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Listeria monocytogenes as an Alternative Vaccine Vector for HIV

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Abstract: The necessity for an HIV vaccine and a brief review of current strategies towards this aim are given here to set into context contemporary studies towards exploiting the bacterium *Listeria monocytogenes* as an HIV vaccine vector. The cell biology and immunology of this unusual intracellular organism are also reviewed, in addition to its application to introducing viral antigens, including HIV antigens, to the immune system.

Key words: HIV vaccine; *Listeria monocytogenes*; vaccine vector.

The Requirement for an AIDS Vaccine

AIDS as a disease was first identified in the early 1980's and its agent was shown to be the HIV virus[6, 54, 85]. HIV primarily infects $CD4^+$ T cells (T-tropic strains) and macrophages (M-tropic strains) and causes a disease characterized by severe immunosuppression, neurologic disease and a high incidence of opportunistic infections and neoplasms[42]. The virus is transmitted through bodily fluids including blood and blood products, sexual contact and mother-fetal interactions in utero and peripartum and postnatally by breast feeding[25]. The epidemic of AIDS has claimed 13.9 million lives since it began. As of December of 1998, it was estimated that 33.4 million people around the world were infected with HIV/AIDS, 1.2 million of them being children under the age of 15[128]. Furthermore, AIDS has been established as the fourth leading cause of death worldwide, and the number one in Africa[129]. Although retroviral therapies have proven effective in controlling viral replication[33], costs of such therapies as well as compliance with a therapeutic regime abundant with side effects and the possible emergence of drug resistant mutants has made the development of a prophylactic vaccine an urgent necessity.

HIV Vaccine Development

Contrary to the early hope of the scientific community, development of a vaccine against HIV has remained elusive due to several factors, including the absence of absolute immune correlates of protection and appropriate animal models. Nonetheless, a significant amount of research has been done on possible approaches to prophylactic vaccination against HIV, including the use of attenuated virus, DNA vaccination and vector vaccines; indeed, several phase I and phase II studies are under way. However, it is important to acknowledge that, although the ideal vaccine should

Abbreviations used: hly – hemolysin; LLO – listeriolysin O; inl – internalin; plcA – phospholipase C; mlp – metalloprotease; LCMV – lymphochoriomeningitis virus; Lm-Gag – *L. monocytogenes* construct expressing HIV-Gag; Lm-NP – *L. monocytogenes* construct expressing influenza nucleoprotein.

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prevent infection, it might be sufficient to develop a vaccine that significantly controls the virus, even in the absence of sterilizing immunity. Thus, inhibition of viral replication during primary infection and decreased steady – state levels of viral replication during chronic stages of infection by means of vaccination may halt the progress of HIV disease as well as the spread of the infection in the community[84, 112].

Vaccine approaches against HIV can be classified in four groups: live attenuated virus vaccines, subunit or peptide vaccines, nucleic acid vaccines and vector vaccines. Live, attenuated virus vaccines have been successful for viral diseases such as measles, mumps and rubella and have been of interest in HIV. Primate studies have shown that an attenuated strain of SIV containing a deletion of the *nef* gene can protect monkeys against challenge with a pathogenic virus[28]. Although in some studies the defective virus was pathogenic in neonatal macaques[114], this observation was not confirmed by other laboratories[130]. Additional support for a *nef*-deficient live attenuated vaccine comes from an isolated non-progressor as well as a cohort of non-progressors in Australia that were infected by the same donor. The viruses obtained from all of these individuals had defective *nef* genes[31, 77, 82]. Nonetheless, safety concerns, with regard to pathogenesis of the deficient viruses, remain with such an approach, pushing for safer alternatives such as subunit vaccines.

Subunit vaccines against HIV consisting of HIV-1 envelope protein preparations were tested in clinical trials and, while proven safe, failed to induce protective humoral immune responses[12, 41, 66, 107]. Nonetheless, studies in primate models have continued in this area, developing subunit vaccines for boosting strategies in combination with DNA and live vectors[4, 16, 68]. In addition, peptide vaccines that could induce CTLs have been studied in both murine and primate models[7, 11, 53, 63, 76]. A major problem with these approaches is the lack of adjuvant properties that result in general inflammatory responses and a better recruitment of multiple arms of the immune response, including cell-mediated responses. In this regard, live vectors and "naked" DNA are more promising.

