Virology & Retrovirology Journal

SCIENTIFIC LITERATURE

Special Issue Article "Plague"

Short Review

Immunoprophylaxis for Plague Infection: An Update

Riyasat Ali[1*], Sudhir Kumar[2] and Rao DN[3]

[1]Department of Pharmacological and Pharmaceutical Science, University of Houston, USA

[2]Department of Zoology, Patliputra University, India

[3]Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), India

ARTICLE INFO

Received Date: March 16, 2020
Accepted Date: April 20, 2020
Published Date: April 24, 2020

KEYWORDS

Plague
Vaccine
MAP
F1 antigen
V antigen

Citation for this article: Riyasat Ali, Sudhir Kumar and Rao DN. Immunoprophylaxis for Plague Infection: An Update. Virology & Retrovirology Journal. 2020; 3(1):125

Corresponding author:
Riyasat Ali,
Department of Pharmacological and Pharmaceutical Science, College of Pharmacy, University of Houston, Health 2, 4849 Calhoun Road, Houston, Texas 77204-5000,
Email: riyasataiims@gmail.com

ABSTRACT

Plague is a vector-borne disease caused by *Yersinia pestis*. It has a high mortality rate with no complete cure. It is a category 'A' agent of bioterrorism as it has caused the highest number of fatalities in human history. It had been used as an agent for bio-warfare in the past and impacted human civilization at large. The world has seen three major pandemics in last two millennia which have similar pattern of infection and spread. Rodent flea, as a reservoir, played a critical role in its initiation from flea to human, and once the disease is established in the lungs (i.e. pneumonic form) it becomes highly contagious since then it does not need the flea anymore and can be transmitted by aerosolized droplets. Initial infections like bubonic form can be treated with broad-spectrum antibiotics but treating the pneumonic form remains still a challenge. Researchers have studied several immunodominant proteins/antigens to develop an effective cure of the disease but complete cure is still awaited. Here we summarize the recent outcomes of antigens and formulations under development.

INTRODUCTION

Plague, a zoonotic disease, is caused by a gram-positive bacillus named *Yersinia pestis*. *Y. pestis*is a non-spore forming coccobacillus measuring 0.5 to 0.8 µm length. There are three primary clinical forms of *Y. pestis* named; bubonic, septicemic and pneumonic plagues. Out of these three forms, the most severe form is pneumonic plague caused by the respiratory droplet route. The route of spread of the pneumonic form and its primary site of infection i.e. lungs make this form the deadliest one and almost impossible to control once infection is established. It is categorized by the Centers for Disease Control and Prevention (CDC) as a category 'A' agent of bioterrorism, the most dangerous and highest priority for public health preparedness [1].

The pathogen primarily resides in rodents from where it gets transmitted to human through the rodent flea. Once the infection is established in humans, it no longer required the host but it can transmit human to human through droplets. The plague has been a constant threat to humanity from centuries as the first reported pandemic of plague known as the "great plague of Justinian" can be traced around 532 AD in Egypt and spread through the Middle East, Greece, Italy and the Mediterranean basin [1,2]. Eventually, human history has faced many plague pandemics; the second major epidemic known as the Black Death started in 14th century in China and spread westward in Europe resulting in millions of deaths and the third started in 1855 in the

Chinese province of Yunnan. The recent increase in the number of human plague cases in countries such as Malawi, Mozambique, Peru, China, Madagascar and India has led the WHO to classify it as a re-emerging infection [3-5].

In the last couple of decades, the possible use of such deadly pathogens as agents of bioterrorism/ biological weapons has been alarming the scientific community and the regulatory agencies. The possible use of *Y. pestis* as a potent biological weapon has been witnessed in history. To counter any possible natural or manmade threat, we need safe and effective prophylactic or therapeutic vaccines against the pathogen. There are various kinds of plague vaccines under development, including live, recombinant protein, subunit vaccine, peptide-based vaccine, multiple antigens based vaccine and also different adjuvants, route of administration, formulations have been used in various animal model. Here, we summarize subunit peptide-based vaccination/formulation strategy as a likely prophylactic plague vaccine.

MAJOR ANTIGENIC FORMULATIONS

Several studies exploited two subunits of *Y. pestis*as the target antigens against the pathogen: the low calcium response protein V (LcrV), a virulent *Y. Pestis* protein, residing at the tip of its type III secretion needles, and a capsule-like antigen, fraction 1 (F1) antigen. Recombinant LcrV alone or in combination with F1 (F1-V hybrid, generated via translational fusion of both antigens) were studied by many groups as potential plague vaccines [6,7].

