引起人们足够的重视。

细小病毒在细胞核内繁殖, 依靠宿主细胞进行复制。细小病毒在自然界的宿主范围非常广, 主要有猫、狗、牛、羊、鹅、猪、水貂等[1], 多数病毒感染宿主后呈长期潜伏感染状态, 是影响畜牧养殖业和小动物健康的一类重要病毒。对该类病毒感染的防治目前仍以接种减毒疫苗和灭活疫苗为主, 但这些疫苗存在一些缺陷。例如: 有一种应用于预防猪细小病毒的灭活疫苗, 它虽然能预防猪细小病毒的感染、发病, 但接种疫苗的母猪并不能避免异源病毒的感染以及排毒, 即使面对同源病

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毒的攻击也不能阻止排毒,这意味着免疫疫苗后的猪仍能携带、传播病毒,这对养殖产业来说仍是一个隐患[2]。同时,灭活疫苗还有制备成本较高、诱导产生抗体慢、不能引起细胞免疫且需要反复接种来避免效果不稳定的可能性等缺陷。减毒疫苗则存在毒力返强的可能性,并且需要低温储存运输等。因此,寻找和发展更加安全有效的疫苗是对细小病毒病免疫预防的重要研究主题。本文对近年来开发的各种细小病毒新型疫苗及新型疫苗的发展进行总结,以期为细小病毒新型疫苗的深入研究及应用于实际生产提供参考。

1　新型疫苗发展概述

随着基因工程技术的发展,研究者们可构建仅含抗原表位部分的表达载体或能表达异源病毒抗原表位的重组病毒,以此为基础开发的新型疫苗相较于传统疫苗具有安全性更好、研制周期更短的优点,可以更快进入临床试验阶段。目前,新型疫苗主要有 DNA 疫苗、亚单位疫苗和重组活病毒载体疫苗。

1.1　DNA 疫苗

核酸疫苗又称基因疫苗,由 Wolff 等[3-4]在 1990 年偶然发现。研究者们通常将抗原表位基因和调控基因克隆到真核表达载体,并将重组质粒直接免疫动物。疫苗进入机体后,在内质网腔与 MHC Ⅰ或Ⅱ类分子结合形成抗原肽 MHC 分子复合物,经 $CD8^+$或 $CD4^+$细胞识别,诱发细胞和体液免疫应答[5~6]。由于质粒的构建是基因工程的成熟技术,所以常规 DNA 疫苗的生产工艺简单且成本低;并且,核酸疫苗仅含抗原表位基因,因此不存在毒力返强的可能性。但是,常规 DNA 疫苗进入细胞的效率往往较低,使得机体免疫应答程度不如传统的减毒疫苗和灭活疫苗,因此研究者们为了提高其免疫效果提出了一些解决方案,如进行密码子优化[7]、利用脂质体为载体进行免疫[8]、实施电穿孔免疫、添加分子佐剂[9]、采用 prime-boost 免疫策略[10~13]等。随着研究的深入,在常规 DNA 疫苗和 RNA 复制子疫苗的基础上研究者们发展出了复制型 DNA 疫苗,DNA 复制子疫苗以具有“自主复制”功能的 RNA 病毒——主要是甲病毒如辛德比斯病毒(Sindbis virus, SBV)、塞姆利基森林病毒(Semliki Forest virus, SFV)等为基础构建而成,包含病毒基因组 5′和 3′末端的顺式作用序列,以及复制酶编码基因的全部非结构蛋白(nonstructural protein, NSP)基因编码区。复制型 DNA 疫苗在复制过程中产生的双链 RNA (double-stranded RNA, dsRNA)能刺激干扰素的产生,增强宿主体液免疫和细胞免疫反应[14],同时能诱导细胞凋亡并最终被机体清除[15]。所以,以此为基础的复制型DNA 疫苗又被称作“自杀性 DNA 疫苗”。猪瘟病毒、牛病毒性腹泻病毒、传染性法氏囊病病毒、狂犬病毒、伪狂犬病毒、口蹄疫病毒等[10, 14, 16~20]常见动物疫病病毒的复制子疫苗的广泛应用显示了其在兽医领域的巨大潜力。

