

Comparative Analysis of Immunostimulatory Activation Mechanisms and Design Strategies for Two Novel Vaccine Platforms: SLP+CpG and mRNA – LNP

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Abstract. With the continuous expansion of the spectrum of immune-related diseases, traditional vaccines and therapies are far from meeting the urgent needs with the required precision and durability. Therefore, there is a requirement for mechanistically programmable immune platforms. Because of their advantages in antigen design and innate immune regulation, synthetic long peptides combined with CpG and messenger RNA-lipid nanoparticles have become research hotspots over the last few years. This review focuses on core elements and the latest development of these two platforms: at the molecular and process level, the review outlines SLP multi-epitope concatenation and auxiliary epitope design, CpG regulation of the TLR9-MyD88 axis, mRNA nucleoside modifications, UTR/ORF optimization, and LNP delivery strategies; at the level of immunological mechanism, it makes a comparison between exogenous "pulsed" antigen presentation and endogenous short-term continuous expression. Generally speaking, SLP+CpG displays epitope-level precision and programmable personalization, while mRNA-LNP has potential in multi-antigen parallel processing and rapid iterative scaling. However, bottlenecks like cross-presentation, "pulsed" antigen availability, expression-innate balance, formulation immunogenicity, and cold chain consistency are still problems to be overcome. Future directions shall focus on high-precision antigen prediction and selection, cDC1-biased and organ-targeted delivery, heterologous sequential and dose-interval optimization, and biomarker-driven personalized combination therapies to further guarantee more reproducible and scalable clinical translation.

1 Introduction

Immunotherapy, which leverages the body's immune system in the fight against disease, has made great strides recently in the treatment of infectious diseases, cancers, and autoimmune disorders[1, 2]. Similarly, new immune delivery platforms focused on the central theme of "programmable antigens-modulatable innate immunity" are continuously emerging, accelerating progress in basic research, translation, and clinical applications[3, 4]. In comparison with conventional protein or inactivated vaccines, vaccines developed based on these platforms highlight targeted reconfiguration of antigen-presenting cells (APCs), rational modulation of innate immune pathways, and precise control of effector and memory responses-quantified by functional potency, including cytotoxic activity, cytokine secretion, neutralization capacity; durability, including maintenance of Tcm/Trm compartments, and antigen specificity, including epitope fidelity and breadth[3-5]. Thus, this approach aims to establish a new optimal balance among immunogenicity, design plasticity, and manufacturability.

Among the current immunization strategies, the SLP+CpG combination and mRNA-LNP platform

represent two of the most important technological pathways that have recently received increasing attention. The first one is able to achieve precise antigen supply through programmable multi-epitope peptides, using the CpG ligand of Toll-Like Receptor 9 (TLR9) as an "innate igniter"[3]. The synergy improves cross-presentation and promotes T helper 1 (Th1) polarization, with amplification of CD8+ and CD4+ T-cell responses. It provides unparalleled value in situations requiring epitope-level precision, such as personalized neoantigen tumor vaccines and chronic viral infections. The other one facilitates effective antigen expression and presentation via endogenous mRNA translation in recipient cells through the Major Histocompatibility Complex class I (MHC-I) pathway. Meanwhile, tissue delivery through lipid nanoparticles (LNP) and programmable activation of innate pathways including TLR7/8, RIG-I/MDA5, and NLRP3 supports rapid iteration and multi-antigen parallel development in such indications as acute infectious diseases and therapeutic cancer vaccines[4-6].

Although both platforms have distinct advantages in design and immunological mechanisms, each has critical challenges: SLP+CpG is constrained by cross-presentation efficiency and "pulsatile" antigen availability, making it highly sensitive to delivery

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systems and dosing schedules[7]; mRNA-LNP requires an optimal balance between expression persistence and innate immune stimulation[7], apart from addressing formulation-related reactogenicity, repeat-dose accessibility, cold chain logistics, and large-scale manufacturing challenges[7, 8]. The current literature mainly focuses on improvements and applications of respective platforms, while most lack systematic comparisons across the integrated continuum of innate immune sensing, topology of antigen presentation, and effector development[3, 7].

