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·综 述·

新型冠状病毒疫苗研究策略分析

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摘 要: 新型冠状病毒 (SARS-CoV-2) 是一种可引起人新型冠状病毒肺炎 (COVID-19) 的新发呼吸道病原体，与重症急性呼吸道综合症冠状病毒 (SARS-CoV) 和中东呼吸综合征冠状病毒 (MERS-CoV) 同属于 β-冠状病毒，具有较高的传染性和一定的致死率。2019 年 12 月在我国武汉被发现，随后蔓延到我国大部分省份，给我国人民健康和经济发展造成巨大损失。疫苗接种是预防和控制传染病的常规和有效手段，国内外多个机构已启动 COVID-19 疫苗研究工作。文中基于 SARS 和 MERS 疫苗研究的经验和教训，对 COVID-19 疫苗的研究策略和需要注意的关键问题进行了阐述，为相关研究人员提供参考。

关键词: 新型冠状病毒肺炎，疫苗，抗体，粘膜免疫，抗体依赖感染增强

Strategies for vaccine development of COVID-19

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Abstract: An epidemic of acute respiratory syndrome in humans, which appeared in Wuhan, China in December 2019, was caused by a novel coronavirus (SARS-CoV-2). This disease was named as “Coronavirus Disease 2019” (COVID-19). SARS-CoV-2 was first identified as an etiological pathogen of COVID-19, belonging to the species of severe acute respiratory syndrome-related coronaviruses (SARSr-CoV). The speed of both the geographical transmission and the sudden increase in numbers of cases is much faster than SARS and Middle East respiratory syndrome (MERS). COVID-19 is the first global pandemic caused by a coronavirus, which outbreaks in 211 countries/territories/areas. The vaccine against COVID-19, regarded as an effective prophylactic strategy for control and prevention, is being developed in about 90 institutions worldwide. The experiences and lessons encountered in the previous SARS and MERS vaccine research can be used for

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reference in the development of COVID-19 vaccine. The present paper hopes to provide some insights for COVID-19 vaccines researchers.

Keywords: COVID-19, vaccine, antibody, mucosal immunization, antibody-dependent enhancement

新型冠状病毒 (SARS-CoV-2) 于 2019 年 12 月初在湖北省武汉市被发现，呈现出高传染性和快速传播特点并可导致一定比例患者出现重症新冠肺炎 (COVID-19)[1-2]。2020 年 1 月 31 日世界卫生组织宣布 COVID-19 疫情为“国际关注的突发公共卫生事件”，于 2 月 28 日将疫情全球风险级别上调为“非常高”,并于 3 月 12 日将其定义为“大流行”。 COVID-19 在临床上表现为发热、干咳、呼吸困难、肌肉或关节痛、腹泻和肺炎，严重时可导致呼吸衰竭甚至死亡[3-4]。SARS-CoV-2 是继 SARS-CoV 和 MERS-CoV 之后第 3 个可引起人体严重急性呼吸窘迫综合征 (ARDS) 的冠状病毒，也是唯一造成全球大流行的非流感病毒。通过高效综合防控我国疫情已被有效控制，截至 2020 年 3 月 17 日，我国已报告 80 894 确诊病例和 3 122 死亡病例，感染人数和传播速度远超前两次冠状病毒疫情，是建国以来最为严重的一次重大突发公共卫生事件。研究证明，SARS-CoV-2 在人体内的病毒载量变化模式不同于 SARS-CoV，与流感病毒更相似[5],且感染性更强,因此 SARS-CoV-2 具有长期在人间存在的潜在风险。随着全球超过 90%的国家和地区出现病例，其已成为全球公共卫生机构的一个巨大挑战。

SARS-CoV-2 为单股正链 RNA 病毒，是第 7 个已发现可感染人的冠状病毒。COVID-19 致死率低于 SARS (9.6%) 和 MERS (34.4%)，但传染性强于两者，且存在一定数量的无症和轻症患者，因此防控难度也更大[6]。作为一种新发传染病，阻隔被证明是最有效的短期防控措施，但从长远来看，一种安全有效的疫苗对于控制疫情和防止再次暴发具有重要意义[7]。SARS-CoV-2 还没有获批的疫苗和治疗性药物，考虑到它与 SARS-CoV 和 MERS-CoV 同属 β-冠状病毒，与 SARS-CoV 同源性达 79.5%，且细胞受体与 SARS-CoV 同为血管紧张素转化酶 2(ACE2)[3]，因此前期 SARS 和 MERS 疫苗研究的经验可用于指导本次 COVID-19 疫苗的研发。多个不同的疫苗平台曾被用于 SARS 和 MERS 疫苗的研发，包括灭活全病毒疫苗、DNA 疫苗、亚单位疫苗、载体疫苗和减毒活疫苗，大部分尚处于临床前研究阶段，少数进入了临床试验阶段。选择一个适合 COVID-19 的疫苗研发策略将直接决定该疫苗研发的成败。