1.2　基因工程亚单位疫苗

基因工程亚单位疫苗是将病毒、致病菌或寄生虫的抗原表位基因与合适的载体结合后在受体(细菌、酵母或动物细胞)中表达,可直接免疫接种或收集表达产物并加入佐剂后再进行免疫的一类疫苗。亚单位疫苗不含有病原体的其他遗传信息,因此具有安全性好的优点。但重组亚单位疫苗缺乏抗原识别需要的病原体相关的分子模式,导致这类疫苗的免疫原性通常比完整病原体差[21~22],需要通过多次免疫、加入佐剂或者使用其他能增强免疫反应的物质来增强免疫效果,才能使机体获得有效保护[23~24]。常规佐剂有铝佐剂[25]、弗氏佐剂[26]等。为进一步发展亚单位疫苗,研究者们尝试使用新型佐剂,如皂甙[27]、含 CpG 基序的寡聚脱氧核苷酸(oligodeoxynucleotides containing CpG motifs, CpG ODNs)[28]、白介素-2 (interleukin-2, IL-2)[29]等。另外,病毒的衣壳蛋白一般具有天然的自我装配能力[30],由其组装而成的介于 15~400 nm 的病毒样颗粒(virus-like particle, VLP)在形态上与真正的病毒粒子相似,可通过和病毒感染一样的途径呈递给免疫细胞,从而有效诱导机体产生免疫保护反应[31],具有开发成为病毒亚单位疫苗的可能性。目前,相关研究已通过细菌表达系统、酵母表达系统、杆状病毒-昆虫细胞表达系统、植物表达系统或哺乳动物表达系统产生猪圆环病毒[32]、口蹄疫病毒[33]、寨卡病毒[34]等多种病毒的 VLPs, VLP 是未来亚单位疫苗的热点研究方向。

1.3　基因工程活病毒载体疫苗

基因工程活病毒载体疫苗是利用基因操作技术将异源性病毒的保护性抗原基因及启动子序列插入到作为载体的另一种病毒如减毒疫苗株的基因组非必需区中而构建的疫苗[35]。由于异源病毒基因已成为载体病毒基因组的一部分,可随载体病毒的繁殖而不停的表达,所以这种疫苗在机体

可同时诱导产生针对载体病毒及异源性病毒的特异性免疫反应, 可达到一针防两病或多病的目的[36~38]。目前, 重组病毒构建时人们主要通过同源重组、fosmid 多片段拯救系统或细菌人工染色体(bacterial artificial chromosome, BAC)系统编辑病毒载体基因组, 编辑后的病毒能正常复制, 免疫原性不受影响, 因此该类疫苗通常不需要再添加佐剂, 但选择插入位点时需要考虑该位点对载体病毒的复制能力及免疫原性的影响, 其次还要考虑插入基因的表达情况, 包括表达盒元件启动子、增强子、起始密码子和终止密码子等影响因素。目前, 常用的病毒载体主要有痘病毒、腺病毒、新城疫病毒(Newcastle disease virus, NDV)和杆状病毒。

2 细小病毒新型疫苗的免疫效果

细小病毒的基因组包含两个开放阅读框(open reading frame, ORF), 左侧 ORF 编码与病毒复制和细胞凋亡有关的非结构蛋白; 右侧 ORF 编码与病毒粒子形成有关的结构蛋白, 又称衣壳蛋白。衣壳蛋白决定着病毒的吸附与进入、胞内转运和定位、外排及其在环境中的稳定性, 含有抗原表位[1], 因此是研制细小病毒新型疫苗的靶抗原。目前, 国内外学者以细小病毒衣壳蛋白为基础进行了各种新型疫苗的研究, 探究了各新型疫苗免疫预防细小病毒的效力(表 1)。