"Naked" DNA has been used as a means of immunization to generate cell-mediated immune responses. The DNA is taken up by cells and HIV genes are transcribed, with the result that the expressed proteins are processed and presented by MHC class I to the $CD8^+$ T cells[18, 23, 71, 80, 126]. Vaccine studies in primates, either with DNA alone or in combination with subunit boosting, have shown promising results in protection against further challenge with virus[13, 48–50, 98]. Phase I trials have also been reported where immune responses against the antigen were observed and a good safety profile was demonstrated[125]. This approach is quite novel and deserves further examination in terms of safety and optimal dosage in humans as well as a better understanding of the mechanisms of DNA uptake and immune recognition.

A better understood vaccination system from the immunological point of view is that of vaccine vectors. In this approach, genes encoding immunodominant antigens from the pathogen of interest are inserted into more benign microorganisms to be delivered to the immune system. Vectors considered for HIV vaccines include well studied viral vectors such as vaccinia, canarypox and adenovirus and some novel bacterial vectors, such as *Salmonella sp.*, BCG, *Shigella* and *Listeria monocytogenes.* Both vaccinia and canarypox vectors have been studied in Phase I trials with good safety reports[60]. In several studies, vaccinia vectors have been shown to induce strong humoral responses[58, 59, 61] and both vaccinia and canarypox have been shown to induce cell-mediated immune responses[40, 43, 45, 106] with or without subsequent boosting. Unfortunately, volunteers immunized with vaccinia expressing gp160 were not protected against later exposure to heterologous HIV virus[111]. On the other hand, CTLs from canarypox immunized volunteers were able to lyse autologous target cells infected with HIV viruses from a different clade[44]. A significant obstacle against the use of poxviruses is the widespread pre-existing immunity to these viruses due to the use of vaccinia as a vaccine for smallpox[22]. Nonetheless, recent studies have shown that the obstacle of pre-existing immunity can be overcome by changing the route of immunization[9]. Novel bacterial vectors such as *Salmonella typhimurium*[121] and *Streptococcus gordonii*[34] have been tested in primate models looking at the ability of these vectors to induce mucosal immune responses. Nevertheless, these novel vectors are still in early stages of development.

Although attempts to develop HIV vaccines that induce humoral responses have been made, they do not provide protection against virus nor decrease the rate of progression. On the other hand, attempts to develop HIV vaccines focusing on approaches that induce strong cell-mediated immunity may be more successful[115]. $CD4^+$ T cells play an essential role in regulating the balance between humoral and cellular immunity. Hence, it has been suggested that in response to HIV-1 infection, Th1 type cells, which induce cell-mediated immunity, are protective, whereas Th2 type cells, which result in humoral immunity, may be detrimental to the induction of protective responses[19]. A vaccine

that targets the induction of Th1 $CD4^+$ T cells with the accompanying cell-mediated responses and strong CTL activity may be the right approach for a successful vaccine.

Our laboratory has been exploring the use of *L. monocytogenes*, an intracellular, facultative, Gram-positive bacterium, as a live vaccine vector for HIV. This bacterium is ubiquitous in nature and has developed an unusual life-style to avoid host humoral immune defenses which results in strong cell mediated immunity to antigens secreted by this organism. The rest of this review will cover the cell biology and immunology of *L. monocytogenes* as well as studies that have exploited it as a vaccine vector.

Cell Biology of *L. monocytogenes*

L. monocytogenes has been intensely studied in rodents. During the 1960's, MACKANESS[87–89] first presented evidence of the existence of T cells and their role in cell-mediated immunity and macrophage activation. It was not until 1972, however, that T cells were shown to protect against challenge upon adoptive transfer[81]. These early studies paved the way for a substantial amount of research on the role of T cells, NK cells, macrophages and cytokines in the immune system, demonstrating that several mechanisms play active roles in protecting the host from disease.