Also, recombinant subunit vaccine comprising the protein antigens rF1+rV demonstrated protection in immunized guinea pigs against exposure to 10 [5] Colony-Forming Units (CFU) of virulent *Y.pestis*. Moreover, the human immune response to recombinant F1 (rF1) and rV antigens, has been assessed in healthy volunteers. All the subjects showed F1 and V antigen-specific IgG titer in serum. Also, the adoptive transfer of the serum showed protection in naïve mice [8]. Monoclonal antibody MAb 7.3 and Mab BA5 showed binding to specific epitope residues 196-225 on LcrV. The monoclonal antibody MAb BA5 neutralized the *Y.pestis* type III secretion system [9,10].

Recently, an F1 antigen variant, a soluble F1V mutant (F1mutV) was generated to overcome the aggregative property of F1 antigen, particularly when expressed in heterologous systems such as Escherichia coli, thus affecting vaccine quality and efficacy. The mutant exhibited similar immunogenicity as wild-type F1V [11]. A new formulation called dual anthrax-plague nanoparticle vaccine was developed using bacteriophage (phage) T4 as a platform. This virus nanoparticle vaccine expressed key antigens of both *B. anthracis* and *Y. pestis* provided complete protection from both anthrax and pneumonia in animal models [12].

Recently, in a clinical trial study Flagellin/F1/V subunit showed a dose-dependent increase in immunogenicity and was well tolerated at all doses vaccine [13]. Flagellin/F1/V antigen formulation showed enhanced inverse caspase-3 level, which is a measure of protective immunity, and also enhanced T cell response in clinical trial samples. Also, Flagellin/F1/V subunit vaccine enhanced protective gene signature [14]. The use of multiple antigens has gain popularity as many studies suggested synergism in the immunogenicity and protection.

Recently, multiple-antigen fusion proteins (MaF1 and MaF2) containing B. anthracis Protective Antigen (PA) and Lethal Factor (LF), and from *Y. pestis* V antigen (LcrV) and Fraction 1 (F1) capsule were produced and showed 100% protection from *Y. pestis* in mice, which was further enhanced by the molecular adjuvant CARD if [15]. Also, a single vector-based vaccine LVS ΔcapB- and Listeria Monocytogenes (Lm)-vectored vaccines express recombinant *B. anthracis*, *Y. pestis* and *F. tularensis* immunoprotective antigens showed antigen-specific humoral and T-cell-mediated immune response and protection against lethal respiratory challenges [16].

A novel recombinant attenuated *Y. pseudotuberculosis* PB1+ strain (χ10069) engineered with Δ*yopK* Δ*yopJ* Δ*asd* triple mutations was used to deliver a *Y. pestis* fusion protein, YopE amino acid 1 to 138-LcrV ($YopE_{Nt138}$-LcrV), to Swiss Webster mice as a protective antigen against infections by yersiniae. Oral immunization of χ10069 induced strong humoral and cellular immune response and showed protection against bubonic and pneumonic plague challenges, with 80% and 90% survival, respectively [17]. The VTnF1 strain, which is a *Y. pseudotuberculosis* strain stably produced the F1 capsule given orally to mice induced a high humoral and cellular response. TheVTnF1 strain provides immunity against both *Y. pestis* and *Y. tuberculosis* after a single oral dose. Moreover, a single oral dose (10^8 CFU) of VTnF1 conferred 100% protection against

pneumonic plague using a high-dose challenge (3,300 LD50) caused by the fully virulent *Y. pestis* CO92 [18-20]. Live attenuated strain of Y. pseudotuberculosis was used because it shares a high **(>95%)** genetic identity and a virulence plasmid with *Y. pestis*. They are different in that *Y. pestis* carries the additional plasmids pPCP1 and pMT1.

Table 1: Vaccine candidates against plague.