2.1 DNA 疫苗

韩新锋等[39]以鹅细小病毒(goose parvovirus, GPV)-CHv 株 *VP3* 为靶基因, 构建含 *VP3* 基因的 pcDNA3.1(+)真核表达载体, 并以此为基因疫苗肌肉注射小鼠和鹅, 结果显示, 接种后 30 d 开始能在小鼠和鹅体内检测到中和抗体, 第 90 天中和抗体水平达到峰值, 105 d 后仍能检测到中和抗体。王印等[40]将猪细小病毒(porcine parvovirus, PPV) *VP2* 基因重组到真核表达载体 pcDNA3.1(+), 并将重组质粒肌肉注射 BALB/c 小鼠, 结果显示: 重组质粒能诱导小鼠产生 PPV 特异性抗体, 但抗体水平略低于 PPV 减毒疫苗组; 重组质粒诱导淋巴细胞增殖能力与减毒疫苗接近, 并且重组质粒组小鼠在免疫后 $CD8^+$细胞数量显著增加, 说明其能有效诱导机体产生免疫反应。需要指出的是, 该重组质粒组小鼠在免疫后 $CD4^+$细胞数量明显低于空白对照组, 具体原因有待进一步探究。此外, 该研究团队在首免 56 d 后对小鼠实质器官的总 DNA 进行了 PCR 检测, 未扩增出 *PPV-VP2* 片段, 表明 *VP2* 基因未整合到小鼠染色体上, 该核酸疫苗无基因整合的风险。孙彦欣等[41]将猪圆环病毒(porcine circovirus type 2, PCV2)中编码 T 细胞表位 P21 多肽的基序与 *PPV-VP2* 基序的 5′端相连插入 pcDNA3.1(+), 并将重组质粒肌肉注射 BALB/c 小鼠, 发现重组质粒能有效诱导小鼠产生 PPV 和 PCV 特异性抗体, 且对淋巴细胞的增殖反应有明显促进作用。Wu 等[42]首先构建了表达GPV-VP2 的重组质粒 pTCY-VP2, 同时通过杆状病毒表达系统获得了重组蛋白 rVP2, 随后以 CpG ODNs 作为佐剂, 在 pTCY-VP2 初次免疫鸭 14 d 后实施 pTCY-VP2 或 rVP2 加强免疫。结果显示, 与空白对照组相比, 两种免疫方案均显著提高了鸭体内特异性抗体的滴度、淋巴细胞增殖指数、$CD4^+$和 $CD8^+$细胞百分比以及 α 干扰素(interferon-α, IFN-α)、IFN-γ、IL-6 和 IL-12 的转录水平。此外, 与 pTCY-VP2+pTCY-VP2 同源 prime-boost 方案相比, pTCY-VP2+rVP2 异源 prime-boost 方案在加强免疫两周后能更显著提高机体外周血淋巴细胞的增殖能力、$CD4^+$和 $CD8^+$细胞百分比、细胞因子的转录水平。

Dahiya 等[43]将犬细小病毒(canine parvovirus, CPV) *VP2* 插入包含 NSP1~4 的 SBV 复制子[14]并转化至 DH5α 大肠杆菌, 随后口服免疫犬只, 初次免疫 21 d 后进行 1 次加强免疫。结果显示: 接种商品化灭活疫苗和复制型 DNA 疫苗后机体淋巴细胞的增殖反应均明显增强, 且复制型 DNA 疫苗接种 21 d 后机体内特异性抗体水平和中和抗体水平均高于商品化灭活疫苗组; 此外, 在体外用灭活 CPV 抗原刺激淋巴细胞后, 复制型 DNA 疫苗组 $CD4^+$和 $CD8^+$淋巴细胞增加的数量高于商品化灭活疫苗组两倍, 表明接种该 DNA 疫苗后犬体内产生了致敏淋巴细胞, 对病毒攻击能更快激发免疫应答, 因此口服复制型 DNA 疫苗接种可能是犬类疫苗接种的有效替代策略, 是一种有潜力的犬类大规模疫苗接种方法。

2.2 基因工程亚单位疫苗

Lee 等[44]利用杆状病毒表达系统表达重组的鸭细小病毒(duck parvovirus, DPV) VP2 蛋白(rVP2), 并将其作为抗原, 以 AI-gel 或 CpG-ODNs 为佐剂免疫母鸭, 初次免疫 14 d 后加强免疫。结果显示, 免疫含 CpG 2 佐剂的 rVP2 的母鸭所孵出的雏鸭的母源抗体水平明显高于其他组且能维持高抗体滴度至少 28 d, 同时 DPV 攻毒后雏鸭不产生特

征病变, 体内免疫器官检测不到病毒。

丁轲等[45]构建了表达 GPV-VP3 的重组乳酸杆菌质粒 pMJ-SP-GPV-VP3, 同时将能增强机体细胞免疫和体液免疫反应的 IL-2[46]与 VP3 串联构建了重组乳酸杆菌质粒 pMJ-SP-GPV-VP3-gIL2, 将两种重组乳酸杆菌质粒转化至乳酸杆菌后口服免疫雏鹅。结果显示, pMJ-SP-GPV-VP3-gIL2 免疫组的保护率、血清 GPV 中和抗体效价和小肠黏膜 sIgA 水平均比 pMJ-SP-GPV-VP3 免疫组高, 但比减毒疫苗组低, 提示重组乳酸杆菌质粒 pMJ-SP-GPV-VP3-gIL2 有开发成口服疫苗的潜力。Xu 等[47]构建了重组乳酸杆菌共表达 PPV-VP2 蛋白和猪瘟病毒(classical swine fever virus, CSFV)细胞毒性 T 淋巴细胞(cytotoxic T lymphocyte, CTL)表位 290 基因, 并在无佐剂的情况下对 2~3 个月大的无 PPV 和 CSFV 母源抗体的仔猪进行口服免疫, 结果显示: 重组乳酸杆菌能引起机体内 CTL 产生强烈的免疫应答; 能诱导机体发生黏膜免疫反应, 并产生高水平的黏膜 IgA, 表明其对 CSFV 攻毒能提供有效的保护。乳酸杆菌是一种肠道益生菌, 重组乳酸杆菌在肠道中的定植能延长其潜在的对健康有益的作用, 并且口服免疫能引起黏膜免疫反应, 有利于增强该类疫苗的免疫效果。