In the mouse model, after intravenous inoculation, *L. monocytogenes* is quickly taken up by the liver (80%) and spleen (10%) [20], presumably as a result of phagocytosis by macrophages. Once engulfed by these cells, the life cycle of *L. monocytogenes* begins (Fig. 1).

L. monocytogenes has a life cycle inside the cell that makes it an interesting organism from both a pathogenic as well as an immunological point of view. This cycle requires the expression of a number of genes contained within the *prfA* regulon region of the chromosome. This region contains at least 5 genes controlled by PrfA, namely *hly, plcA, plcB, mpl,* and *actA*[36, 47, 83, 93]. In addition, *inlA* and *inlB,* which encode the internalins required for cell entry, are also regulated by PrfA. All of these genes, including *prfA,* are important virulence factors for the organism and, in fact, constitute the main virulence factor cluster of the bacteria.

The bacterium enters host cells, primarily hepato-

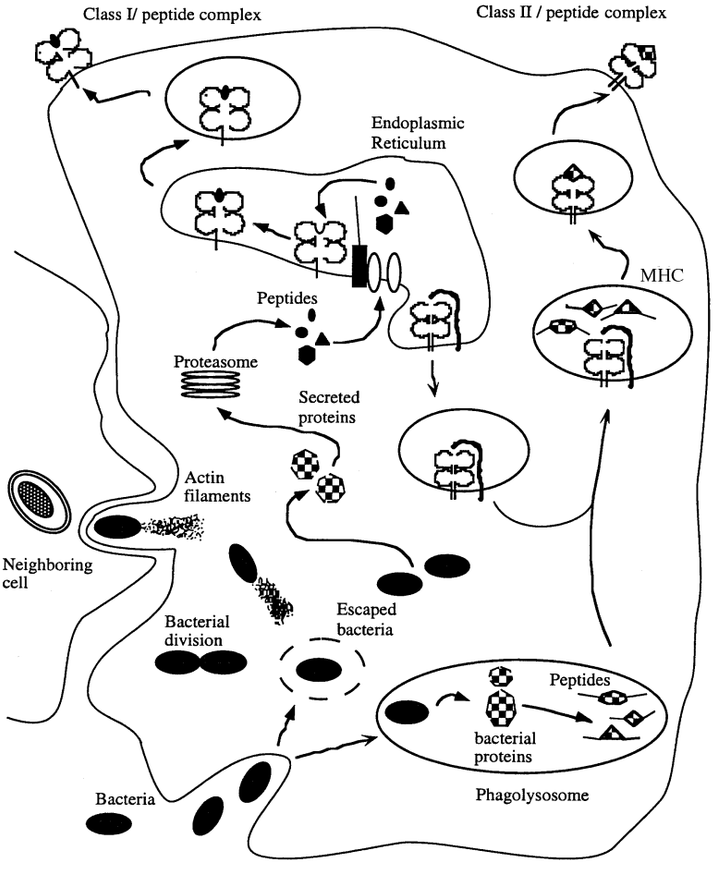

Fig. 1. The life cycle of *Listeria monocytogenes* allows access of listerial secreted antigens to both the MHC class I and class II pathways for antigen processing. After phagocytosis into an antigen presenting cell *L. monocytogenes* is either destroyed within a lysosomal compartment, where peptides may be loaded onto MHC class II molecules, or it escapes into the cytoplasm of the cell. In the cytosolic compartment *L. monocytogenes* can grow and any protein it secretes can be processed by proteosomes into peptides which will be transported to the endoplasmic reticulum for loading onto MHC class I molecules. In the cytosolic compartment *L. monocytogenes* reorganizes the host cells actin into "tails" which propel it around the cell and to the cell's periphery. There it can be internalized by a neighboring cell and, by breaking through two plasma membranes, colonize this cell

