Liu and Ostrowski *AIDS Res Ther (2017) 14:43*
DOI 10.1186/s12981-017-0165-8

AIDS Research and Therapy

REVIEW

Open Access

Development of targeted adjuvants for HIV-1 vaccines

Jun Liu[1*] and Mario Ostrowski[1,2,3*]

Abstract

Finding new adjuvants is an integrated component of the efforts in developing an effective HIV-1 vaccine. Compared with traditional adjuvants, a modern adjuvant in the context of HIV-1 prevention would elicit a durable and potent memory response from B cells, $CD8^+$ T cells, and NK cells but avoid overstimulation of HIV-1 susceptible $CD4^+$ T cells, especially at genital and rectal mucosa, the main portals for HIV-1 transmission. We briefly review recent advances in the studies of such potential targeted adjuvants, focusing on three classes of molecules that we study: TNFSF molecules, TLRs agonists, and NODs agonists.

Keywords: HIV, Vaccine, Adjuvant, TNFSF, TLRs, NODs

Background

More than three decades after human immunodeficiency virus 1 (HIV-1) was identified as the cause of AIDS, we still do not have an effective vaccine to stymie its global spread [1]. Barriers to developing an effective HIV-1 vaccine include the following: (1) HIV-1 mutates rapidly and has a tremendous genetic diversity. In this regard, broadly neutralizing antibodies (bNAbs) can neutralize a broad range of HIV-1 isolates, but we do not know how to induce such bNAbs with a vaccine [2]. Vaccines that induce non-broadly neutralizing HIV-1 Env-binding antibodies can afford partial protection against HIV-1/SHIV infection, but their efficacy needs to be substantially improved for clinical use [3, 4]. (2) All HIV-1 envelope (Env) based vaccine candidates can only induce a short-lived antibody response. This is in striking contrast to vaccines currently in clinical use and may severely limit the long-term efficacy of HIV-1 vaccines [5–8]. The mechanisms underlying this short duration of Env-antibody responses are not clear yet, but might be due to the failure of the Env glycoprotein to induce long-lived plasma cells [9, 10]. (3) HIV-1 is a rapidly replicating lentivirus that can establish latent infection soon after infection [11]. Thus an effective HIV-1 vaccine should elicit memory immune responses that can be mobilized fast (probably within a few days of infection) and sufficiently to block HIV-1 transmission through genital and rectal mucosa. Cytomegalovirus (CMV)-vectored HIV-1 vaccine might be able to elicit such a persistent and strong immune response [12], but we do not know if and how other vaccine platforms can elicit such immune responses, especially at genital and rectal mucosa. (4) $CD4^+$ T cells play a pivotal role in forming memory immune response but are also target cells of HIV-1. An effective HIV-1 vaccine should induce potent cellular and humoral memory immune responses but avoid or limit stimulation of HIV-1 susceptible $CD4^+$ T cells, which is highlighted by the Step and Phambili clinical trials results [13, 14]. Overcoming these barriers requires a multidisciplinary and multipronged approach, such as design of novel immunogens, development of better adjuvants, testing of multiple vaccination routes/schedules, and invention of novel delivery vehicles. Recent advances in immunology should be able to replace traditional adjuvants, such as alum, with an adjuvant that can preferentially promote protective responses from B cells, $CD8^+$ T cells, and/or natural killer cells (NK), but not activate $CD4^+$ T cells. Here, we will briefly review recent advances in the studies of such potential targeted adjuvants for HIV-1 vaccines. A thorough review is out of the scope of this short paper, and we will focus on three

*Correspondence: junut.liu@gmail.com; mario.ostrowski@gmail.com
[1] Clinical Sciences Division, University of Toronto, Room 6352, Medical Sciences Building, 1 King's College Circle, Toronto, ON M5S1A8, Canada
Full list of author information is available at the end of the article

classes of molecules that we are studying: tumor necrosis factor superfamily (TNFSF) molecules, toll-like receptors (TLRs) agonists, and nucleotide-binding oligomerization domain-containing proteins (NODs) agonists.