Vaccine candidate	Protective efficacy	Reference
F1-LcrV-HSP70(II) fusion protein	Complete protection in mice against i.p. challenge with 100 LD50(105CFU) of *Y. pestis* S1 strain	[28]
rF-V1 adjuvanted with a novel TLR4 ligand, BECC438	complete protection in mice against i.p. challenge with ~20 × LD50 of *Y. pestis* CO92 Δpgm	[29]
Flagellin/F1/V	a dose dependent increase in immunogenicity and was well tolerated at all doses in human	[13]
F1mutV-PA	Complete protection of mice, rat and rabbit against simultaneous challenge with 200 LD50 *Y. pestis* CO92 (i.n.)	[30]
VypVaxDuo	full protection for BALB/c mice against the s.c. challenge with 2 × 104 LD50 of *Y. pestis* CO92	[31]
F+ rV	Induced a robust immune response in both Cynomolgus macaques and Human adults	[32,33]
VTnF1	Conferred 100% protection against pneumonic plague caused by the fully virulent *Y. pestis* CO92.	[18,19]
Adenovirus (Ad5) expressing fusion gene YFV (ycsF, caf1, and lcrV)	Complete protection for mice against aerosolized *Y. pestis* CO92	[34]
Sylvatic plague vaccine [RCN-F1/V307])	Partially Protects for Prairie Dogs (Cynomys spp.) in Field Trials	[35]
ΔnlpD*Y. pestis* Kimberley53	Provided complete protection against s.c. challenge with *Y. pestis* Kimberley53 and 82% protection against i.n. challenge with *Y. pestis* Kimberley53	[36]
Live attenuated S. Typhimurium mutant strain, χ12094(pYA5383) delivering three protective antigens (LcrV, F1 and Psn)	complete protection against s.c. challenge with 5700 CFU (~570 LD50) of Y. pestis CO92 and 60% protection against intranasal challenge with 5000 CFU (~50 LD50) of *Y. pestis* CO92	[37]
F. tularensis LVS capB/Yp	50% protection against intranasal challenge with 1900 CFU of Y. pestis CO92	[16]
dual anthrax-plague nanoparticle vaccine employing bacteriophage (phage) T4	Complete protection against inhalational anthrax and/or pneumonic plague in three animal challenge models, namely, mice, rats, and rabbits.	[12]
ΔnlpD*Y. pestis* 231 ΔnlpD Y. microtus I-3455 and ΔnlpD Y. microtus I-2359	provided potent immunity against plague in the mouse model	[38]

Y. pseudotuberculosis is less virulent (typically causes a limited enteric disease in human and animals) therefore, recombinant attenuated *Y. pseudotuberculosis* strains as a plague vaccine would be safer alternatives. After a comprehensive screening of *Y. pseudotuberculosis*, one strain (IP32680) was used as a vaccine agent as it harbors minimum virulence genes as compared to the others. Oral delivery of IP32680 showed partial protection in mice [21]. Also, a new strain V674pF1 was generated from IP32680, by deletion of three essential virulence factors and insertion of the *Y. pestis* F1 antigen. Oral immunization of V674pF1 induced both humoral and cellular responses. A single oral dose conferred 100% protection against a lethal pneumonic plague CO92 strain [22].

DNA vaccines, a plasmid encoding the gene of interest, also used to induce protection against a variety of infections. The plasmids expressing the F1 antigen [23,24], or V antigen [25] provide partial protection. The rF1-V formulation adjuvanted with Alhydrogel generates a strong Th2 response. A formulation incorporating SA-4-1BBL, an agonist of the CD137 costimulatory pathway and a potent inducer of Th1 response, enhanced Th1 response to rF1-V formulation [26]. Also, alumadjuvant further enhanced the immunogenicity of SA-4-1BBL [27]. The SA-4-1BBL showed a gender biased protection in the mice as male mice showed better protection that female [26].

Other Yops (Yersinia outer membrane proteins) have also been studied. *Y. pestis* YadB and YadC are two new outer membrane proteins related to its pathogenicity. Mice immunized with *Salmonella* encoding YadC, YadC810, or YadBC develop enhanced humoral response and showed partial protection against the intranasal challenge of *Y.pestis* CO92 [39]. Other Yops like HmuR, Psn and modified forms of

LcrV196 or LcrV5214 delivered by live attenuated Salmonella strain showed high humoral response and showed protection against *Y.pestis* CO92 [40]. PsaA, an adhesion, synthesized inside macrophage. BALB/c mice immunized with Salmonella based PsaA formulation elicited systemic immune response but limited protection against lethal challenge with the *Y.pestis* CO92 [41].

