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• 综述 •

重组狂犬病毒疫苗的研制现状*

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【摘要】 狂犬病是一种由狂犬病毒(*Rabies virus*, RABV)感染导致的急性中枢神经系统病变的病毒性传染病,可用 RABV 的减毒株进行免疫预防。这些减毒株可诱导宿主产生细胞、体液和粘膜免疫应答,是一种理想的疫苗载体,可表达病毒、细菌和寄生虫的多种蛋白。这些疫苗包括 I 型人类免疫缺陷病毒(rRABV-gag/env)、埃博拉病毒(rRABV-GP)、尼帕病毒(rRABV-G/F)、西尼罗病毒(rRABV-prME)、丙型肝炎病毒(rRABV-E2^Δ CD4G)、水疱性口炎病毒(rRABV-GP)、淋巴细胞脉络膜炎病毒(rRABV-GPC)、犬瘟热病毒(rRABV-H/F)、猪细小病毒(rRABV-VP2)、狂犬病毒(rRABV-tRVG)、细菌(rRABV-Fla)和细粒棘球绦虫(rRABV-Eg95)等,本文就重组 RABV 疫苗的研制现状进行概述。

【关键词】 狂犬病毒;疫苗;综述

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The status in the research of recombinant *Rabies virus* vaccine

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【Abstract】 *Rabies* caused by *Rabies virus* is one type of zoonotic, infectious diseases of central nervous system. it may be controlled by immunoprophylaxis with attenuated RABV strains, this strains may induce the host to produce cellular, humoral and mucosal immune responses, it may become ideal vaccine vector for expressing many proteins of viruses bacteria and patasites. These vaccines include *Human immunodeficiency virus* (rRABV-gag/env), *Ebola virus* (rRABV-GP), *Nipah Virus* (rRABV-G/F), *West Nile virus* (rRABV-prME), *Hepatitis C virus* (rRABV-E2^Δ CD4G), *Vesicular stomatitis virus* (rRABV-GP), *Lymphocytic choriomeningitis virus* (rRABV-GPC), *Canine distemper virus* (rRABV-H/F), *Porcine parvovirus* (rRABV-VP2), *Rabies virus* (rRABV-tRVG), *Bacteria* (rRABV-Fla) and *Echinococcus granulosus* (rRABV-Eg95). The review outlines the status in the research of recombinant *Rabies virus* vaccine.

【Keywords】 *Rabies virus*; vaccine; review

***狂犬病是一种由狂犬病毒(*Rabies virus*, RABV)感染导致的急性中枢神经系统病变的病毒性传染病,可用 RABV 的 ERA 株、SAD 株和 SAG2 株等减毒株进行免疫预防。狂犬病毒的糖蛋白(*Rabies virus* glycoprotein, RVG)是一种典型的跨膜糖蛋白,能与乙酰胆碱受体结合使病毒具有神经毒性。通过多位点变异或基因编辑技术可减弱 RABV 的毒性,使其成为疫苗载体^[1-4]。pHEP-GFP、pCW111-GFP、pRV-eGFP 和 pBSK-GLuc 能表达绿色荧光蛋白(GFP)、增强型绿色荧光蛋白(EGFP)或高斯荧光素酶(Gluc),这为制备重组狂犬病毒提供了有利的工具^[5-7]。

目前许多重组 RABV 疫苗已制备,这些疫苗包括 I 型人类免疫缺陷病毒(rRABV-gag/env)、埃博拉病毒(rRABV-GP)、尼帕病毒(rRABV-G/F)、西尼罗病毒(rRABV-prME)、丙型肝炎病毒(rRABV-E2^Δ CD4G)、水疱性口炎病毒(rRABV-GP)、淋巴细胞脉络膜炎病毒(rRABV-GPC)、犬瘟热病毒(rRABV-H/F)、猪细小病毒(rRABV-VP2)、狂犬病毒(rRABV-tRVG)、细菌(rRABV-Fla)和细粒棘球绦虫(rRABV-Eg95)等,本文概述 rRABV 疫苗的研制现状。