我国已对 COVID-19 的检测、疫苗和治疗性药物的研发进行了重要部署，多个研究机构在抓紧进行相关研究开发。本文基于前期 SARS 和 MERS 疫苗研究过程中积累的经验和教训，将从疫苗研发平台的选择、研发中需着重注意的几个关键问题入手，分析探讨 COVID-19 疫苗研发策略，旨在为正在进行疫苗研发的同仁提供一些参考。

1 疫苗研发平台

SARS 和 MERS 候选疫苗诱导的中和抗体滴度与试验动物肺部感染指数和存活率直接相关[8]，但仅靠血清中和抗体并不能产生足够保护[9-10]，细胞免疫对于病毒和感染细胞的清除起到至关重要的作用[11-12]，而且记忆性 T 细胞在 SARS 康复病人体内的维持时间明显长于中和抗体[13]，提示细胞免疫是实现疫苗长效保护不可或缺的。因此 COVID-19 疫苗的设计需兼顾体液免疫和细胞免疫。另外, COVID-19 主要通过呼吸道和接触传播[3]，因此粘膜免疫在防止病毒感染中的作用要引起足够重视。病毒包含 4 个结构蛋白：棘突蛋白 (Spike，S)、包膜蛋白 (Envelope，E)、膜蛋白 (Membrane/matrix，M) 和核衣壳蛋白(Nucleocapsid，N)，S 蛋白通过位于 S1 亚基上的受体结合区(Receptor-

binding domain，RBD) 与特异受体结合导致病毒感染细胞[14-16]，针对 S 蛋白的中和抗体可阻断这一过程从而防止病毒侵入[17]，S 蛋白还可有效刺激 T 细胞免疫应答[18]，因此是疫苗设计最重要的靶抗原，而 N 和 M 也被证明可诱导机体产生高效细胞免疫反应[19-21]。前期多个疫苗平台被用于 SARS 和 MERS 疫苗的研发，其中灭活病毒疫苗、DNA 疫苗和载体疫苗已进入临床试验[22-26]，尽管还没有疫苗上市，但依然为 COVID-19 疫苗的设计积累了丰富经验，如何选择适合 COVID-19 的研发平台是疫苗研发人员需要面临的首个问题。

1.1 灭活病毒疫苗

灭活疫苗是最为经典的疫苗形式，易于制备且能高效引起体液免疫应答，往往是新发传染病的首选疫苗方案。灭活疫苗主要通过甲醛、β-丙内酯和紫外 3 种灭活方式获得。SARS 和 MERS 灭活疫苗可引起小鼠、仓鼠、雪貂和猴子产生高滴度中和抗体[10,27-34]，而且 SARS 灭活疫苗已完成Ⅰ期临床试验，证明了在人体上是安全的，且能诱导生成中和抗体[23]。但是灭活疫苗引起的 T 细胞免疫应答普遍偏弱，前期有研究证明 SARS 和 MERS 灭活疫苗无法有效刺激机体产生细胞免疫应答[35-36]，即使产生高滴度血清中和抗体，保护效力也并不满意[10,28]，而且还有研究发现 MERS 灭活疫苗会导致小鼠肺部过敏性病理反应[37]，另外疫苗生产需要操作高浓度活病毒，具有一定的生物安全风险，因此该疫苗策略需谨慎考虑。