SYNTHETIC ANTIGENS/PEPTIDES

With the advancement of peptide synthesis technologies, improved productivity, and reduced metabolism of peptides, a large number of synthetic peptides have been designed and studied for their efficacy, therapeutic and prophylactic properties. Synthetic peptides have been used as agonists, antagonists, inhibitors in various disease models. Also, epitope identification approaches that identify immunodominant epitopes on an entire protein become popular. With the epitope identification approach, entire antigens/proteins can be fragmented into various small peptides called epitopes [42]. With the advancement of bioinformatics, a large number of resources are available that can predict the putative MHC class I and class II epitopes [43,44]. Using these online resources, B and T cell epitopes on F1, V and YscF antigen were identified and their immunogenicity was tested in murine model [45-47]. Also, HLA-DR1 restricted epitope of CaF1 was identified in humanized mice.

Moreover, for an optimum immune response which exhibits both Th1 and Th2 type of immune profile, the identified epitopes were used in various combination using B and T epitope conjugated or mixed and using a suitable adjuvant and micro particle delivery system. This approach showed enhanced humoral and cellular immune response against epitopes and showed partial protection in murine model [6,48-51]. Moreover, a large number of CTL epitopes were identified using a combined high throughput computational and experimental. A total of 1532 peptides were identified using IFNγ release approach from splenocytes isolated from vaccinated mice [52].

Multiple-antigenic peptide approaches have been exploited to enhance the immunogenicity of antigens/epitopes and improved protection. F1-V fusion protein has been used in combination with adjuvants in several studies. Instead of using whole antigen, delineated epitopes have been used either by physical mixing or chemical conjugation. Advancement in synthetic chemistry makes it possible to synthesize multiple epitopes (chimeric) or multiple copies of an epitope using single start point [42,53,54]. Multiple Antigenic Peptides (MAP) have been designed and synthesized using immunodominant B and T epitopes of F1, V and YscF antigen. The MAPs were encapsulated in Poly DL-Lactide-Co-Glycolide (PLGA) micro particles and administered to out bred mice with CpGas an adjuvant through intranasal route. The intranasal route has an advantage as it generates systemic as well as mucosal immunity. The MAPs showed enhanced B and T cell-specific immune response in murine model. Moreover, the formulation exhibited mucosal immune response as measured by secretory antibody and secretory component level [4,55-60].

CONCLUSION

Plague has been a high priority infectious disease for researchers because of its virulence, mode of transmission, outstanding skill to escape the human immune system, and indelible history of its pandemics that caused enormous loss. Due to constant fear of the use of the pathogen as an agent of biowarfare/bioterrorism, development of therapeutics and/or prophylactics has been in progress using various combinations and formulations. F1 and V antigens have been highly exploited. Moreover, other Yopslike YscF, Pla, YadC have also been used to develop effective prophylactics. Multiple antigen/peptide/epitope approach needs to be explored more vigorously to develop an effective prophylactic agent. Multiple antigen/peptide approach generates a variety of antibodies, antigen-specific T cells and cytokine response. This approach might provide advantages over single antigen.

AUTHOR CONTRIBUTIONS

The manuscript was written by Riyasat Ali, Sudhir Kumar, and D.N.Rao.

COMPETING INTERESTS

The authors declare no competing interests

REFERENCES

1. Zietz BP, Dunkelberg H. (2004). The history of the plague and the research on the causative agent Yersinia pestis. Int J Hyg Environ Health. 207: 165-178.
2. Perry RD, Fetherston JD. (1997). Yersinia pestis--etiologic agent of plague. Clin Microbiol Rev. 10: 35-66.