1 重组狂犬病毒抗病毒

1.1 I 型人类免疫缺陷病毒(HIV-1) HIV-1 是获得性免疫

缺陷综合征的病原体。HIV 的结构基因包括 gag、pol 和 env 基因。其中 Gag 基因编码核衣壳蛋白 P24、P7 和内膜蛋白 P14,env 基因编码一种前体蛋白 gp160,gp160 可分解为 gp120 和 gp41。这些蛋白的重组抗原或 DNA 疫苗可诱导恒河猴(*Macaca mulatta*)产生有效的免疫应答^[8-9]。将 Gag 基因克隆入表达载体 pSPBN^Δ CD333 得到重组质粒 pSPBN-Gag,将其转染 BSR T7 细胞,筛选培养后通过免疫印渍证实患者血清识别重组病毒表达的 Gag 蛋白;采取腹腔注射的途径将 3.4×10^8 FFU 重组病毒接种 BALB/c 鼠,在接种后 5 周显示血清的中和抗体提升,脾细胞 CTL 反应增强;此时将 10^7 FFU 重组 VV-Gag 株进行肌肉注射攻击,在攻击后 5 d 经 ELISPOT 显示脾 IFN- γ^+ SFC 的数目增多^[10-11]。

猴/人类免疫缺陷病毒(*Simian/human immunodeficiency virus*, SHIV89.6P)的基因组结构类似 HIV-1。将 rRABV-

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env/gag 疫苗肌肉注射恒河猴,在第1次接种后7和19周加强2次,在第1次接种后25周肌肉或静脉注射50MID₅₀的SHIV89.6P株进行攻击,在攻击后4周显示血清的病毒负荷显著减少;血清的中和抗体升高,滴度为1:1414,流式细胞仪显现外周血单核细胞(PBMC)的CD4⁺T细胞的数目增加,ELISPOT提示PBMC的IFN- γ ⁺SFC的数目增多^[12-13]。

1.2 埃博拉病毒(Ebola virus, EBOV) EBOV感染可诱发埃博拉出血热。将GP1/GP2蛋白的核酸疫苗接种小鼠可部分对抗EBOV的攻击^[14]。将 5×10^5 FFU重组EBOV-GP1/GP2疫苗肌肉注射BALB/c鼠,在第1次接种后2周重复1次,在第1次接种后4周显示血清IgG升高;此时将 1×10^7 FFU重组VV-GP株进行腹腔注射攻击,在攻击后5d经ELISPOT提示脾IFN- γ ⁺SFC增多^[15-16]。随后,将 5×10^5 FFU重组病毒加GLA-SE佐剂肌肉注射恒河猴,在第1次注射后6周重复1次,在第1次注射后10~12周显现血清的中和抗体升高;此时将 10^3 FFU埃博拉病毒CO5株或Mayinga株进行肌肉注射攻击,在攻击后4周发现免疫组的保护力可达100%^[17-18]。

1.3 尼帕病毒(NiV) NiV常诱发呼吸道感染,重症伴发病毒性脑炎。将糖蛋白(G)和融合蛋白(F)接种仔猪可诱导一定的保护力^[19]。将人工合成的G/F基因克隆入表达载体pCI-ERA333E得到重组质粒pERA333E-G/F,加辅助质粒转染HEK293T细胞,经过筛选培养后,使用免疫荧光证实重组病毒可表达融合蛋白的分子;借助口服途径将 $10^{6.5-8}$ FFU重组病毒免疫BALB/c鼠或仔猪,显现血清的中和抗体在第1次免疫后3~11周提升,在第1次免疫后9周达较高水平^[20]。

1.4 西尼罗病毒(WNV) WNV是西尼罗热的病原体,将前膜蛋白(prM)和包膜蛋白(E)聚合的异二聚体蛋白(prME)接种宿主可对抗WNV的攻击感染^[21-22]。将prME基因克隆入表达载体pSAETOPO得到重组质粒pSAETOPO-prME,加pH-N/P/L/G辅助质粒转染BHK-21细胞,经过筛选培养后,通过免疫荧光证实重组病毒可呈现融合蛋白的分子^[23],但未进行接种动物的免疫试验。