cytes and macrophages, by a variety of mechanisms controlled by the pathogen or the host cell. *L. monocytogenes* can invade host cells through the expression of one of a number of wall surface molecules called internalins (encoded by *inlA* and *inlB*) [51] that have been shown to bind to E cadherin on epithelial cells[94]. In addition, phagocytosis by macrophages has been demonstrated to be mediated by type I scavenger receptors recognizing lipoteichoic acid in the bacteria[39] and by components of the complement cascade C3b[37] and C1q[3]. Once within the phagolysosome of the cell, the bacterium can escape this vacuole by the low-pH-dependent secretion of a hemolysin (hly) protein, listeriolysin O (LLO), that lyses the membrane. Hemolysin has been shown to be essential for *L. monocytogenes* pathogenesis, since in its absence the bacteria remain within vacuoles, fail to grow intracellularly, and are avirulent[52, 110]. Furthermore, complementation of the hemolysin mutant with the *hly* gene restores the wild type phenotype[24]. In addition, phospholipase C (plcA) and metalloprotease (mpl) may also contribute to escape from the phagosome[17, 90].

Once in the nutrient-rich cytosol of the cell, *L. monocytogenes* replicates with a doubling time of approximately one hour. In addition, an interesting characteristic of this pathogen is its ability to use host cell actin to mobilize itself. *L. monocytogenes* expresses a cell wall surface protein, ActA, that polymerizes the actin filaments of the cells[78], forming a tail structure similar to a comet on one end of the bacterium[122, 123]. This tail structure allows for the propulsion of the pathogen through the cytoplasm in a random fashion. When the bacteria reach the host cell membrane, cellular projections, or pseudopods, are formed and engulfed by neighboring cells, thus starting the cycle again[109]. While this cell-to-cell spreading mechanism allows the pathogen to avoid the humoral branch of the immune response, this dissemination pathway makes it a perfect target for cell-mediated immune responses. While some organisms may escape the phagolysosome, 90% of the bacteria are killed in this compartment and the remaining proteins are easily targeted to the class II pathway of antigen presentation. On the other hand, the small percentage that reaches the cytosol secretes proteins directly into the cytoplasm where they can be delivered to the class I pathway and presented to $CD8^+$ T cells.

Innate immune responses

Following uptake by resident macrophages of *L. monocytogenes* and escape into the cytoplasm, logarithmic multiplication of the bacteria takes place, reaching a peak by days 2 or 3 after infection[21, 108]. During these early stages, a strong innate immune response controls the bacteria growth and spread through several mechanisms. For example, macrophages play a critical role through both oxygen-dependent and oxygen-independent mechanisms of killing[1, 8, 35]. Macrophages also secrete a number of proinflammatory cytokines required for the recruitment and activation of other cell types. In addition, neutrophils are another important component of the early response. Massive infiltration of polymorphonuclear cells in a response to proinflammatory cytokines secreted by the infected cells is seen[21]. Control of bacteria by neutrophils takes place by direct lysis of the infected hepatocytes[2, 27].

Another important element of the innate immune response is the NK cell. Depletion of NK cells results in exacerbation of infection after 24 h suggesting that NK cell activation is important during the early stages of the immune response[124]. NK cells mediate anti-*Listeria* immunity by secreting IFN-γ, which in turn activates macrophages[38]. The result is a listericidal macrophage with increased MHC expression on its surface. Production of IFN-γ by NK cells is dependent on IL-12 and TNF-α produced by macrophages[38], forming a positive feedback loop.

All the activity observed early during infection has a dual purpose. First, this response is strong enough to control initial bacteremia, although not sufficient to eliminate the infection. Second, the interaction between cells at this early stage results in the secretion of cytokines important for the recruitment and activation of the antigen-specific arm of the immune response.

Acquired immune responses

Although the innate immune response is sufficient to contain the early stages of infection, the cellular immune response is required for sterilizing immunity and memory responses. $CD8^+$ T cells are thought to be the major effector subset in controlling and clearing the listerial murine infection while $CD4^+$ T cells are important for cytokine secretion, DTH responses and granuloma formation.