1.2 核酸疫苗

核酸疫苗包括 DNA 疫苗和 mRNA 疫苗两种。由于研发周期短，每当出现新发疫情时采用该策略可最快速地获得候选疫苗[40-43]。目前已有包括 SARS 和 MERS 在内的多个 DNA 疫苗进入临床试验阶段[24,26,44-45]，而且部分 DNA 疫苗已经上市，包括动物用流感、西尼罗病毒等[46]。相比 DNA 疫苗需要进入细胞核，mRNA 疫苗仅需进入细胞质即可实现靶抗原的表达，因此理论上更为安全。近些年 mRNA 疫苗得到迅速发展，狂犬病毒和流感病毒 mRNA 疫苗已完成Ⅰ期临床评价[45-46]，但免疫效果并不令人满意，接种人员出现了较高比例的头痛、疲劳和肌肉痛等副反应，疫苗生成的免疫保护在一年内即迅速下降，而且未检测到细胞免疫应答，因此还需进一步改善 mRNA 疫苗的免疫效力和长期保护力。截止目前还没有 mRNA 疫苗上市。SARS 和 MERS DNA 疫苗可使小鼠和猴子产生体液和细胞免疫反应[19-21,38-39,47-52]，而且临床试验证明在人体上也是安全有效的[22,24]。国内外多个机构已迅速启动了 COVID-19 DNA 疫苗和 mRNA 疫苗的研发工作，美国国家过敏和传染病研究所 (National Institute of Allergy and Infectious Diseases，NIAID) 与 Moderna 公司合作开发的 mRNA 疫苗已率先启动了Ⅰ期临床试验。DNA 疫苗可像病毒感染一样进入细胞利用宿主蛋白翻译系统生成靶抗原，作为一种内生免疫原可同时诱导体液和细胞免疫应答，而且生产成本低廉、容易量产、无需冷链运输，但相比蛋白疫苗方便的接种方式，DNA 疫苗的接种方式限制了其应用。接种 DNA 疫苗主要包括 3 种方式：直接肌肉注射、基因枪接种和电穿孔仪接种。尽管肌肉注射操作方便，但由于疫苗接种后主要分布于细胞间隙，仅有极少量能够进入胞内生成蛋白免疫原，因此免疫效果大打折扣。而基因枪和电穿孔仪接种增加了免疫成本，且临床试验报告显示受试者普遍反映痛感较高，因此当前 DNA 疫苗应用存在瓶颈。本团队前期在研究埃博拉和拉沙热病毒 DNA 疫苗过程中开发了一种无针皮下接种 DNA 疫苗的方式，免疫效果与基因枪和电穿孔仪接种无显著差异，且成本低廉易于推广。该技术可用于 COVID-19 DNA 疫苗的接种，从而打破 DNA 疫苗的接种瓶颈。尽管核酸疫苗可有效诱导全身性免疫应答，但由于免疫原性偏弱，且不易产生粘膜免疫应答，与其他疫苗联合使用会达到更好的免疫效果[53]。鉴于核酸疫苗的优势，

推荐其单独使用或与其他平台的疫苗联合使用。

1.3 亚单位疫苗

亚单位疫苗由纯化的重组蛋白构成，被认为是最安全的疫苗，目前已有多个亚单位疫苗上市，包括乙型肝炎、戊型肝炎和人乳头瘤病毒疫苗。SARS 和 MERS 亚单位疫苗可使小鼠产生高滴度中和抗体，通过鼻腔或口腔接种还可以诱导粘膜免疫反应，从而更有效地阻断病毒通过呼吸道传播，数据也证明了粘膜接种途径保护效力优于肌肉接种[54-68]。然而作为一种非内生抗原，亚单位疫苗不能通过 MHC-Ⅰ递呈，也就不能有效产生致敏细胞毒性 T 细胞 (CTL)，考虑到细胞免疫在清除冠状病毒感染中的关键作用，针对 COVID-19 的亚单位疫苗最好与其他平台疫苗配合使用，而且建议包含鼻腔、口腔粘膜接种途径以激活粘膜免疫应答。另外亚单位疫苗免疫原性相对较弱，将其设计成多聚体或病毒样颗粒 (Virus like particle，VLP) 结构可有效增强其免疫原性。合适的疫苗佐剂对于增强免疫效率也是至关重要的，铝佐剂是一种应用广泛的人用疫苗佐剂，但是该佐剂不能有效诱导 Th1 型免疫应答 (介导细胞免疫)，而 Th1 型免疫反应在清除病毒感染中处于重要地位。基于角鲨烯成分的佐剂，如 MF59、AS03、AF03，被证明可更均衡地诱导亚单位疫苗的体液及细胞免疫应答，而且能诱导更广泛的交叉反应[69]，相对更适合于 COVID-19 亚单位疫苗。