TNFSF molecules-CD40L, BAFF, and APRIL

TNFSF molecules are type II transmembrane proteins that have a conserved tumor necrosis factor homology domain at their C-termini [15]. Many TNFSF members are immune costimulatory molecules, among which CD40 ligand (CD40L), B cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) are pivotal for B cell costimulation. CD40L expressed on activated $CD4^+$ T cells binds CD40 on B cells to promote B cell proliferation and survival, antibody isotype switching, and antibody affinity maturation. BAFF and APRIL are two closely related TNFSF molecules that are important for B cell development and differentiation [16, 17]. BAFF binds to three receptors on B cells: BAFF receptor (BAFFR), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA) while APRIL binds to TACI and BCMA. BAFF–BAFFR interaction provides a key survival signal for mature B cells [16, 17]. The APRIL–BCMA pathway is essential for long-term survival of bone marrow plasma cells [18, 19]. BAFF and APRIL can also induce antibody isotype switching independent of CD40L [20]. Notably, BAFF and APRIL were shown to be essential for IgA production. The CD40L-CD40 pathway is also important for promoting the $CD8^+$ T cell response. Binding of CD40 on immature DC by CD40L activates and matures them, which are "licensed" to activate $CD8^+$ T cells.

Many reports have been published on testing CD40L as adjuvant for HIV-1 and Simian immunodeficiency virus (SIV) vaccines. We reported CD40L expressed from a canarypox vector (ALVAC) enhanced memory polyfunctional cytotoxic T cell (CTL) responses elicited by an ALVAC HIV-1 vaccine in mice [21]. Kwa et al. found CD40L augmented SIV-specific humoral and cellular immune responses, improved protection against SIV infection, and strengthened control of SIV replication in rhesus macaques receiving DNA prime/Modified Vaccinia Ankara (MVA) boost SIV vaccine [22, 23]. We recently found CD40L mainly enhanced SIV Env-specific antibody responses elicited by an ALVAC prime-Env protein boost SIV vaccine in monkeys (Liu et al. manuscript in preparation). Although further study is required, these results indicate CD40L could be a potential adjuvant capable of targeting B cells and $CD8^+$ T cells.

BAFF and APRIL were also reported to enhance immunogenicity of HIV-1 vaccines. Gupta et al. found plasmid expressing multimeric soluble BAFF or APRIL, when co-administered with plasmid expressing IL-12, increased titer and avidity of gp120-binding antibodies and titer of neutralizing antibodies against a tier-1 and an autologous tier-2 HIV-1 virus in mice receiving a DNA prime/protein boost HIV-1 gp140 vaccine [24]. Melchers et al. made trimeric fusion constructs of HIV-1 gp140 with CD40L, BAFF, and APRIL and found only the gp140-APRIL construct significantly enhanced Env-binding antibodies in rabbits [25]. These previous reports just tested antibodies in blood. We found BAFF and APRIL increased HIV-1 Env-binding antibodies at mucosa in mice (Liu et al. manuscript in preparation).