Ju 等[48]利用杆状病毒表达系统在昆虫细胞中分别表达 GPV 的 VP1、VP2 和 VP3 重组蛋白。电镜观察结果显示, 重组蛋白(rVPs)能在昆虫细胞中自发组装成与天然 GPV 病毒颗粒相似的 VLPs。将不同 VLPs 加入矿物油佐剂后接种雏鹅, 结果显示, 每种 VLP 都能诱导机体产生高水平的中和抗体, 其中 rVP2-VLP、rVP3-VLP 诱导机体产生抗体的水平明显高于灭活疫苗组和减毒疫苗组。Chen 等[49]将野生株 GPV *VP2* 的密码子优化为昆虫细胞中更常见的密码子, 利用昆虫杆状病毒表达系统产生 VLPs, 收集 VLPs 后加入矿物油佐剂并免疫雏鹅, 发现其可诱导机体产生高水平的中和抗体。Xu 等[50]在大肠杆菌中表达融合了小分子泛素相关修饰物蛋白(small ubiquitin-related modifier protein, SUMO)的 CPV-VP2 蛋白, SUMO 裂解后 VP2 蛋白自组装成 VLPs, 将这些 VLPs 皮下接种小鼠后发现, 其能有效诱导小鼠产生高水平的中和抗体, 促进淋巴细胞增殖。Nan 等[51]将序列优化后的 CPV-VP2 克隆到 pET-30a 载体并转化至能表达触发因子的 BL21(DE3)进行共表达, 发现获得的 VLPs 能诱导豚鼠产生高水平的中和抗体、IFN-γ 和 IL-4。

2.3　基因工程活病毒载体疫苗

细小病毒基因组较小, 为 4.5~5.5 kb, 因此人们通常选择其结构蛋白基因作为插入基因构建重组病毒。Wang 等[52]将 *GPV-VP3* 基因重组到对 F 蛋白进行修饰致弱[53~55]的新城疫病毒(NDV) NA-1 型菌株构建 rmNA-VP3, 将该活病毒载体疫苗接种雏鹅后发现, 雏鹅无明显病征, 疫苗能诱导机体产生高水平的 GPV 和 NDV 中和抗体, 且高水平的中和抗体能维持至少 12 周而不出现明显下降。此外, 进一步的研究发现, 重组病毒在鸡胚中连续传代 10 次后 F 蛋白修饰位点和插入的 *VP3* 基因仍然很稳定, 提示该重组病毒活载体疫苗的遗传稳定性良好。陈柳等[56]将 GPV-VP2 表达框插入鸭肠炎病毒(duck enteritis virus, DEV) *US7* 和 *US8* 基因之间构建重组病毒 rDEV-VP2, 并将重组病毒感染鸡胚成纤维细胞(chicken embryo fibroblast, CEF), 结果显示其增殖滴度与亲本株无显著差异, 且在 CEF 中能正常表达 GPV-VP2 蛋白; 进一步将重组病毒接种 7 日龄雏番鸭, 结果显示其能诱导机体产生 GPV-VP2 抗体, 3 周后抗体阳性率为 50%, 但免疫保护力有待进一步研究。Luo 等[57]使用基于 HEP-Flury 株狂犬病毒(rabies virus, RABV)改造的携带双糖蛋白(G)基因的狂犬病毒 rHEP-dG 和 RAVB HEP-Flury 株, 构建表达 CPV-VP2 蛋白的两种重组病毒 rHEP-dG-VP2 和 rHEP-VP2, 发现两种重组病毒的增殖速率均高于亲本株, 同时两种重组病毒均能诱导小鼠产生高水平的犬细小病毒和狂犬病毒特异性抗体, 并且免疫保护率均超过 90%。