1.4 载体疫苗

载体疫苗是利用病毒或细菌为载体，将疫苗靶基因整合入载体基因组中制备的疫苗，分为复制型和非复制型两种。载体疫苗可感染细胞并在细胞质内表达靶抗原，因此可高效诱导机体产生体液及细胞免疫应答，由于具有天然粘膜嗜性，通过鼻腔或口腔接种还能诱导粘膜免疫反应，是近几年发展最为迅速的疫苗研发平台。有多个载体可供选择，包括腺病毒、水泡性口炎病毒、痘苗病毒、麻疹病毒、副流感病毒、新城疫病毒、狂犬病毒以及减毒沙门氏菌等。其中基于水泡性口炎病毒的埃博拉载体疫苗被用于非洲刚果金埃博拉疫情的防控，展示了良好的安全性和保护效力[70]，而我国也已批准了首个埃博拉腺病毒载体疫苗。基于多个不同载体研制的 SARS 和 MERS 疫苗可使小鼠、雪貂和猴子产生高效体液和细胞免疫应答，尤其是通过鼻腔或口腔接种可以诱导良好的粘膜免疫反应[10,71-95]。研究显示 SARS 腺病毒载体疫苗鼻腔接种产生的血清中和抗体水平低于肌肉接种途径，但由于可在粘膜表面生成高滴度 IgA 抗体，保护效力反而优于肌肉接种[10,73]，提示单靠高滴度血清中和抗体的保护效力是不完整的。两个分别基于痘苗病毒和腺病毒的 MERS 体疫苗也已完成Ⅰ期临床研究[25-26]，确认了其安全性和免疫原性。但是设计腺病毒载体疫苗时需着重考虑其预存免疫影响，建议选用在人群中低血清阳性率的人源 (如 Ad26、Ad35) 或非人灵长动物源腺病毒载体。另外采用多途径接种方式 (肌肉、鼻腔接种)，或增加接种剂量和次数在动物实验中证明可避开预存免疫影响，但在人体上还没有相关证据。总体来说，载体疫苗策略在 COVID-19 疫苗开发中具有较大优势。

1.5 减毒活疫苗

减毒活疫苗是一种通过病毒关键蛋白点突变或缺失引起病毒毒力降低，但不影响其免疫原性和复制能力的疫苗，该疫苗方案具有非常好的免疫原性，可诱导全身性免疫和粘膜免疫应答，且免疫力持久。已有多个减毒活疫苗上市，包括黄热病、天花、麻疹、脊髓灰质炎、腮腺炎、风疹、水痘等。SARS-CoV E 蛋白缺失减毒活疫苗证明可在小鼠和仓鼠上诱导产生体液和细胞免疫应答，而且可实现攻毒部分保护[96-101]，有研究人员通过缺失 E 蛋白或突变 NSP16 蛋白制备了 MERS 减毒活疫苗。然而，研究证明 SARS 减毒活疫苗在细胞或小鼠体内连续传代后会恢复毒力[101]，提

示该疫苗方案存在较大的生物安全风险。在没有足够证据确保减毒活疫苗不会返强的情况下，该策略暂不建议用于 COVID-19 疫苗开发。

2 疫苗设计需注意的问题

前期 SARS 疫苗研究发现如果候选疫苗诱导产生针对病毒的非中和抗体，会引起抗体依赖感染增强效应 (Antibody-dependent enhancement, ADE)，机制是病毒特异抗体 Fc 段通过与巨噬细胞 Fc 受体结合，从而使得 SARS-CoV 可以感染不含 ACE2 受体的巨噬细胞，增强了病毒的感染性，出现疫苗接种反而加重病毒感染的问题，而中和抗体水平越低感染越严重[102-106]，该现象在其他冠状病毒中较为常见[107]，而且也在登革病毒、人类免疫缺陷病毒 (HIV) 和埃博拉病毒等病原上存在[108-111]。考虑到 SARS-CoV-2 与 SARS-CoV 同源度较高，因此 COVID-19 疫苗设计时必须高度关注 ADE 问题，这将直接关系到疫苗的临床安全性。防止发生 ADE 效应的一个关键措施就是选择合适的靶抗原，尽量减少非中和抗体诱导区。冠状病毒 S 蛋白由 S1 和 S2 两个亚基组成，S1 包含 RBD 区，相比全长 S 蛋白，单独免疫 S1 区段或者 RBD 区同样可以获得很好的免疫保护[53,112]。因此应选择优势中和抗体诱导区 (S1 或 RBD 区)，排除可能产生非中和抗体的抗原区段，同时优化免疫策略产生高滴度中和抗体以降低 ADE 发生风险，从而保证疫苗的安全性。细胞免疫反应在清除冠状病毒和被感染细胞过程中起着关键作用，而且粘膜免疫反应相比血清中和抗体在机体保护中更具优势。因此，COVID-19 疫苗的设计研发策略需要兼顾全身性免疫 (体液和细胞免疫) 和粘膜免疫。SARS 疫苗研究证明单一疫苗策略无法有效形成对实验动物的完全保护，为了提高疫苗的免疫保护效力，我们可以考虑采用多个不同平台的疫苗联合免疫，同时采用多种途径接种，这样可充分利用不同平台疫苗的优势，补齐技术短板，有望获得更好的免疫保护效果。