Several lines of evidence support the role of $CD8^+$ T cells. Depletion of $CD8^+$, but not $CD4^+$, T cells abolishes the protective effect of immune splenocytes in primary infection[95] and during adoptive transfer[26]. In addition, nude, SCID and β2-microglobulin knockout mice, which lack class I restricted $CD8^+$ T cells, behave in a similar way in that they control but are unable

to clear the infection[100, 108, 113]. Finally, transfer of $CD8^+$ T cell clones specific for listerial class I epitopes have been shown to protect against subsequent challenge[64, 65]. $CD8^+$ T cells appear to control infection by lysing *L. monocytogenes*-infected cells and by secreting cytokines such as IFN-γ[101]. $CD8^+$ T cells have been shown to lyse cells, *in vitro*, by two different mechanisms, namely the Fas-FasL pathway[99] and the perforin pathway[75, 79, 86, 127]. *In vivo*, both CD95 (FasL) and perforin deficient mice were shown to have deficient CTL responses but were able to clear infection[74]. Thus, a third yet unidentified mechanism, independent of Fas and perforin, may be involved in protection against *L. monocytogenes*.

In the murine model, several antigens have been shown to be presented by both class Ia and class Ib molecules and recognized by CTLs. The immunodominant antigen appears to be LLO and a large amount of work on the H-$2K^d$-restricted epitope of LLO, aa 91-99, has been carried out to understand antigen processing and immunodominance. LLO is also required for CTL responses because of its role in *L. monocytogenes* escape to the cytosol and consequent access to the class I pathway[10, 15].

$CD4^+$ T cells appear to be less critical for the control of the *L. monocytogenes* infection in the murine model. Depletion of $CD4^+$ T cells during adoptive transfer of splenocytes from an immunized mouse or during primary infection had only a marginal effect on controlling *L. monocytogenes*[26, 95]. Nevertheless, depletion of $CD4^+$ T cells during primary infection severely diminished delayed type hypersensitivity responses and granuloma formation[95]. Another important role of $CD4^+$ T cells during listeriosis is cytokine production. Several *in vitro* and *in vivo* lines of evidence suggest that the immune response to *L. monocytogenes* is of a type 1 phenotype with the induction of IL-12 and IFN-γ but not IL-4 or IL-10 production[30]. In addition, adoptive transfer of a Th1 p60 specific $CD4^+$ T cell clone was shown to induce protection against *L. monocytogenes* infection[55].

γδ T cells appear to be involved in the early stages of immunity. An increase in the absolute numbers of γδ T cells is observed within 3 days and depletion of γδ T cells results in an exacerbation of the disease between days 3 and 5 after intraperitoneal inoculation of *L. monocytogenes*. Nonetheless, these mice eventually clear the bacteria[67]. Using αβ T cell deficient mice, γδ T cells were able to mediate protection against primary challenge although clearance was delayed; this protection was abolished after depletion with anti-TCR-δ monoclonal antibody or in mice deficient for both αβ and γδ T cell subsets[96]. These data suggested that γδ T cells play a role in the early stages of primary infection. In addition, the liver lesions of γδ T cell deficient mice had altered morphology and increased size, suggesting some regulatory role for γδ T cells in later stages as well. Finally, γδ T cells recognizing LLO have been identified in humans[62].

Human Listeriosis

L. monocytogenes can cause a food borne disease in humans. While *L. monocytogenes* can be found everywhere in nature, including soil, dust, fertilizer, stream water and plants, it is its presence in processed foods, including raw meat, poultry and unpasteurized dairy products, among others, that has been associated with a number of outbreaks. Regardless of its widespread distribution, little is known about the level of exposure of humans to this pathogen. Some studies have shown that both symptomatic (26%) and asymptomatic humans (1–5 %) excreted the organism[5], but these data fail to determine whether infection was systemic, in the case of the asymptomatic individuals.

Although exposure to this pathogen could potentially be high, it is remarkable that the incidence of listeriosis is only about 0.7 cases per 100,000[14]. Most of the patients in this group are immunocompromised individuals, including transplant patients under corticosteroid therapy, neonates and pregnant women in their third trimester and the elderly[56, 117]. While the incidence of listeriosis is higher in HIV^+ patients than in the general population[73], it is not as frequently seen as in some of the other susceptible groups[29, 72]. A possible explanation is that the high levels of TNF-α in HIV patients play a role in control of infection[32].