TLRs agonists

TLRs are type I transmembrane proteins belonging to pattern recognition receptors (PRRs), a large family of molecules that can sense "danger signals" (pathogen-associated molecular patterns and damage-associated molecular patterns) to activate innate immune cells, which then initiates adaptive immune responses through production of cytokines and chemokines and antigen presentation. Ten TLRs have been identified in human and 12 in mouse, each of which has distinct ligands [26]. Synthetic TLRs agonists, especially TLR7, TLR8, and TLR9 agonists, have been tested as adjuvants for HIV-1/SIV vaccines in animal studies. Moody et al. compared the effect of TLR4 agonist (lipid A), TLR7/8 agonist (R848), and TLR9 agonist (oCpG), either alone or in pairwise combination, on antibody responses elicited by a gp140 protein vaccine in monkeys [27]. They found combination of R848 and oCpG helped the vaccine induce the strongest Env-binding antibodies, including neutralizing antibodies and antibodies mediating antibody-dependent cell-mediated cytotoxicity (ADCC). Based on previous studies, the authors suggested combination of R848 and oCpG might enhance antibody responses by suppressing type 1 T helper cells (Th1). Kasturi et al. used combination of TLR4 and TLR7/8 agonist (MPL and R848) encapsulated in poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles as adjuvant for SIV Env plus Gag protein vaccine or SIV virus-like particle (VLP) vaccine [28]. They reported that PLGA (MPL + R848) helped the SIV vaccine elicit persistently higher SIV Env binding IgG and IgA in blood and at mucosa, more long-lived Env-specific plasma cells in bone marrow and draining lymph nodes, and higher Env-specific $CD4^+$ T cell responses than alum. Only PLGA (MPL + R848) adjuvanted SIV vaccines significantly protected monkeys expressing a restrictive tripartite motif-containing protein 5α (TRIM5α) allele from a heterologous SIV intravaginal challenge, and the protection correlated with SIV Env-binding IgG in blood and vaginal secretion.

We recently reported that self-assembling peptide nanofibers could co-deliver an HIV-1 $CD8^+$ T cell epitope, SL9, and TLR7/8 agonist R848 to activate human monocyte-derived dendritic cells (MDDCs) in vitro and elicited stronger SL9-specific $CD8^+$ T cells in HLA-A2 transgenic mice [29]. EAK16-II is a 16mer peptide that can self-assemble to form nanofibers in aqueous solution. We found SL9-EAK16-II fusion peptide could co-assemble with R848 and TLR7 agonist R837 to form nanofibers. The nanofibers were taken up by MDDCs into endosomes, where TLR7 and TLR8 are localized. Consequently, SL9-EAK16-II nanofibers with R848 or R837 activated MDDCs, which elicited stronger SL9-specific $CD8^+$ T cell responses in vitro than non-nanoformed SL9 peptide. R848 was more potent than R837 in helping the nanofibers to induce the SL9-specific $CD8^+$ T cell responses in vitro, possibly due to its synergistic activation of both TLR7 and TLR8 in DCs. The mechanisms underlying the enhanced SL9-specific $CD8^+$ T cell induction by SL9-EAK16-II nanofiber in vitro and in vivo are still under investigation, but are possibly related to its increased stability due to resistance to extracellular and intracellular proteinases and peptidases (Liu et al. unpublished data).

NODs agonists

NODs are intracellular PRRs [30]. There are two closely related NODs, NOD1 and NOD2, all of which containing N-terminal caspase recruitment domain(s) (CARD) (one for NOD1 and two for NOD2) to activate downstream signaling molecules, a C-terminal leucine-rich repeat domain to recognize microbial molecules, and a central nucleotide-binding oligomerization domain to bind nucleoside triphosphate. The ligands of NODs are components of peptidoglycan in bacterial cell wall. NOD1 ligand is γ-D-glutamyl-mesodiaminopimelic acid (iE-DAP) present in some Gram-positive bacteria and all Gram-negative bacteria. NOD2 ligand is muramyl dipeptide (MDP) found in all Gram-positive and Gram-negative bacteria. These ligands bind and activate NODs, which finally activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1), leading to autophagy and production of pro-inflammatory cytokines, chemokines, and anti-microbial factors. Activation of NOD1 and NOD2 primes a Th2-polarized adaptive immune response with potent antibody responses in mice [31], which makes NODs agonists attractive as adjuvants for HIV-1 vaccines, since Th2 cells are much less susceptible to HIV-1 infection than Th1 and Th17 [32]. Pavot et al. reported NOD1 and NOD2 agonists encapsulated in polylactic acid (PLA) nanoparticles enhanced mucosal antibody responses elicited by HIV-1 p24 coated on PLA nanoparticles in mice [33]. Both NOD1 and NOD2 agonists augmented p24-specific IgG in feces after subcutaneous vaccination, compared with p24-alum or PLA-p24. Only NOD2 agonist significantly enhanced p24-specific IgA in feces and vaginal lavage after oral or intranasal vaccination, respectively, and p24-specific IgG in vaginal lavage after intranasal vaccination. These findings suggest NOD2 agonist may be better than NOD1 agonist as an adjuvant to elicit mucosal antibody responses. We found MDP could enhance mucosal gp140-specific antibody response in mice (Liu et al. unpublished data).