3. Cornelis GR. (2000). Molecular and cell biology aspects of plague. Proceedings of the National Academy of Sciences of the United States of America. 97: 8778-8783.
4. Shreewastav RK, Ali R, Uppada JB, Rao DN. (2012). Cell-mediated immune response to epitopic MAP (multiple antigen peptide) construct of LcrV antigen of Yersinia pestis in murine model. Cellular immunology. 278: 55-62.
5. Christopher GW, Cieslak TJ, Pavlin JA, Eitzen EM Jr. (1997). Biological warfare. A historical perspective. Jama. 278: 412-417.
6. Gupta G, Ali R, Khan AA, Rao DN. (2012). Evaluation of CD4+/CD8+ T-cell expression and IFN-gamma, perforin secretion for B-T constructs of F1 and V antigens of Yersinia pestis. International immunopharmacology. 12: 64-73.
7. Quenee LE, Ciletti NA, Elli D, Hermanas TM, Schneewind O. (2011). Prevention of pneumonic plague in mice, rats, guinea pigs and non-human primates with clinical grade rV10, rV10-2 or F1-V vaccines. Vaccine. 29: 6572-6583.
8. Williamson ED, Flick-Smith HC, Lebutt C, Rowland CA, Jones SM, et al. (2005). Human immune response to a plague vaccine comprising recombinant F1 and V antigens. Infection and immunity. 73: 3598-3608.
9. Quenee LE, Berube BJ, Segal J, Elli D, Ciletti NA, et al. (2010). Amino acid residues 196-225 of LcrV represent a plague protective epitope. Vaccine. 28: 1870-1876.
10. Sun W, Singh AK. (2019). Plague vaccine: recent progress and prospects. npj Vaccines. 4: 1-9.
11. Tao P, Mahalingam M, Rao VB. (2016). Highly effective soluble and bacteriophage T4 nanoparticle plague vaccines against Yersinia pestis. Vaccine Design: Springer. 1403: 499-518.
12. Tao P, Mahalingam M, Zhu J, Moayeri M, Sha J, et al. (2018). A bacteriophage T4 nanoparticle-based dual vaccine against anthrax and plague. MBio. 9: e01926-2018.
13. Frey SE, Lottenbach K, Graham I, Anderson E, Bajwa K, et al. (2017). A phase I safety and immunogenicity dose escalation trial of plague vaccine, Flagellin/F1/V, in healthy adult volunteers (DMID 08-0066). Vaccine. 35: 6759-6765.
14. Hamzabegovic F, Goll JB, Hooper WF, Frey S, Gelber CE, et al. (2020). Flagellin adjuvanted F1/V subunit plague vaccine induces T cell and functional antibody responses with unique gene signatures. npj Vaccines. 5: 1-10.
15. Gallagher TB, Mellado-Sanchez G, Jorgensen AL, Moore S, Nataro JP, et al. (2019). Development of a multiple-antigen protein fusion vaccine candidate that confers protection against Bacillus anthracis and Yersinia pestis. PLoS neglected tropical diseases. 13: e0007644.
16. Jia Q, Bowen R, Dillon BJ, Masleša-Galić S, Chang BT, et al. (2018). Single vector platform vaccine protects against lethal respiratory challenge with Tier 1 select agents of anthrax, plague, and tularemia. Scientific reports. 8: 1-20.
17. Singh AK, Curtiss R, Sun W. (2019). A Recombinant Attenuated Yersinia pseudotuberculosis Vaccine Delivering a Y. pestis YopENt138-LcrV Fusion Elicits Broad Protection against Plague and Yersiniosis in Mice. Infection and immunity. 87: e00296-219.
18. Derbise A, Hanada Y, Khalifé M, Carniel E, Demeure CE. (2015). Complete protection against pneumonic and bubonic plague after a single oral vaccination. PLoS neglected tropical diseases. 9.
19. Demeure CE, Derbise A, Carniel E. (2017). Oral vaccination against plague using Yersinia pseudotuberculosis. Chemico-biological interactions. 267: 89-95.
20. Demeure CE, Derbise A, Guillas C, Gerke C, Cauchemez S, et al. (2019). Humoral and cellular immune correlates of protection against bubonic plague by a live Yersinia pseudotuberculosis vaccine. Vaccine. 37: 123-129.
21. Blisnick T, Ave P, Huerre M, Carniel E, Demeure CE. (2008). Oral vaccination against bubonic plague using a live avirulent Yersinia pseudotuberculosis strain. Infection and immunity. 76: 3808-3816.
22. Derbise A, Marín AC, Ave P, Blisnick T, Huerre M, et al. (2012). An encapsulated Yersinia pseudotuberculosis is a highly efficient vaccine against pneumonic plague. PLoS neglected tropical diseases. 6.
23. Grosfeld H, Cohen S, Bino T, Flashner Y, Ber R, et al. (2003). Effective protective immunity to Yersinia pestis infection conferred by DNA vaccine coding for derivatives

of the F1 capsular antigen. Infection and immunity. 71: 374-383.