3　总结与展望

通常, 一款兽用疫苗的研制程序需要经历毒种分离、种子批建立与鉴定、培养材料选择、生产工艺(灭活、冻干)选择、效果评价、试制及临床试验等过程, 新型疫苗相较于传统疫苗最大的优势就是利用成熟的技术直接构建疫苗, 免去了毒种筛选、分离和鉴定的过程, 开发时间更短; 同时, 没有致病基因存在, 安全性更好。目前, 国内外已有许多种病毒的新型疫苗成功商品化, 但针对细小病毒的新型疫苗尚未成功商品化, 国内近期只有两种猪细小亚单位疫苗处于临床试验阶段。不过, 目前处于研制阶段的各种细小病毒新型疫苗

表 1 细小病毒新型疫苗
Table 1 Novel vaccines against parvoviruses

Target virus	Type of vaccine	Adjuvant and immunization strategy	Immune response and efficacy	Reference
GPV	DNA vaccine	–	Virus-neutralizing antibodies remained high until 105 days	[39]
GPV	DNA vaccine	CpG ODNs; prime-boost	Increased the titer of antigen-specific antibodies, lymphocyte proliferation index, percentage of $CD4^+$ and $CD8^+$ cells	[42]
GPV	Subunit vaccines	IL-2; prime-boost	Induced higher titers of virus-neutralizing antibodies, effective protection against GPV challenge	[45]
GPV	Subunit vaccines	Mineral oil emulsions	Induced higher titers of virus-neutralizing antibodies	[48]
GPV	Subunit vaccines	Mineral oil emulsions; prime-boost	Induced higher titers of virus-neutralizing antibodies	[49]
GPV, NDV	Recombinant viral vector vaccines	Prime-boost	Induced higher titers of virus-neutralizing antibodies	[52]
GPV, DEV	Recombinant viral vector vaccines	–	Induced specific antibodies	[56]
CPV	DNA vaccine	Prime-boost	Induced higher titers of virus-neutralizing antibodies, increased lymphocyte proliferation	[43]
CPV	Subunit vaccines	Prime-boost	Induced higher titers of virus-neutralizing antibodies	[50]
CPV	Subunit vaccines	Oil emulsions; prime-boost	Induced higher titers of virus-neutralizing antibodies	[51]
CPV, RABV	Recombinant viral vector vaccines	–	Induced higher titers of antibodies, effective protection against RABV challenge	[57]
PPV	DNA vaccine	Prime-boost	Induced specific antibodies, increased lymphocyte proliferation	[40]
PPV, PCV	DNA vaccine	Prime-boost	Induced higher titers of antibodies, increased lymphocyte proliferation	[41]
PPV, CSFV	Subunit vaccines	Prime-boost	Induced higher titers of virus-neutralizing antibodies, effective protection against CSFV challenge	[47]
DPV	Subunit vaccines	AI-gel, CpG-ODNs; prime-boost	The titer of maternal VP2 specific antibodies remained high until 28 days	[44]

大部分能诱导机体产生高水平的中和抗体，保护动物免受强毒的攻击，显示出了继续开发的潜力。核酸疫苗和亚单位疫苗的主要优势在于仅含能诱导机体产生免疫反应的基因，对机体无致病性，比较安全，但核酸疫苗所用的载体可能存在对机体有害的隐患，而亚单位疫苗大多情况下都需要加入佐剂等才能增强免疫反应以提高免疫原性，因此开发新型佐剂、优化表达载体是发展这两种疫苗所需解决的关键问题。重组活病毒载体疫苗的主要优势是与自然病毒感染刺激机体产生免疫应答的效果接近，不需要为了加强免疫原性而再进行处理。另外，细小病毒重组活载体疫苗在开发时通常选择同样对该种动物危害大的病毒作为载体，具有病毒载体容量更大、能容纳多个异源片段、一苗防多病的优势。当然，该类疫苗也还存在一些问题有待进一步研究和解决，比如：使用鸭瘟病毒这类研究还不够全面的载体病毒时，插入位点对外源基因表达和抗原递呈的影响、外源基因对载体病毒免疫原性的影响等问题是未来研究的关键。总之，各种新型疫苗各有优劣，在开发新型疫苗时可以根据实际情况选择合适的疫苗类型及疫苗配方，未来对于细小病毒的防控还可以从疫苗免疫途径及免疫周期等与免疫程序相关的方面进行探索，找到更理想、更有效、更适合于生产实际的疫苗及免疫程序。

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