To address these research questions, we perform parallel mechanistic comparisons between the SLP+CpG and mRNA-LNP platforms. First, we examine innate immune recognition and signaling pathways to elucidate their distinct programming in receptor involvement and temporal regulation.

Subsequently, we compare antigen processing and presentation pathways to analyze their shaping effects on CD8⁺/CD4⁺ T cells and the Tfh-GC axis. Third, we evaluate the risk-benefit trade-offs of immune escape and tolerance alongside avoidance strategies. Finally, from a methodological perspective, we propose design and reporting recommendations including dose-interval optimization, heterologous sequential immunization, targeted delivery to conventional dendritic cell 1 (cDC1), and predictable biomarkers. Through this integrated examination of the “innate-presentation-effector-kinetics” pathway, we aim to establish an actionable design-validation-translation framework for subsequent mechanism studies, process optimization, and clinical development. This framework will also provide a comparable, reusable evidence base for selecting and combining immune strategies across diverse disease spectra.

2 SLP+CpG immunostimulatory mechanism

2.1 Antigen presentation pathways and parametric design of SLP

SLPs delivered in local tissues through subcutaneous or intramuscular administration are initially captured by APCs, primarily dendritic cells (DCs) and tissue-resident macrophages[1, 9]. Within the endosomal-lysosomal compartment, SLPs undergo proteolytic cleavage into short peptide fragments. These fragments are presented via Major Histocompatibility Complex class II (MHC-II) molecules to activate CD4⁺ T cells[9]. Some antigens translocate into the cytoplasm and are processed by proteasomes. The resulting peptides are then loaded onto MHC-I via the endoplasmic reticulum-TAP pathway, enabling cross-presentation and initiating CD8⁺ T-cell responses[9]. Against this processing backdrop, cDC1 subsets are pivotal *in vivo*. Because they combine efficient cross-presentation with CCR7-mediated homing to draining lymph nodes (LNs), they consequently shape the magnitude and the quality of CD8⁺ T-cell responses[1, 10].

At the molecular design level, cytotoxic T lymphocyte epitopes (CD8 epitopes) are typically

concatenated with helper T cell epitopes (CD4 epitopes), with broad-range DR epitopes (pan-DR epitopes, PADRE) introduced when necessary to enhance co-stimulatory signals[11]. Key parameters—including peptide length (typically 20–35 amino acids), linker sequences, and N-/C-terminal modifications—are deliberately tuned to steer proteolytic processing and promote endosomal escape for efficient cross-presentation[11]. Delivery can follow either co-formulation, in which synthetic peptides and adjuvants are combined within a single vehicle such as nanoparticles or oil-in-water adjuvants, or co-administration, in which they are provided separately or sequentially. The biological aim is to direct payloads to cDC1 subsets and to optimize tissue distribution and the intracellular sequence of uptake, processing, and antigen presentation, thereby enhancing cross-presentation and T-cell priming[3, 9]. These delivery strategies consequently improve the magnitude and functional quality of CD8⁺ and CD4⁺ T-cell responses elicited through the MHC-I and MHC-II pathways[9].

2.2 Innate immune programming of CpG oligonucleotides

CpG oligonucleotides serve as ligands for TLR9, a pattern recognition receptor (PRR), primarily expressed in plasmacytoid dendritic cells (pDCs), B cells, and some conventional dendritic cells. After endocytosis into endosomes, CpG engages TLR9 and signals via MyD88, sequentially activating IRAK4 and TRAF6 to initiate downstream pathways. This cascade induces Type I interferons (IFN-I) and inflammatory cytokines, such as IL-12 and TNF- α , through the NF- κ B and IRF7 signaling pathways, respectively. This upregulates CD80/CD86, CD40, and CCR7 on the DC surface, enhancing lymph node-directed migration and T cell activation capacity[3, 12]. In vaccinology, distinct CpG subtypes (A/B/C) exhibit preferential type-I interferon and proinflammatory profiles, allowing fine-tuned modulation of innate-adaptive immune coupling through dose, formulation, and timing parameters[3, 12].

