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Recombinant *Salmonella* Bacteria Vectoring HIV/AIDS Vaccines

Nyasha Chin'ombe[*,1,2] and Vurayai Ruhanya[1]

[1]*Department of Medical Microbiology, University of Zimbabwe, Harare, Zimbabwe*

[2]*Division of Medical Virology, University of Cape Town, Cape Town, South Africa*

Abstract: HIV/AIDS is an important public health problem globally. An affordable, easy-to-deliver and protective HIV vaccine is therefore required to curb the pandemic from spreading further. Recombinant *Salmonella* bacteria can be harnessed to vector HIV antigens or DNA vaccines to the immune system for induction of specific protective immunity. These are capable of activating the innate, humoral and cellular immune responses at both mucosal and systemic compartments. Several studies have already demonstrated the utility of live recombinant *Salmonella* in delivering expressed foreign antigens as well as DNA vaccines to the host immune system. This review gives an overview of the studies in which recombinant *Salmonella* bacteria were used to vector HIV/AIDS antigens and DNA vaccines. Most of the recombinant *Salmonella*-based HIV/AIDS vaccines developed so far have only been tested in animals (mainly mice) and are yet to reach human trials.

Keywords: *Salmonella*, bacteria, vectoring, HIV/AIDS, antigens, DNA, vaccines.

INTRODUCTION TO *SALMONELLA* AND STRATEGIES FOR VECTORING VACCINES

Salmonella bacteria infect their host *via* the intestine by crossing the epithelial barrier through the M cells overlying lymphoid follicles [1.2]. The translocation of *Salmonella* into the Peyer's patches of the intestine through the M cells is important for the systematic spread of the bacteria to distant organs such as the spleen and liver [3,4]. The bacteria are engulfed by the macrophages, dendritic cells and neutrophils, into which replication occurs [5,6]. To achieve this, the bacteria utilize the type-III secretion systems, (TTSS) designed to transport pathogenic proteins through both the bacterial and target host cell membranes [7]. *Salmonella* encode at least 2 virulence-associated TTSS. The first, *Salmonella* Pathogenicity Island 1 (SPI-1), is important for epithelial cell invasion and enteric pathogenesis [8,9]. The second pathogenicity island, SPI-2, is critical for intracellular pathogenesis and systemic infection by the bacteria [8,10]. After contact with target cells, TTSS-mediated translocation of effector proteins occurs and this results in successful bacterial invasion [11]. SPI-2 gene expression is induced by phagocytosis and SPI-2 mutants cannot replicate efficiently within host cells [12]. Several other *Salmonella* pathogenicity islands such as SPI-3, SPI-4, SPI-5, SPI-6, SPI-7, SPI-8, SPI-9 and SPI-10 have also been identified and play various roles in intracellular survival and virulence of the bacteria [13-18]. Since most *Salmonella enterica* virulence genes are encoded on known pathogenicity islands, it is now possible to systematically attenuate the bacteria using genetic engineering tools in order to develop vaccines. These vaccines can be harnessed to carry foreign genes and can be used as recombinant vaccines. Other mutants of *Salmonella* generated or identified by traditional methods can also be used as vaccine vectors although some of them may be unsafe for use [19-21]. Several reviews have been written on the feasibility of using attenuated recombinant *Salmonella enterica* to vector foreign antigens or DNA vaccines [22-28].

Two fundamentally different approaches are employed when using *Salmonella* to vector vaccines. The first approach involves the expression of the foreign antigens by the engineered recombinant bacterium. The foreign antigen genes are cloned on plasmid or onto the bacterial chromosome and the expression is driven by a prokaryotic promoter. The foreign antigens are expressed by the bacteria and presented to the immune system of the host after infection. In general, MHC Class II restricted and immunoglobulin responses are recorded in a number of studies [29-32]. However, other groups have also demonstrated the generation of MHC Class I-restricted CD8+ T cells after vaccination of animals with recombinant *Salmonella* expressing the foreign antigens [33-39]. The second approach to vector vaccines using recombinant *Salmonella enterica* involves the delivery of DNA vaccines by the bacteria [40,41]. The recombinant bacteria carrying the recombinant plasmid DNA harbouring foreign antigen gene under eukaryotic promoter enter the host cells for delivery. The host cells will use the host machinery to express the foreign antigens that can ultimately be presented to immune system [42,43]. The two approaches have already been used in development of candidate bacteria-vectored HIV-1 vaccines.