L. monocytogenes as a Vaccine Vector

It has long been recognized that live vaccines result in long-lasting cellular immunity, unlike killed preparations or soluble proteins in adjuvant. In the search for appropriate live vectors for vaccination, the ability of *L. monocytogenes* to induce strong cell-mediated responses has made it a prime candidate for delivering foreign antigens as a vaccine strategy. The advantages of *L. monocytogenes* as a vaccine vector can be divided into 3 groups: biological, safety and practical advantages. Biological advantages are related to the efficiency of the vaccine to confer adequate protection against the pathogen. The most important advantage of using

L. monocytogenes is clearly the ability of this pathogen to induce an immune response to the antigen with strong CTL and Th1 mediated responses as well as the secretion of high levels of inflammatory cytokines. Not only does *L. monocytogenes* deliver the antigen, but it also works as an adjuvant. Another factor that may prove useful is that *L. monocytogenes*, being a food-borne pathogen, may be able to induce mucosal immune responses at the site of entry as well as in other mucosal areas. Indeed, we have shown that *L. monocytogenes* administered orally can induce sterilizing immunity[116].

Since a prophylactic vaccination strategy is aimed at a healthy population, it is important to think of the safety of a vaccine. *L. monocytogenes* offers several advantages in this area. *L. monocytogenes*, as a Gram-positive organism, does not produce endotoxin, which Gram-negative vectors, such as *Salmonella sp.*, produce. As a bacterium, *L. monocytogenes*, unlike viral vectors, is highly susceptible to a wide variety of antibiotics *in vitro*, including penicillin G, ampicillin, erythromycin, chloramphenicol, rifampin, the tetracyclines, gentamycin, sulfamethoxazole and trimethoprim[56], that can be used for patients that are allergic to any antibiotic group, should this be necessary.

In a more practical sense, *L. monocytogenes* has not been previously used for vaccination, unlike some of the other vectors currently under study for HIV vaccination. Therefore, widespread pre-existing immunity to the vector might not represent a problem, unlike the case of vaccinia virus or BCG, previously used for smallpox and tuberculosis vaccination campaigns, respectively. It is important to mention that prior exposure with a resulting memory response to *L. monocytogenes* in healthy individuals[97] has been documented, but it is unclear how this exposure would impact the efficacy of *L. monocytogenes* based vaccines. Finally, unlike BCG, *L. monocytogenes* grows rapidly and well outside the cell in inexpensive media, remains viable on refrigeration and freezing, and can be stored indefinitely. These qualities are important when thinking about cost efficiency and fieldwork applicability.

L. monocytogenes delivers foreign antigens to the immune response efficiently

The first studies to evaluate the ability of *L. monocytogenes* to deliver foreign antigens to the immune system were done using a *L. monocytogenes* mutant strain transgenic for the β-galactosidase gene of *Escherichia coli*[116]. In this work, infection of mice either orally or intraperitoneally resulted in the induction of antigen specific CTL responses against β-galactosidase. Drawbacks of this early system were low levels of antigen expression and the lack of secretion of this antigen, resulting in weak CTL responses. Thus, secretion of antigen, although beneficial to inducing strong CTL responses, is not always required. In addition, SHEN at al.[118], using a *L. monocytogenes* recombinant expressing a lymphocytic choriomeningitis virus (LCMV) nucleoprotein (NP) epitope, showed that $CD8^+$ T cell responses were primed even in the absence of secretion of the antigen. Nonetheless, secretion was required for recognition of target cells by CTL.

A second generation of constructs used a fusion protein strategy, utilizing the first 420 amino acids of the listerial protein LLO, including the promoter and signal sequence, fused to the NP gene of influenza[69]. As a result, the NP would be induced and secreted in a pattern similar to LLO. The fusion gene cassette was inserted into a plasmid containing the gene for the transcriptional activator PrfA. This plasmid was then used to transform a replication defective *prfA*$^-$ *L. monocytogenes* strain to ensure stability of the plasmid. Infection of mice with this construct (Lm-NP) resulted in strong CTL responses against the NP.

A third approach used was to insert antigen-LLO fusion proteins directly into the chromosome of the *L. monocytogenes* for further stability. One such construct expressed the HIV Gag protein (Lm-Gag) with the resulting induction of CTLs *in vivo* against the foreign antigen[46].