Furthermore, CpG can synergize with other PRR pathways, such as TLR7/8 or STING, to jointly shape the expression patterns of chemokines (e.g. CXCL9 and CXCL10) and the functional programming of dendritic cells, thereby influencing germinal center (GC) responses and the differentiation of memory T cells[12, 13]. Such combined adjuvant strategies have been demonstrated to facilitate an optimal balance between reactogenicity and immunogenicity[12].

2.3 The synergistic immune activation mechanism of SLP and CpG

Under co-formulation or co-administration delivery conditions, SLP+CpG accomplishes temporal/spatial coordination between antigen supply and innate activation[3, 11]. In this context, CpG quickly induces an IFN-I- and IL-12-conducive microenvironment to promote DC maturation and cross-presentation efficiency, while SLP facilitates initial clonal expansion

by multi-epitope concatenation and CD4⁺ helper activation, enhances effector-to-memory formation, and augments generation of tissue-resident memory (Trm) T cells[9-12]. Relative to protein or short peptide approaches, SLPs confer distinct process advantages in antigen sequence programmability, personalized neoantigen production and cold-chain accessibility[11]. In chronic viral infection and tumor neoantigen settings, they serve as functional partners to immune checkpoint inhibitors, enhancing the functional quality and persistence of T cells[11].

The immunogenic enhancement of SLP + CpG is restricted by cross-presentation bottlenecks and pulsed antigen availability[9]. Therefore, the dosing regimen should aim at synergistically optimizing variables such as dose-interval, injection site and delivery system to achieve high-quality CD8⁺/Th1 responses consistently[3, 11].

In summary, SLP+CpG is best conceived as a platform that integrates programmable antigen design with calibrated innate stimulation to elicit predominantly Th1/CD8⁺ responses, while its performance is bounded by cross-presentation efficiency and the pulsed nature of antigen availability[9, 11, 12]. Practically, its value lies in epitope-level precision and personalization, provided that dose-interval, delivery route, and cDC1-oriented targeting are rationally optimized[3, 10, 11]. Building on this baseline, the next section examines mRNA-LNP as a contrasting paradigm that relies on endogenous antigen expression with programmable innate sensing, allowing a side-by-side comparison of activation logic, design levers, and translational implications[3-5, 7]

3 mRNA-LNP immunostimulatory mechanism

3.1 Platform architecture and innate immune perception framework

The mRNA-LNP platform encapsulates in vitro-transcribed (IVT) mRNA within LNP and is typically administered intramuscularly or subcutaneously to achieve in vivo delivery[7, 8]. After entering local tissues, LNPs undergo endocytosis into endosomes. In the acidic environment, lipids ionize to become positively charged and interact with the endosomal membrane, facilitating endosomal escape. The mRNA is then released into the cytoplasm and translated by ribosomes into the target antigen protein.

At the innate sensing level, RNA sensing involves Toll-like receptors 7/8 (TLR7/8) detecting single-stranded RNA and RIG-I/MDA5 recognizing 5' signatures and double-stranded intermediates, with Protein Kinase R (PKR) and the OAS-RNase L axis modulating translation and RNA stability. Carrier sensing ensues from LNP lipid composition and particle attributes, which can activate inflammasome pathways, including NLRP3. These signals collectively shape the IFN-I and proinflammatory factor landscape at the injection site and draining lymph nodes. This promotes antigen-presenting cell maturation and migration while

potentially inhibiting mRNA translation when excessive[4, 5, 12].