RATIONALE OF USING RECOMBINANT *SALMONELLA* AS HIV-1 VACCINE VECTORS

One of the key features of mucosal sites is the presence of M cells that play important roles in uptake and transport

*Address correspondence to this author at the Department of Medical Microbiology, University of Zimbabwe, P O Box A178, Avondale, Harare, Zimbabwe; Tel: +263-4-791-631, Ext. 2419; Fax: +263-4-792-245;
E-mail: nyasha.chinombe@gmail.com

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of pathogens [2,44,45]. HIV-1 and *Salmonella* utilize these M cells in the mucosal surfaces as their gateways for systemic transmission [2,44,45]. Besides mucosal transmission, HIV-1 and *Salmonella* replication also occurs in the mucosal lymphoid tissue before systemic spread [3,45,46]. The MALT is rich in immune cells such as dendritic cells, macrophages, CD4+ and CD8+ T cells which play important roles in provoking mucosal immunity to HIV-1 or *Salmonella* [6,47]. The rationale of using recombinant *Salmonella* to vector candidate HIV-1 vaccines is therefore based on the fact that the two pathogens use the same mode of infection. The two pathogens provoke the same type of immune responses, that is, innate, mucosal and systemic cellular and humoral immune responses [28,48-51]. In recent years, the innate immune responses to HIV, especially the role of NK cells have attracted a lot of interest in the development of HIV/AIDS vaccines. Recombinant *Salmonella* vectoring HIV/AIDS vaccines are capable of activating such immune responses that are also key in the induction of the adaptive responses [28]. Therefore *Salmonella* vectors can be exploited to deliver HIV-1 antigens for induction of protective mucosal and systemic immune responses to potentially block the invasion of HIV after sexual intercourse.

There are a number of advantages of using *Salmonella* as vectors for HIV-1 vaccines. The recombinant bacteria can be used as oral vaccines that can be delivered easily. Vaccine-induced immune responses or protection can be achieved at mucosal surfaces as well as in the systemic compartments [52]. The bacteria are capable of stimulating broad humoral as well as cell-mediated immune responses in both mucosal and systematic lymphoid tissues [52,53]. The bacteria are fast growing and vaccines can therefore be manufactured expeditiously. Several genetic engineering techniques developed for *E. coli* manipulation can be applied to *Salmonella* easily as the two bacteria are closely related [52,54]. Attenuation of specific *Salmonella* genes to generate vaccines can now easily be done using recombinant DNA technology. *Salmonella* also possess intrinsic adjuvantic properties that will make the vaccines very immunogenic [55]. Despite disarmed virulence, attenuated recombinant *Salmonella* can still colonize the lymphoid tissues after inoculation, but they do not cause disease. They are therefore safe for use and can even be used in HIV-positive patients [54,56].

RECOMBINANT *SALMONELLA* VECTORS EXPRESSING HIV-1 VACCINE ANTIGENS

Recombinant *Salmonella* expressing HIV-1 antigens have a great potential in the development of vaccines to curb AIDS globally. A number of studies have already been done in which recombinant *Salmonella*-vectored HIV-1 antigens were tested, mostly in small animals such as mice. Generally, HIV-1 antigens can be expressed by the recombinant *Salmonella* bacterium either from its chromosome harboring the HIV-1 gene or from a plasmid carrying the gene. Chromosomal integration has the advantage of increasing the stability of foreign antigens and also protects them against proteolytic degradation [52,57]. Expression of genes from the plasmid has the advantage of increasing gene dosage [58]. The expressed antigens can be presented to the immune system after uptake of the bacterium by the host.