24. Alvarez ML, Cardineau GA. (2010). Prevention of bubonic and pneumonic plague using plant-derived vaccines. Biotechnology advances. 28: 184-196.
25. Garmory HS, Freeman D, Brown KA, Titball RW. (2004). Protection against plague afforded by immunisation with DNA vaccines optimised for expression of the Yersinia pestis V antigen. Vaccine. 22: 947-957.
26. Bowen W, Batra L, Pulsifer AR, Yolcu ES, Lawrenz MB, et al. (2019). Robust Th1 cellular and humoral responses generated by the Yersinia pestis rF1-V subunit vaccine formulated to contain an agonist of the CD137 pathway do not translate into increased protection against pneumonic plague. Vaccine. 37: 5708-5716.
27. Dinc G, Pennington JM, Yolcu ES, Lawrenz MB, Shirwan H. (2014). Improving the Th1 cellular efficacy of the lead Yersinia pestis rF1-V subunit vaccine using SA-4-1BBL as a novel adjuvant. Vaccine. 32: 5035-5040.
28. Verma SK, Batra L, Tuteja U. (2016). A Recombinant Trivalent Fusion Protein F1–LcrV–HSP70 (II) Augments Humoral and Cellular Immune Responses and Imparts Full Protection against Yersinia pestis. Frontiers in microbiology. 7: 1053.
29. Gregg KA, Harberts E, Gardner FM, Pelletier MR, Cayatte C, et al. (2018). A lipid A-based TLR4 mimetic effectively adjuvants a Yersinia pestis rF-V1 subunit vaccine in a murine challenge model. Vaccine. 36: 4023-4031.
30. Tao P, Mahalingam M, Zhu J, Moayeri M, Kirtley ML, et al. (2017). a Bivalent anthrax–Plague Vaccine That can Protect against Two Tier-1 Bioterror Pathogens, *Bacillus anthracis* and *Yersinia pestis*. Frontiers in immunology. 8: 687.
31. Moore B, New R, Butcher W, Mahood R, Steward J, et al. (2018). Dual route vaccination for plague with emergency use applications. Vaccine. 36: 5210-5217.
32. Liu L, Wei D, Qu Z, Sun L, Miao Y, et al. (2018). A safety and immunogenicity study of a novel subunit plague vaccine in cynomolgus macaques. Journal of applied toxicology. 38: 408-417.
33. Hu J, Jiao L, Hu Y, Chu K, Li J, et al. (2018). One year immunogenicity and safety of subunit plague vaccine in Chinese healthy adults: An extended open-label study. Human vaccines & immunotherapeutics. 14: 2701-2705.
34. Sha J, Kirtley ML, Klages C, Erova TE, Telepnev M, et al. (2016). A replication-defective human type 5 adenovirus-based trivalent vaccine confers complete protection against plague in mice and nonhuman primates. Clin Vaccine Immunol. 23: 586-600.
35. Rocke TE, Tripp DW, Russell RE, Abbott RC, Richgels KL, et al. (2017). Sylvatic plague vaccine partially protects prairie dogs (Cynomys spp.) in field trials. EcoHealth. 14: 438-450.
36. Tidhar A, Flashner Y, Cohen S, Levi Y, Zauberman A, et al. (2009). The NlpD lipoprotein is a novel Yersinia pestis virulence factor essential for the development of plague. PloS one. 4.
37. Sanapala S, Rahav H, Patel H, Sun W, Curtiss R. (2016). Multiple antigens of Yersinia pestis delivered by live recombinant attenuated Salmonella vaccine strains elicit protective immunity against plague. Vaccine. 34: 2410-2416.
38. Dentovskaya S, Ivanov S, Kh KP, Shaikhutdinova R, Kombarova T, et al. (2015). Selective protective potency of Yersinia pestis ΔnlpD mutants. Acta Naturae. 7.
39. Sun W, Olinzock J, Wang S, Sanapala S, Curtiss 3rd R. (2014). Evaluation of YadC protein delivered by live attenuated Salmonella as a vaccine against plague. Pathogens and disease. 70: 119-131.
40. Branger CG, Sun W, Torres-Escobar A, Perry R, Roland KL, et al. (2010). Evaluation of Psn, HmuR and a modified LcrV protein delivered to mice by live attenuated Salmonella as a vaccine against bubonic and pneumonic Yersinia pestis challenge. Vaccine. 29: 274-282.
41. Torres-Escobar A, Juarez-Rodriguez MD, Branger CG, Curtiss R, 3rd. (2010). Evaluation of the humoral immune response in mice orally vaccinated with live recombinant attenuated Salmonella enterica delivering a secreted form of Yersinia pestis PsaA. Vaccine. 28: 5810-5816.
42. Ali R, Rani R, Kumar S. (2013). New peptide based therapeutic approaches. Advances in Protein Chemistry Jeddah: OMICS Group eBooks.