To balance expression duration with the intensity of the initial innate stimulus, tuning should be conducted along two coordinated axes. On the mRNA side, programmable levers include nucleoside modifications like N1-methylpseudouridine, in concert with 5' cap1, UTR architecture, and poly(A) length to modulate translation efficiency and transcript stability[7, 12]. Meanwhile, formulation variables on the LNP side, such as ionizable-lipid pKa, the cholesterol-to-structural-lipid ratio, PEG-lipid density, and particle size and surface characteristics, govern endosomal escape, biodistribution, and reactogenicity[14, 15].

3.2 Antigen presentation topology and immune response programming

If the antigen is designed as a secreted or membrane-anchored protein, the secreted product or antigen released from dead/apoptotic cells can be taken up by APCs, cross-presented, and further amplified to elicit a CD8⁺ response[9]. Simultaneously, exogenous antigens that enter the endosomal-lysosomal compartment are presented via MHC-II, thus driving the differentiation of CD4⁺ and T follicular helper (Tfh) cells and inducing GC responses that improve antibody affinity and give rise to memory B cells[12].

mRNA expression normally demonstrates a peak only for a short period of time with significant innate stimulation[4, 7]. Depending on dosage, dosing intervals, and formulation parameters, the amplitude and quality of initial clonal expansion, effector differentiation, and tissue migration can be modulated[14, 15]. The ratio of antigen topology to signal intensity defines the relative weighting of CD8⁺/CD4⁺ T cells versus the Tfh-GC axis, thereby allowing control over the cellular/humoral balance of immunity[12].

3.3 Application scenarios and strategy optimization

The mRNA-LNP platform integrates mechanistic and engineering advantages. Driven by endogenous antigen expression, there is direct MHC-I loading and robust CD8⁺ T-cell priming; decoupling formulation from sequence allows for rapid multi-epitope design and parallel antigen coverage within a single product[7, 15]. Rapid and modular manufacturing supports consistent scale-up, while antigen-topology engineering more effectively engages the Tfh-GC axis to enhance affinity maturation and memory establishment[7, 8, 15]. These features together yield a programmable composite response: CD8⁺-dominant cellular immunity with tunable humoral quality[4, 7, 12].

With these properties, mRNA-LNP lends itself particularly well to applications in fast-evolving antigenic landscapes and time-critical applications: accelerated emergency development for acute infectious diseases; multi-neoantigen loading in therapeutic tumor vaccines; and rapid iteration for variant updates through

simple coding-sequence replacement. Across indications, the platform enables functional balancing of CD8⁺ potency with Tfh-GC-driven humoral durability.

However, translational constraints require upfront management. Cold-chain and formulation sensitivity impose strict control of storage conditions, lipid ratios, and particle-size distribution to maintain expression kinetics and reactogenicity[7, 8]. Repeat dosing may be limited by anti-PEG/anti-lipid immunity, suggesting lower-reactogenicity chemistries and optimized schedules[5]. Population heterogeneity and IFN-I-driven reactogenicity necessitate biomarker-guided personalization, as excessive IFN-I can suppress translation and induce response attenuation[4, 12].

4 Mechanism comparison

4.1 Antigen supply and innate pathways

The SLP-CpG platform initiates immune responses with exogenous antigens. After uptake by APCs, the SLPs are processed in the endosomal-lysosomal pathway[9]. Critically, a portion of the antigen can access the cross-presentation pathway, leading to the loading of peptides onto MHC-I molecules[9]. This mechanism results in a relatively narrow time of antigen availability for T cell priming. In innate immune responses, the TLR9-MyD88-IRF7/NF- κ B axis drives rapid secretion of IFN-I and IL-12, promoting the establishment of a Th1-biased immune landscape[3, 12].

mRNA-LNP drives endogenous antigen synthesis within transfected cells, which is processed by proteasomes and loaded onto MHC-I, thereby priming CD8⁺ T cells[7, 15]. Antigen that is secreted or released from dying transfected cells becomes exogenous cargo for APCs: it is processed in endo-lysosomes for MHC-II presentation to CD4⁺/Tfh cells and can in parallel be cross-presented onto MHC-I, amplifying the CD8⁺ response[12]. Functionally, this topology yields a short-lived but coherent expression pulse that couples direct MHC-I access with MHC-II/Tfh-GC engagement via antigen release and uptake, without re-stating upstream innate-sensing details already covered[4, 7].