A recombinant *Salmonella enterica* serovar Typhi constitutively expressing HIV-1 gp120 from the bacterial chromosome was previously constructed [59]. High levels of the gp120 were shown to be expressed by the vector [59]. The same antigen was also cloned chromosomally and expressed in recombinant *Salmonella enterica* serovar Typhimurium and its immunogenicity evaluated in mice [60]. Another recombinant *Salmonella enterica* serovar Typhimurium expressing the antigen episomally from a multicopy plasmid was constructed by the same group [60]. The recombinant *Salmonella* expressed more gp120 from the plasmid than from the chromosome [60]. Oral vaccination of mice with the two recombinant *Salmonella* vaccines did not induce HIV-1 env-specific CD8+ T cell responses or a significant antibody response [60]. However, there was some gp120-specific Th1 response in mice vaccinated with the *Salmonella* vector expressing gp120 from the plasmid [60]. This was the first study to demonstrate that a recombinant *Salmonella* expressing cytoplasmic HIV-1 gp120 could induce an immune response in mice. Later, recombinant *Salmonella* expressing the HIV-1 gp120 on the bacterial surface was constructed and found to be more immunogenic than the one expressing the antigen in the bacterial cytoplasm [61]. Another recombinant *Salmonella* expressing a truncated derivative of HIV-1 gp120 and displayed in the bacterial periplasm was later constructed and found to be highly immunogenic after only a single inoculation of mice [62]. The vector induced systemic gp120-specific splenic CD4+ Th1and Th2 responses in the spleen after vaccination of mice [62]. The vector also induced strong mucosal gp120-specific IgA antibody-secreting cell responses [62]. This study clearly demonstrated that recombinant *Salmonella*-vectored secreted HIV-1 antigens could induce better T cell responses than those expressed cytoplasmically. More recently, a recombinant *Salmonella enterica* serovar Typhi expressing HIV-1 gp120 from a plasmid and HIV-1 Gag from the bacterial chromosome was constructed and tested [63]. Mice inoculated intranasally with the recombinant vector elicited high titers of gp120-specific IgG in the sera and gp120-specific IgA in fecal washes [63]. Systemic Gag-specific and gp120-specific CD8+ T cell responses were also induced in vaccinated animals [63]. This study further highlighted that recombinant *Salmonella* bacteria could potentially be used to vector HIV/AIDS vaccines. It is now generally accepted that induction of HIV-1 envelope-specific humoral responses is very critical in developing successful HIV vaccines. Although recombinant *Salmonella* may induce Env-specific humoral responses as shown by the studies mentioned above, the antibodies are unlikely to be neutralizing since the Env expressed by the bacteria lacks post-translational modifications found in nature. However, the Env-specific CD8+ and CD4+ T cell responses elicited by the recombinant bacteria should have the potential to protect against HIV infection. The non-neutralizing Env-specific antibodies induced by the bacteria may also have the potential to protect against HIV infection through antibody-dependent cell-mediated pathway.

Salmonella enterica vaccine vectors have also been used to express HIV-1 Gag antigens [reviewed in 28]. Most of the studies have shown that Gag expressed in *Salmonella* could

provoke HIV-specific immune responses in mice [28]. Briefly, recombinant *Salmonella* expressing HIV-1 Subtype C Gag elicited Gag-specific CD4+ Th1 and Th2 cytokine responses as well as Gag-specific IgG1 (Th1) and IgG2a (Th2) responses [29]. Recombinant *Salmonella enterica* serovar Typhimurium vaccine secreting HIV-Gag (p24) using the hemolysin secretorial signal of *E. coli* was also shown to elicit Gag-specific humoral and T cell responses in mice [64]. A recombinant *Salmonella* expressing HIV-1 Gag fused to the secretable bacterial Type III secretion system SopE protein elicited *Salmonella*-specific mucosal immune responses in vaccinated human volunteers, but no HIV-Gag specific responses were detected after a single dose [65]. Boosting with the same recombinant vector could have induced HIV-Gag-specific responses. Recombinant *Salmonella enterica* serovar Typhi expressing HIV-1 Gag from the bacterial chromosome also elicited systemic Gag-specific cytotoxic T lymphocyte responses in mice vaccinated intranasally [63]. Recombinant *Salmonella* vaccine vector expressing codon optimized HIV-1 Gag induced Gag-specific mucosal CD8+ T cell and humoral responses in mice vaccinated orally [66]. Other HIV-1 antigens have also been expressed in recombinant *Salmonella* vaccine vectors for delivering to the immune system. In our previous study, we successfully overexpressed codon-optimized HIV-1 Tat and Nef in *Salmonella enterica* serovar Typhimurium [67]. Animal studies to investigate the immunogenicity of the recombinant *Salmonella* expressing the two antigens are underway. All these studies showed that recombinant *Salmonella* could vector an HIV antigen, Gag, to induce specific immune responses. So far the target antigens for expression in recombinant *Salmonella* have been Env and Gag. These antigens have most of the B- and T-cell epitopes which are important in induction of protective immune response against HIV/AIDS infection [68-70].