43. Rammensee H-G, Bachmann J, Emmerich NPN, Bachor OA, Stevanović S. (1999). SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics. 50: 213-219.
44. Bhasin M, Raghava G. (2004). SVM based method for predicting HLA-DRB1* 0401 binding peptides in an antigen sequence. Bioinformatics. 20: 421-423.
45. Khan AA, Babu JP, Gupta G, Rao D. (2008). Identifying B and T cell epitopes and studying humoral, mucosal and cellular immune responses of peptides derived from V antigen of Yersinia pestis. Vaccine. 26: 316-332.
46. Ali R, Kumar S, Naqvi RA, Rao DN. (2013). B and T cell epitope mapping and study the humoral and cell mediated immune response to B-T constructs of YscF antigen of Yersinia pestis. Comparative immunology, microbiology and infectious diseases. 36: 365-378.
47. Sabhnani L, Rao DN. (2000). Identification of immunodominant epitope of F1 antigen of Yersinia pestis. FEMS Immunology & Medical Microbiology. 27: 155-162.
48. Uppada JB, Khan AA, Bhat AA, Deshmukh R, Rao DN. (2009). Humoral immune responses and protective efficacy of sequential B-and T-cell epitopes of V antigen of Yersinia pestis by intranasal immunization in microparticles. Medical microbiology and immunology. 198: 247-256.
49. Sabhnani L, Manocha M, Sridevi K, Shashikiran D, Rayanade R, et al. (2003). Developing subunit immunogens using B and T cell epitopes and their constructs derived from the F1 antigen of Yersinia pestis using novel delivery vehicles. FEMS Immunology & Medical Microbiology. 38: 215-229.
50. Gupta G, Khan A, Rao D. (2010). Cell-Mediated Immune Response and Th1/Th2 Cytokine Profile of B-T Constructs of F1 and V Antigen of Yersinia pestis. Scandinavian journal of immunology. 71: 186-198.
51. Musson JA, Ingram R, Durand G, Ascough S, Waters EL, et al. (2010). Repertoire of HLA-DR1-restricted CD4 T-cell responses to capsular Caf1 antigen of Yersinia pestis in human leukocyte antigen transgenic mice. Infection and immunity. 78: 4356-4362.
52. Zvi A, Rotem S, Zauberman A, Elia U, Aftalion M, et al. (2017). Novel CTL epitopes identified through a Y. pestis proteome-wide analysis in the search for vaccine candidates against plague. Vaccine. 35: 5995-6006.
53. Partidos C, Obeid O, Steward M. (1992). Antibody responses to non-immunogenic synthetic peptides induced by co-immunization with immunogenic peptides. Immunology. 77: 262.
54. Tam JP. (1988). Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proceedings of the National Academy of Sciences. 85: 5409-5413.
55. Ali R, Kumar S, Naqvi RA, Sheikh IA, Rao DN. (2013). Multiple antigen peptide consisting of B- and T-cell epitopes of F1 antigen of Y. pestis showed enhanced humoral and mucosal immune response in different strains of mice. International immunopharmacology. 15: 97-105.
56. Ali R, Naqvi RA, Kumar S, Bhat AA, Rao DN. (2013). Multiple antigen peptide containing B and T cell epitopes of F1 antigen of Yersinia pestis showed enhanced Th1 immune response in murine model. Scandinavian journal of immunology. 77: 361-371.
57. Rai R, Das B, Choudhary N, Talukdar A, Rao DN. (2015). MAP of F1 and V antigens from Yersinia pestis astride innate and adaptive immune response. Microbial pathogenesis. 87: 13-20.
58. Uppada SB, Bhat AA, Sah A, Donthamshetty RN. (2011). Enhanced humoral and mucosal immune responses after intranasal immunization with chimeric multiple antigen peptide of LcrV antigen epitopes of Yersinia pestis coupled to palmitate in mice. Vaccine.; 29: 9352-9360.
59. Klinman DM. (2004). Immunotherapeutic uses of CpG oligodeoxynucleotides. Nature Reviews Immunology. 4: 249-259.
60. Klinman DM, Xie H, Little SF, Currie D, Ivins BE. (2004). CpG oligonucleotides improve the protective immune response induced by the anthrax vaccination of rhesus macaques. Vaccine. 22: 2881-2886.