Conclusions and perspectives

Recent advances in the development of targeted adjuvants should help HIV-1 vaccines elicit potent and durable memory responses of B cells, $CD8^+$ T cells, NK cells, etc. while avoiding generation of abundant HIV-1 susceptible $CD4^+$ T cells at genital and rectal mucosa. An ideal adjuvant should preferentially activate B cells, $CD8^+$ T cells, and NK cells other than $CD4^+$ T cells. Using targeted delivery vehicles, such as nanoparticles coated with specific ligands for the receptors on these cells, may further increase the targeting of the adjuvants. More studies are still needed to find the best targeted adjuvant for HIV-1 vaccine before clinical trials.

Abbreviations

ADCC: antibody-dependent cell-mediated cytotoxicity; AP-1: activator protein 1; APRIL: a proliferation-inducing ligand; BAFF: B cell activating factor; BAFFR: BAFF receptor; BCMA: B cell maturation antigen; bNAbs: broadly neutralizing antibodies; CARD: caspase recruitment domain; CD40L: CD40 ligand; CMV: cytomegalovirus; CTL: cytotoxic T cell; CXCL10: C-X-C motif chemokine 10; DCs: dendritic cells; Env: HIV-1 envelope protein; HIV-1: human immunodeficiency virus 1; iE-DAP: γ-D-glutamyl-mesodiaminopimelic acid; MDDC: monocyte-derived dendritic cells; MDP: muramyl dipeptide; MVA: Modified Vaccinia Ankara; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; NK: natural killer cells; NODs: nucleotide-binding oligomerization domain-containing proteins; PLA: polylactic acid; PLGA: poly(lactic-*co*-glycolic acid); PRRs: pattern recognition receptors; SHIV: Simian-human immunodeficiency virus; SIV: Simian immunodeficiency virus; TACI: transmembrane activator and calcium modulator and cyclophilin ligand interactor; TLRs: toll-like receptors; TNFSF: tumor necrosis factor superfamily; TRIM5α: tripartite motif-containing protein 5α; VLP: virus-like particle.

Authors' contributions

JL and MO wrote the manuscript. Both authors read and approved the final manuscript.

Author details

[1] Clinical Sciences Division, University of Toronto, Room 6352, Medical Sciences Building, 1 King's College Circle, Toronto, ON M5S1A8, Canada. [2] Department of Immunology, University of Toronto, Toronto, ON, Canada. [3] Keenan Research Centre for Biomedical Science of St. Michael's Hospital, Toronto, ON, Canada.

Acknowledgements

We apologize to our colleagues whose work cannot be cited here due to space limit. Authors' work is supported by Ontario HIV Treatment Network and Canadian Institutes of Health Research.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials
Not applicable.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

Funding
Ontario HIV Treatment Network (OHTN) and Canadian Institutes of Health Research (CIHR). Funders have no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Publisher's Note

Received: 22 March 2017 Accepted: 11 August 2017
Published online: 12 September 2017