1.5 丙型肝炎病毒(HCV) 丙型肝炎是HCV感染引起的,E2糖蛋白存在大量B细胞和CTL表位,具有一定的免疫原性^[24]。E2 Δ CD4G是删除跨膜结构域CD4G的E2蛋白,将质粒pTM1-E2₆₆₁-CD4用作模板克隆E2 Δ CD4G基因,插入表达载体pSPBN得到重组质粒pSPBN-E2 Δ CD4G,加辅助质粒转染BSR-T7细胞,筛选重组病毒;将 10^7 FFU重组病毒腹腔注射BALB/c鼠,在初次注射后5周肌肉注射重组E2 Δ CD4G蛋白进行强化,显示血清的中和抗体在第1次注射后45d升高,脾CTL反应增强^[25]。

1.6 水疱性口炎病毒(VSV) VSV诱口水疱性口炎,将糖蛋白(glycoprotein, GP)接种宿主可抵抗VSV有毒株的攻击感染^[26]。采用质粒pVSV-XN1作为模板克隆G基因,插入质粒pSN得到重组质粒pSN-G,加辅助质粒转染BSR-T细胞,筛选重组病毒;借助肌肉注射途径将 10^6 FFU重组病毒接种BALB/c鼠,显现血清的中和抗体在初次注射后9d提升;此时通过肌肉注射途径将 5.5×10^6 FFU狂犬病毒CVS-N2C株进行攻击,在攻击感染后4周提示免疫组的小鼠全部死亡,表明该疫苗的免疫效果差^[27]。

1.7 淋巴细胞脉络膜炎病毒(LCMV) LCMV基因组的S节段基因编码糖蛋白前体(glycoprotein precursor, GPC),GPC可裂解为GP1和GP2,两者聚合为成熟的GP蛋白具有较好的免疫原性^[28]。将GPC基因插入p3.1-defp得p3.1-defp-GPC,加pH-N/P/L/G辅助质粒转化HEK293T细胞,筛选重组病毒;采取腹腔注射将 10^6 FFU重组病毒接种C57BL/6鼠,在初次接种后1周重复1次,显示血清的中和抗体在初次接种后1~7周提升,在初次接种后5周达较高水平;此时通过脑内注射途径将10 PFU的WE株进行感染攻击,在攻击感染后3周计算存活率,发现免疫组和对照组的存活率分别为88.2%(15/17/10)和10%(1/10)^[29]。

1.8 犬瘟热病毒(CDV) CDV是犬瘟热的病原体。血凝素H和融合蛋白F是主要的表面糖蛋白,CDV通过H蛋白吸附到细胞表面的受体上,起细胞趋向作用,而F蛋白介导CDV与感染细胞以及感染细胞与非感染细胞的融合,使CDV具有在宿主体内扩散的能力。将其DNA疫苗免疫幼犬可诱导较强的保护力^[30-31]。将H基因插入质粒pLBSNE得到pLBSNE-H,转化BSR细胞,筛选重组病毒;经肌肉注射途径将 10^6 FFU重组病毒免疫幼犬,在免疫后3周发现血清的中和抗体提升;此时采用滴鼻途径将 10^7 TCID₅₀犬瘟热病毒ZJ7株进行攻击感染,在攻击感染后2周显示血液的病毒负荷显著减少,脑和肺组织的病理显著减轻。随后,将重组病毒肌肉注射雪貂(*Mustella putorius furo*)也可完全对抗犬瘟热病毒5804PEH株的滴鼻攻击^[32-33]。