RECOMBINANT *SALMONELLA* VECTORS DELIVERING HIV DNA VACCINES

The use of naked DNA as vaccines was originally demonstrated about two decades ago [71]. Since then, naked DNA vaccines have been used for induction of potent immune responses, especially cell-mediated [72,73]. The DNA inoculated by intradermal or intramuscular injection is taken by professional antigen presenting cells such as macrophages and dendritic cells which express the foreign antigen for presentation and induction of specific immune response [74]. In recent years, it has been demonstrated that *Salmonella* vaccines can vector naked DNA vaccines to deliver these DNA vaccines directly to the antigen-presenting cells such as macrophages and dendritic cells [23,74-78]. A number of studies have already demonstrated that recombinant *Salmonella* vectors can deliver HIV/AIDS DNA vaccines. Most of the studies have been done in animal models.

HIV-1 DNA vaccines delivered by recombinant *Salmonella* have been constructed. Most of the vaccines developed so far were based on the HIV-1 *env (gp160)* gene. A recombinant *Salmonella* Env DNA vaccine vector was constructed and used to vaccinate mice intragastrically [78]. Both mucosal and systemic HIV-1 Env-specific CD8+ T-cell responses were induced in vaccinated mice [78]. The plasmid HIV-1 Env DNA vaccine delivered intramuscularly only generated systemic but not mucosal CD8+ T cell responses in the vaccinated animals [78]. Such vaccine-induced T cell responses are very important in protecting or controlling sexual HIV transmission. This study was the first to demonstrate the feasibility of using recombinant *Salmonella* in vectoring HIV-1 DNA vaccine to induce specific cellular immune responses. Later, recombinant *Salmonella* and *Shigella* were compared in the capacity to vector the HIV-1 Env DNA vaccine [79]. Better cellular immune responses were generated by the recombinant *Shigella* than by the *Salmonella* [79]. This could be because the *Shigella* normally escapes the phagosome into the cytosol while the *Salmonella* remains confined in the phagosome. To further improve the immunogenicity of recombinant *Salmonella* vector, the HIV-1 Env (gp120) DNA vaccine was constructed with the gp120 and cholera toxin catalytic domain (CTA1) genes co-expressed [80]. Mice vaccinated with recombinant *Salmonella* vectoring the DNA vaccine co-expressing Env and CTA1 induced HIV-1 gp120-specific IgG responses that were more than 1000-fold greater than in mice vaccinated with a vector delivering the DNA vaccine only expressing the gp120 antigen [80]. The mice vaccinated with recombinant *Salmonella* delivering the DNA vaccine expressing gp120 and CTA1 also generated significantly more gp120-specific IFN-gamma ELISPOTs than mice vaccinated with the Salmonella carrying DNA expressing gp120 DNA vaccine alone [80]. These results demonstrated that HIV-1 DNA vaccines could be vectored more efficiently by recombinant *Salmonella* when adjuvants such as the cholera toxin were used. Recombinant *Salmonella enterica* serovar Typhimurium delivering an HIV-1 DNA vaccine expressing a polyepitope protein (composed of more than 80 CTL epitopes from HIV-1 subtype A, B and C proteins) was previously evaluated. The recombinant *Salmonella* elicited better HIV specific serum antibody, proliferative and CTL responses than by naked DNA vaccine in vaccinated mice [81]. The ability of recombinant *Salmonella enteritidis* to vector the same HIV-1 DNA vaccine was also later evaluated by the same research group [82]. Mice vaccinated with the *Salmonella* vector elicited both HIV-1 –specific humoral and cellular responses [82]. This study further demonstrated the capacity of recombinant *Salmonella* bacteria in vectoring even HIV-1 DNA vaccines expressing polyepitopes. HIV-1 Gag (p24) DNA vaccine was successfully delivered using recombinant *Salmonella* in mice which elicited HIV-specific immune responses [64]. An adjuvant, MCP3 was used to improve the immunogenicity of the *Salmonella* vector [64].