References

1. Fact sheet November 2016. http://www.unaids.org/en/resources/fact-sheet. Accessed 14 Mar 2017.
2. Burton DR, Hangartner L. Broadly neutralizing antibodies to HIV and their role in vaccine design. Annu Rev Immunol. 2016;34:635–59.
3. Barouch DH, Stephenson KE, Borducchi EN, Smith K, Stanley K, McNally AG, Liu J, Abbink P, Maxfield LF, Seaman MS, et al. Protective efficacy of a global HIV-1 mosaic vaccine against heterologous SHIV challenges in rhesus monkeys. Cell. 2013;155(3):531–9.
4. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. 2009;361(23):2209–20.
5. Lewis GK, DeVico AL, Gallo RC. Antibody persistence and T-cell balance: two key factors confronting HIV vaccine development. Proc Natl Acad Sci USA. 2014;111(44):15614–21.
6. Klasse PJ, Sanders RW, Cerutti A, Moore JP. How can HIV-type-1-Env immunogenicity be improved to facilitate antibody-based vaccine development? AIDS Res Hum Retrovir. 2012;28(1):1–15.
7. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang ZY, Seaton KE, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. Sci Transl Med. 2014;6(228):228.
8. Robb ML, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Kunasol P, Khamboonruang C, Thongcharoen P, Morgan P, Benenson M, et al. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post hoc analysis of the Thai phase 3 efficacy trial RV 144. Lancet Infect Dis. 2012;12(7):531–7.
9. Bonsignori M, Moody MA, Parks RJ, Holl TM, Kelsoe G, Hicks CB, Vandergrift N, Tomaras GD, Haynes BF. HIV-1 envelope induces memory B cell responses that correlate with plasma antibody levels after envelope gp120 protein vaccination or HIV-1 infection. J Immunol. 2009;183(4):2708–17.
10. Sundling C, Martinez P, Soldemo M, Spangberg M, Bengtsson KL, Stertman L, Forsell MN, Hedestam GBK. Immunization of macaques with soluble HIV type 1 and influenza virus envelope glycoproteins results in a similarly rapid contraction of peripheral B-cell responses after boosting. J Infect Dis. 2013;207(3):426–31.
11. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc Natl Acad Sci USA. 1998;95(15):8869–73.
12. Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, Whizin N, Oswald K, Shoemaker R, Swanson T, et al. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature. 2011;473(7348):523–7.
13. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, Gilbert PB, Lama JR, Marmor M, Del Rio C, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008;372(9653):1881–93.
14. Moodie Z, Metch B, Bekker LG, Churchyard G, Nchabeleng M, Mlisana K, Laher F, Roux S, Mngadi K, Innes C, et al. Continued follow-up of Phambili phase 2b randomized HIV-1 vaccine trial participants supports increased HIV-1 acquisition among vaccinated men. PLoS ONE. 2015;10(9):e0137666.
15. Liu J, Ostrowski M. Development of TNFSF as molecular adjuvants for ALVAC HIV-1 vaccines. Hum Vaccine. 2010;6(4):355–9.
16. Kalled SL. Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production. Semin Immunol. 2006;18(5):290–6.
17. Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, function and signaling. Semin Immunol. 2006;18(5):263–75.
18. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, Lin LL, Mantchev GT, Bram RJ, Noelle RJ. BCMA is essential for the survival of long-lived bone marrow plasma cells. J Exp Med. 2004;199(1):91–8.
19. Belnoue E, Pihlgren M, McGaha TL, Tougne C, Rochat AF, Bossen C, Schneider P, Huard B, Lambert PH, Siegrist CA. APRIL is critical for plasmablast survival in the bone marrow and poorly expressed by early-life bone marrow stromal cells. Blood. 2008;111(5):2755–64.
20. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, Cerutti A. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol. 2002;3(9):822–9.