1.9 猪细小病毒(PPV) CPV诱发初孕母猪繁殖障碍综合症,将结构蛋白VP2免疫仔猪可诱导有效的免疫应答^[34]。将VP2基因克隆入质粒pHEP-Flury得到重组质粒pHEP-VP2,转染BSR细胞,筛选重组病毒;经肌肉注射途径将 10^5 FFU重组病毒接种昆明鼠,在初次免疫后3周发现血清IgG提升,滴度可达1:80;此时将50 MILD₅₀狂犬病毒CVS-24株进行脑内注射攻击,在攻击后3周发现免疫组产生80%(8/10)的保护力。重复实验表明重组病毒接种小鼠诱导的血清IgG滴度可达1:256,浓度>0.6 IU/mL^[35-36]。

1.10 RABV 狂犬病毒的糖蛋白(RVG)具有较强的免疫原性,将重组RVG抗原或核酸疫苗接种幼犬可产生中和抗体^[37-38]。tG是RVG的三聚体编码序列,将人工合成的tG基因克隆入质粒pS1-Ig得到重组质粒pS1-tG,加RABV减毒株转染HEK293T细胞,筛选重组病毒;将1 μ g重组病毒加Matrix-MTm佐剂肌肉注射Swiss鼠,在初次注射后28d重复1次,在初次注射后7周显示血清IgG升高,滴度可达1:12.56;此时经脑内注射途径将 10^4 TCILD₅₀狂犬病毒CVS-11株进行攻击感染,在攻击感染后4周发现脑组织的病毒负荷下降4个数量级;免疫组产生100%(10/10)的保护效果^[39]。

2 重组狂犬病毒抗菌

细菌鞭毛素(flagellin, Fla)是TLR5的配体,促进树突状细胞的成熟,是一种免疫佐剂,提高机体的免疫应答^[40-41]。将Fla基因克隆入质粒pLBNSE得到重组质粒pLBNSE-Fla,加pH-N/P/L/G辅助质粒转染BSR-T细胞,挑选重组病毒。采用口服或肌肉注射途径将 10^6 FFU重组菌苗接种ICR鼠,在第1次接种后3周强化1次,显示血清的中和抗体在第1次接种后4

周达较高水平;此时将 50 MLD₅₀ 狂犬病毒 CVS-24 株进行脑内注射攻击,在攻击后 3 周计数存活,结果表明口服组、肌肉注射免疫组和对照组的存活率分别为 80%(8/10)、70%(7/10)和 0(0/10)^[42]。

3 重组狂犬病毒抗细胞棘球绦虫

细胞棘球绦虫(Eg)的续绦期幼虫是囊型棘球蚴病的病原体。将 Eg95 抗原接种绵羊可对抗 Eg 虫卵的口服攻击,产生 96%~98%的减绦率^[43]。经肌肉注射途径将 $1 \times 10^{6.5}$ TCID₅₀ 重组 RABV-Eg95 疫苗免疫 BALB/c 鼠,在免疫后 2 周经 ELISPOT 显示 IFN- γ ⁺ 脾细胞和 IL-4⁺ 脾细胞数目增加;流式细胞仪提示脾 DC 细胞和 B 细胞数目增多;在免疫后 4 周显现血清的中和抗体和 IgG 升高;在免疫后 8 周采取肌肉注射途径将 100 MLD₅₀ 狂犬病毒进行感染攻击,在攻击感染后 3 周显示免疫组生存可产生 100%(8/8)的保护力^[44-45]。

4 结语

重组狂犬病毒具有自然靶向神经元的特性,是中枢神经系统感染治疗的优选载体之一;宿主范围广,能感染几乎所有的哺乳动物细胞,能诱导宿主产生较强的体液和细胞免疫应答,体内并不预先存在抗病毒的抗体,使用相对安全。在重组 RABV 的构建过程中要研究启动子和起始密码子的距离以及启动子之间相互作用可否影响外源基因的表达效率;挑选不同的启动子构建载体可否提高重组 RABV 的遗传稳定性;不同启动子的反向串联可否降低外源基因在转录水平上的干扰,提高外源基因的表达;重组 RABV 共表达细胞因子的探索;重组 RABV 共表达多种病原蛋白的探索;新型佐剂和疫苗载体的研制;这些研究可为重组 RABV 疫苗的研发提供理论基础。

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