The use of bacterial ghosts as a vaccine delivery platform has gained momentum in recent years [83-85]. Some of them have been found to be immunogenic [86]. Recently, recombinant *Salmonella* typhi Ty21a carrying HIV-1 gp140 DNA vaccine has been used as bacterial ghosts (BGs) [87]. It was demonstrated that the Ty21a BGs carrying the HIV-1 gp140 DNA vaccine could be taken up and efficiently expressed by macrophages [87]. Mice inoculated with BGs-DNA vaccine elicited significantly higher immune responses than those inoculated with the plasmid DNA vaccine alone [87]. The results demonstrated that recombinant *Salmonella* bacterial ghosts could be used to vector HIV-1 DNA vaccines.

The use of heterologous prime-boost vaccination strategies to induce better cellular and humoral responses to vaccination has become popular in development of HIV vaccines in recent years. This has been successful especially when DNA and viral vectors are used to deliver HIV vaccines. In literature, we did not come across studies in which recombinant *Salmonella* vectoring was used in heterologous prime-boost strategy. We hope future *Salmonella*-based vaccines should employ this strategy since it may improve the immune responses elicited.

ANTI-*SALMONELLA* PRE-EXISTING IMMUNITY AND VECTORING OF HIV-1 VACCINES

The existence of prior immunity to vaccine vectors needs to be considered when developing vaccines for HIV/AIDS. This comes after one of the HIV/AIDS vaccines vectored by a recombinant adenovirus failed to induce the expected immune responses, but enhanced infection [88]. The impact of pre-existing anti-vector immunity on the efficiency of recombinant *Salmonella* vaccine vectors in delivering foreign antigens is still poorly understood. It is currently not clear whether prior exposure to *Salmonella* enhances or decreases the immune response to vectored antigens. In early studies, it has been found that pre-existing *Salmonella* immunity in mice improved their subsequent humoral and mucosal immune responses [89]. These observations were later supported by studies which showed that antibody responses to a viral antigen expressed by a recombinant *Salmonella* were enhanced in mice that were previously primed with the vector alone [90]. It was also demonstrated that secondary immune responses to a recombinant *Salmonella* expressing *Streptococcus mutans* antigen were not affected by pre-existing immunity [91]. In humans vaccinated with recombinant *Salmonella enterica* serovar Typhi Ty21a expressing *Helicobacter. pylori* ureases A and B, it was shown that that prior vector immunity enhanced immune responses to the foreign antigens [92]. It was also shown that animals previously exposed to the vector induced much higher CD8+ T cell responses when compared with animals that did not have any pre-existing *Salmonella* immunity [93]. These studies, though limited, suggest that prior exposure to the vector enhances immunogenicity to the heterologous antigens. The mechanisms of this enhancement of immune responses are not yet clear. However, it has been suggested that this could be due to enhanced uptake of antibody-coated vector by antigen-presenting cells such as macrophages and dendritic cells during antigen presentation [94]. However, contrasting results have been found in other studies. One study showed that pre-existing *Salmonella* immunity reduced memory serum responses and further interfered with immune response to the foreign antigen [95,96]. It was also shown that subsequent rapid clearance of the vector was due to better *Salmonella*-specific CD8+ T cell responses induced after initial priming [97]. After inoculation, the vector was cleared within 7 days, and after the second booster inoculation, the clearance was within 4 days [97]. Whether rapid clearance of the vector affects the immune response to the delivered antigen, is not clear. Some studies also showed that ability of the recombinant *Salmonella* vector to colonize was significantly compromised in animals that had previously been exposed to the vector [98]. A recent review of the literature has however concluded that recombinant bacterial vaccine vectors such as *Salmonella*, in most cases, enhance immunogenicity, mainly humoral immune responses, while for viral vectors such as adenovirus, pre-existing immunity hinders subsequent induction of cell-mediated responses [99]. Further research needs to be done to clarify whether pre-existing vector immunity will have any impact on the use of *Salmonella* bacterial vaccines vectors, especially in Africa, where the wild-type *Salmonella enterica* serovars are endemic.

CONCLUSIONS

This review shows that there is a growing body of literature on the use of recombinant *Salmonella* bacterial vaccines as potential delivery vectors for HIV/AIDS antigens and DNA vaccines. So far a number of *Salmonella*-based HIV/AIDS vaccines have already been tested in animal trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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