Other groups have made similar constructs that expressed the NP from LCMV with comparable results[57, 119]. GOOSSENS et al.[57] made a recombinant *L. monocytogenes* using a plasmid approach, but no attempts were made to force the retention of the plasmid *in vivo*, as done by IKONOMIDIS et al.[69]. Although loss of antigen expression *in vivo* was likely, mice immunized with this construct had strong LCMV-specific CTL responses. The same viral antigen model was employed by SHEN et al.[119], who made stable mutants by direct integration of the transgene into the chromosome similar to the approach developed by our laboratory[46]. This group utilized a new site within transcription regulons, thereby allowing the generation of stable mutants without disrupting expression of listerial genes. Immunization with this construct resulted in strong $CD8^+$ T cell responses against LCMV. In addition, elevated numbers of CTL precursors were observed for as long as 170 days after immunization with recombinant *L. monocytogenes*[120].

Cell mediated immune responses and Th1 cytokines that enhance antigen-presenting cells have long been

associated with clearance of tumors[105]. Therefore, the idea that *L. monocytogenes* could be useful to deliver tumor antigens to the immune system has also been explored. Using tumor cells retrovirally transduced to express NP, the Lm-NP construct was tested for its ability to protect against a tumor challenge with positive results. More importantly, infection with Lm-NP led to the regression of established tumors in an antigen-specific manner[102–104]. The elimination of macroscopic tumors in the absence of additional chemotherapeutic treatment was an unparalleled result, supporting the possibility of using *L. monocytogenes* as a therapeutic cancer vaccine.

L. monocytogenes as a vaccine for viral infections including HIV

The rationale of using *L. monocytogenes* as a vaccine vector against viral infections comes from findings that indicate that similar immune responses are elicited when protection is achieved against either pathogen. Both viruses and *L. monocytogenes* reach the cytoplasm of the host cell and thus, their antigens are presented by Class I pathway, resulting in a CTL response. Lm-NP was designed as a vaccine against influenza virus. While the finding that NP epitopes for H-2^k, H-2^b and H-2^d were presented by Lm-NP[69] is interesting, the most exciting finding was the protection observed when mice previously immunized with Lm-NP were challenged with the influenza virus at the mucosal surface of the lungs[70]. Moreover, the level of protection was comparable to that induced by a vaccinia virus carrying the influenza NP gene. Similarly, recombinant *L. monocytogenes* mutants that expressed NP from LCMV were protected from an intracerebral lethal challenge with LCMV[57]. Using the same viral antigen model, SHEN et al.[119] demonstrated that protection against LCMV was mediated by CD8$^+$ T cells against NP. The ability of *L. monocytogenes* to effectively induce protection against viral pathogens support the idea that *L. monocytogenes* could successfully be utilized as a vaccine vector for HIV infection.

In order to characterize the immune response to HIV proteins delivered by *L. monocytogenes*, we have expressed HIV Gag in *L. monocytogenes* in secretable form. We have used these recombinant organisms (Lm-Gag) to generate HIV specific immune responses in the mouse, which we have characterized *in vivo* and *in vitro*. We have shown the induction of strong CTL[46, 92] and Th1 helper responses that appear to be directed to the p24 gene product and have identified epitopes in two different strains of mice[91]. Furthermore, immunization of mice with Lm-Gag protects them from a challenge with a vaccinia construct expressing Gag (MATA et al., manuscript in preparation). Such protection is abrogated upon depletion of both CD4$^+$ and CD8$^+$ T cells, suggesting that the Gag-specific T cell immune response induced by the Lm-Gag vaccine is protective. These data support the hypothesis that *L. monocytogenes* can effectively deliver HIV antigens to the immune system to induce strong Th1 type cell mediated responses that would protect individuals against the HIV virus.

As we have shown in this review, there is now a wealth of studies that document the potential power of using *L. monocytogenes* as a vaccine vector against viral diseases. Our laboratory, in collaboration with Dr. Michael Murphey-Corb at the University of Pittsburgh, is currently exploring the immunogenicity and efficacy of SIV antigen expressing *Listeria* vectors in a non-human primate model. We hope that these pre-clinical studies will also prove the safety of this live vector approach and pave the way for its future clinical use.

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