3 展望与思考

COVID-19 是新中国成立以来我们遇到的传播速度最快、感染范围最广、防控难度最大的重大突发公共卫生事件。疫情在全球肆虐，全世界各研究机构也全力开展了疫苗和药物的研发工作。一个好的疫苗需要同时具备安全和有效两个特性，在设计之初就要尽量避免潜在的安全风险。考虑到灭活病毒疫苗可能存在的 ADE 效应，且短期内不易被发现，选择该策略需加倍谨慎。而减毒活疫苗由于存在毒力返强风险，不建议用于 COVID-19 疫苗研发。DNA 疫苗、亚单位疫苗和载体疫苗策略被证明是安全的。前期 SARS 和 MERS 疫苗研究经验证明，高滴度血清中和抗体并不能提供足够保护，需要 CTL 协助清除病毒和感染细胞，而且通过鼻腔接种诱导的 IgA 抗体在冠状病毒感染中表现出的保护效力优于其他接种途径诱导的血清中和抗体 IgG[12,68,73]。因此 COVID-19 疫苗设计应能够激发机体广泛的免疫应答：全身免疫 (体液免疫和细胞免疫) 和粘膜免疫。载体疫苗能够满足这个要求，可惜已上市的载体疫苗数量非常有限，大范围人群接种的长期安全性仍需观察，而且载体的选择需要考验疫苗设计人员的智慧，既要达到高效的免疫效力又要规避预存免疫。DNA 疫苗可诱导生成体液和细胞免疫，亚单位疫苗可诱导体液免疫和粘膜免疫，这两个策略也非常适合用于 COVID-19 疫苗研发，尽管两者可被各自开发为单独的疫苗，但如果能够将两者结合起来，有望弥补各自不足，高效激发全身免疫和粘膜免疫应答使机体获得最佳保护。前期研究发现感染 SARS 康复人员体内的中和抗体水平仅能维持较短时间[13]，因此期待通过感染而获得群体免疫的策略是缺乏理论依据的，只有接种安全有效的疫苗才能更好地获得免疫保护。

中国采取的史无前例的综合非药物性干预措施有效控制了疫情传播，为研制疫苗及验证治疗方法争取到宝贵的时间。病毒没有国界，全世界不同研究机构根据各自所长分别选用了不同疫苗研发策略，这是一场与时间赛跑的研究，最终会使我们对冠状病毒的研究水平上升到一个新的高度，杜绝类似悲剧的再次发生。尽管 SARS-CoV-2 与 SARS-CoV 的受体相同，但是两者 RBD 区多个关键氨基酸存在差异，而且针对 SARS-CoV 的绝大部分中和抗体不能识别 SARS-CoV-2，因此前期研究的 SARS 候选疫苗并不能用于 COVID-19 的防疫。美国已将一个尚未完成临床前动物实验的 mRNA 疫苗提前启动临床实验，这种打破常规疫苗研发程序的方式是为了应对当前疫情紧急态势的应急措施。但是将一种还没有在动物上证明安全性和有效性的疫苗仓促用于人体测试是否存在伦理问题，还需疫苗从业人员的进一步深入探讨。鉴于近些年我国多个机构建立了相对成熟的疫苗研发平台，COVID-19 疫苗是非常有希望能够研发成功的。考虑到冠状病毒变异较为频繁，且目前已发现病毒开始出现少量变异，尤其是病毒 RBD 区出现了一些氨基酸变异，因此建议将研制成功的疫苗作为一个类似季节性流感疫苗的平台技术产品，即使病毒发生变异，我们也可在最短时间内对现有疫苗进行调整后快速应用。前期的疫苗研发能力储备为本次疫情应急响应提供了有力技术支撑，后续我们仍需高度关注病毒可能的变异，必要时调整疫苗方案，用一种安全有效的疫苗打赢这场抗疫战争。

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