21. Liu J, Yu Q, Stone GW, Yue FY, Ngai N, Jones RB, Kornbluth RS, Ostrowski MA. CD40L expressed from the canarypox vector, ALVAC, can boost immunogenicity of HIV-1 canarypox vaccine in mice and enhance the in vitro expansion of viral specific $CD8^+$ T cell memory responses from HIV-1-infected and HIV-1-uninfected individuals. Vaccine. 2008;26(32):4062–72.
22. Kwa S, Lai L, Gangadhara S, Siddiqui M, Pillai VB, Labranche C, Yu T, Moss B, Montefiori DC, Robinson HL, et al. CD40L-adjuvanted DNA/modified vaccinia virus Ankara simian immunodeficiency virus SIV239 vaccine enhances SIV-specific humoral and cellular immunity and improves protection against a heterologous SIVE660 mucosal challenge. J Virol. 2014;88(17):9579–89.
23. Kwa S, Sadagopal S, Shen X, Hong JJ, Gangadhara S, Basu R, Victor B, Iyer SS, LaBranche CC, Montefiori DC, et al. CD40L-adjuvanted DNA/modified vaccinia virus Ankara simian immunodeficiency virus (SIV) vaccine enhances protection against neutralization-resistant mucosal SIV infection. J Virol. 2015;89(8):4690–5.
24. Gupta S, Clark ES, Termini JM, Boucher J, Kanagavelu S, LeBranche CC, Abraham S, Montefiori DC, Khan WN, Stone GW. DNA vaccine molecular adjuvants SP-D-BAFF and SP-D-APRIL enhance anti-gp120 immune response and increase HIV-1 neutralizing antibody titers. J Virol. 2015;89(8):4158–69.
25. Melchers M, Bontjer I, Tong T, Chung NP, Klasse PJ, Eggink D, Montefiori DC, Gentile M, Cerutti A, Olson WC, et al. Targeting HIV-1 envelope glycoprotein trimers to B cells by using APRIL improves antibody responses. J Virol. 2012;86(5):2488–500.
26. Gay NJ, Gangloff M. Structure and function of Toll receptors and their ligands. Annu Rev Biochem. 2007;76:141–65.
27. Moody MA, Santra S, Vandergrift NA, Sutherland LL, Gurley TC, Drinker MS, Allen AA, Xia SM, Meyerhoff RR, Parks R, et al. Toll-like receptor 7/8 (TLR7/8) and TLR9 agonists cooperate to enhance HIV-1 envelope antibody responses in *Rhesus macaques*. J Virol. 2014;88(6):3329–39.
28. Kasturi SP, Kozlowski PA, Nakaya HI, Burger MC, Russo P, Pham M, Kovalenkov Y, Silveira EL, Havenar-Daughton C, Burton SL et al. Adjuvanting a simian immunodeficiency virus vaccine with toll-like receptor ligands encapsulated in nanoparticles induces persistent antibody responses and enhanced protection in TRIM5α restrictive macaques. J Virol. 2017;91(4):e01844–16.
29. Ding Y, Liu J, Lu S, Igweze J, Xu W, Kuang D, Zealey C, Liu D, Gregor A, Bozorgzad A, et al. Self-assembling peptide for co-delivery of HIV-1 $CD8^+$ T cells epitope and Toll-like receptor 7/8 agonists R848 to induce maturation of monocyte derived dendritic cell and augment polyfunctional cytotoxic T lymphocyte (CTL) response. J Control Release. 2016;236:22–30.

30. Feerick CL, McKernan DP. Understanding the regulation of pattern recognition receptors in inflammatory diseases—a 'Nod' in the right direction. Immunology. 2017;150(3):237–47.
31. Fritz JH, Le Bourhis L, Sellge G, Magalhaes JG, Fsihi H, Kufer TA, Collins C, Viala J, Ferrero RL, Girardin SE, et al. Nod1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. Immunity. 2007;26(4):445–59.
32. Gosselin A, Salinas TRW, Planas D, Wacleche VS, Zhang Y, Fromentin R, Chomont N, Cohen EA, Shacklett B, Mehraj V, et al. HIV persists in CCR6^{+} CD4^{+} T cells from colon and blood during antiretroviral therapy. AIDS. 2017;31(1):35–48.
33. Pavot V, Climent N, Rochereau N, Garcia F, Genin C, Tiraby G, Vernejoul F, Perouzel E, Lioux T, Verrier B, et al. Directing vaccine immune responses to mucosa by nanosized particulate carriers encapsulating NOD ligands. Biomaterials. 2016;75:327–39.