In summary, the two platforms embody fundamentally different design principles: SLP+CpG relies on pulsed delivery of exogenous antigens and cDC1-driven CD8⁺ T cell responses, whereas mRNA-LNP induces more durable parallel cellular and humoral immune responses through endogenous expression and trans-presentation mechanisms, coupled with modulated innate immune responses[7, 12].

4.2 Immune escape and immune tolerance

The major liabilities of SLP+CpG include limited epitope breadth and HLA-restricted presentation. When compounded by cross-presentation bottlenecks, it can increase the probability of immune escape under high mutational pressure[9, 11]. By comparison, SLPs enable sequence-level programming suitable for rational multi-epitope concatenation and patient-specific neoantigen incorporation. This design supports the targeted

mobilization of desired epitopes, coupled with the provision of CD4⁺ helpers and cDC1-oriented delivery, and provides depth and durability to T-cell responses[10, 11].

A core risk of mRNA-LNP is the need to balance antigen-expression kinetics against innate immune responses, which, if driven too strongly by IFN-I, for instance, can inhibit translation, whereas stringency in dosing regimens may also induce a state of exhaustion [4, 12]. Production constraints include formulation immunogenicity and cold chain-process consistency[7, 8]. Corresponding benefits include direct access to MHC-I presentation, rapid parallel multivalent antigen capability, and manufacturing-iteration speed. These features therefore favor applications requiring timeliness and accommodate the rapidly evolving antigen spectra[7, 15].

5 Conclusion

This work focused on two major immunological platforms, SLP+CpG and mRNA-LNP, with the aim of establishing a parallel comparative framework covering “innate recognition-antigen presentation topology and effect-immunodynamics”[3, 12]. With respect to the risk-benefit profile, the emphases are different for the two platforms: the limitations of SLP+CpG mainly emanated from the epitope coverage/HLA constraints and presentation bottlenecks[9, 11], while for mRNA-LNP, the key constraints lay in the expression-innate balance, formulation immunogenicity, and cold chain[4, 5].

The study provides a methodological basis for emergency R&D in infectious diseases and personalized immunotherapy of cancer : On one hand, mRNA-LNP offers an effective route to achieving high-speed iteration for updating variant strains and meeting urgent needs by parallel multi-antigenism[7]. On the other hand, good epitope-level programmability and clear safety profile are strong tools in SLP+CpG for precise neoantigen mobilization and quality consolidation[11].

Future priority should be given to relieving cross-presentation bottlenecks and stabilizing response quality for SLP+CpG-through cDC1-biased delivery, rational dose-interval schedules, and composite adjuvanting to tune the IFN-I/IL-12 axis[9, 10], whereas for mRNA-LNP, it involves defining the operating window between expression and innate tone through tuning nucleoside chemistry, cap/UTR/poly(A), and low-reactogenicity LNP formulations[14,15]. Programmatic evaluation of heterologous prime-boost and dose-interval regimens is warranted to map effects on Tcm/Trm partitioning and Tfh-GC quality across platforms[12]. In indications with pronounced epitope heterogeneity, including personalized neoantigens or chronic viral variants, SLP+CpG should emphasize multi-epitope concatenation with CD4⁺ helper provisioning[11]; conversely, the rapid design-manufacture cycle and multivalent capacity position mRNA-LNP for time-critical settings and multi-neoantigen oncology. Overall, platform selection and sequencing should be guided by

biomarker-anchored readouts and secured by robust cold-chain and repeat-dose accessibility.

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