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Review article

Escheriosome: A potential antigen carrier

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Abstract

Escheriosomes are one kind of liposomes grafted from polar lipids extracted from *Escherichia coli*. These vesicular form elicit high cytotoxic T lymphocyte (CTL) responses. Escheriosomes have shown to deliver their entrapped molecules right into the cytosol of the APCs (Antigen Presenting Cells) that leads to the processing of entrapped antigen via endocytic pathway leading to the antigen presentation by MHC Class I mode. Expression via MHC class I molecules results in $CD8^+$ T cell activation. Escheriosomes may function as a novel immunoadjuvants and emerge as an effective tool for generating protective immunity. It proves to be attractive niche for the scientist in the delivery of vaccine. This review is grafted to highlight its potential as antigenic carrier along with its characterization.

Keywords: Escheriosomes; *Escherichia coli*; immunoadjuvants; fusogenic liposomes

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Introduction

Traditional vaccines have mainly consisted of live attenuated pathogens, whole inactivated organisms, or inactivated bacterial toxin [1]. It induces antibody production. Somehow it neutralizes viruses or bacterial toxins, inhibit the binding of microorganisms to cells, or promote their uptake by phagocytes. It seems easy on paper but in actual scenario it is very much difficult against pathogens that often establish chronic infections, e.g., HIV, hepatitis C virus (HCV), tuberculosis, and malaria, the induction of potent and focused cellmediated immunity (CMI) will be necessary and may require the induction of cytotoxic T lymphocytes (CTL), which kill host cells infected with intracellular organisms [2]. Such issues have served to highlight the urgent need for the development of new and improved vaccines. Targeted delivery of adjuvants and vaccines to specific cell types or tissues may reduce potential toxic effects, or help to achieve a specific desired response. Currently viral lipid based vesicles proves to be good as vaccine adjuvant. Escheriosome is one of those examples. These are lipoidal vesicles, prepared from polar lipids extracted from *Escherichia coli* (like phosphatidyl ethanolamine, cardiolipin, and phosphatidyl glycerol). These are classes of phospholipid, present in *Escherichia coli* [3]. *E. coli* contain an altered fatty acid and phospholipid composition when grown in the presence of sublethal concentrations of a variety of organic solvents and food additives [4]. When it comes to exponential growth phase to the stationary growth phase, the phospholipid composition of the cell was altered. Unsaturated

fatty acids were converted to cyclopropane fatty acids, and phosphatidyl glycerol appears to have been converted to cardiolipin [5]. Also, ethanol was found to decreased the level of lipids in *E. coli.* These novel fusogenic liposomes have strong tendency to fuse with the plasma membrane of target cells and thereby delivering the entrapped contents into their cytosol. Escheriosomes are helpful in controlling intracellular pathogens by expression of particular enzymes. Escheriosomes-encapsulated antigen elicited strong humoral immune response in immunized animals. But in general, Escheriosomes are considered as potential candidate vaccine carrier system capable of eliciting both cell-mediated as well as humoral immune responses.

Evaluation

Once the escheriosomes are prepared with appropriate method the next step come is the characterization of prepared vesicles for its physical, chemical and biological stability. The characterization elements of niosome are depicted in Table 1.

Table 1. Characterization of Escheriosome [6-11]

Assay		Methodology
Physical Characterization/Stability		
1	Vesicle size, surface morphology and size distribution	Transmission electron microscopy (TEM), freeze fracture electron microscopy Dynamic light scattering, TEM, zeta sizer, Laser light scattering, gel permeation, gel exclusion, Cryo electron microscopy, Confocal Microscopy
2	Osmotic pressure	Osmometer
3	Phase behavior	Differential scanning calorimetry
4	Lamellarity	Small angle X-ray scattering, ^{31}P-NMR
5	Surface charge	Free-flow electrophoresis
6	Entrapment efficiency	Exhaustive dialysis, gel filtration and centrifugation.
7	Relevant body fluid induced leakage	Protamine precipitation and GEC
8	Dilution dependent drug release	Retention loss on dilution
9	*In vitro* release	Hu's method, Dialysis through a semipermeable membrane and its measurement using suitable analytical method
Chemical Characterization/Stability		
1	pH	pH meter
2	Cholesterol Concentration	Cholesterol oxidase assay
3	Cholesterol Auto oxidation	HPLC, TLC
4	Electric surface potential and surface pH	Zeta potential measurements and pH sensitive probes
Biological Characterization/Stability		
1	Sterility	Aerobic and Anaerobic culture
2	Pyrogenicity	SAM or LAL test
3	Test specific to vaccine delivery	Antigen expression in Vero cell line, SDS-PAGE and Western blot analysis, Quantification of bacteria in the spleen, In vitro assay for cytokine production by spleen cells, Splenocyte culture and lymphocyte proliferation, Immune response, ELISA
4	Animal toxicity	Monitor survival, histology and pathology, Protective efficacy

Potential of escheriosomes in vaccine delivery

Development of protective immunity against many pathogens, particularly viruses, requires a handy coordination of both humoral- and cell mediated-immunity in the body. Encapsulation of antigen in egg phosphatidyl-choline (egg PC) liposomes fails to generate antigen-specific CD8(+) cytotoxic T cell response due to inefficient access to the cytosolic pathway of MHC I-dependent antigen presentation. In other words using liposome only humoral immune response could be achieved not cell mediated response which is crucial against fungal vaccination [12, 13]. Activation of a cell-mediated immune response against exogenous antigens has always been a challenge, requiring special strategies [14]. Candida albicans (C. albicans) cytosolic antigens (cAg) were encapsulated using escheriosomes so as to generate protective immunity against systemic C. albicans infection in BALB/c mice [12]. Results justifies the production of humoral as well as cellular immunization. The strong antigen-specific CD8(+) T-cell responses in the immunization using escheriosomes were markedly higher than that observed in mice immunized with IFA-antigen emulsion, or antigen encapsulated in egg PC liposomes. Apart from such response it also serves as a cogent immunoadjuvent. As a matter of fact; immunization with cAg delivered in escheriosomes leads to complete elimination of C. albicans infection in Balb/c mice. It was concluded from the study that escheriosomes could be successfully delivered the antigen simultaneously to the cytosolic as well as endosomal processing pathways of antigen presenting cells, leading to the generation of both CD4(+) T-helper and CD8(+) cytotoxic T cell response [13]. Some added advantages of escheriosomes includes rise in the level of IL-2, IFN-gamma and IL-4 in the immunized animals [13]. The vaccine antigen is Brucella L7/L12 protein, which was delivered through escheriosome [15]. Combinations containing rL7/L12 protein and different adjuvants were formed for vaccination using escheriosome which can elicit strong immunological responses in the Balb/c mice. However, egg PC/Chol liposome entrapped rL7/L12 was found to impart relatively poor immune response. Escheriosome-entrapped rL7/L12 protein elicited high IgG2a isotype response, suggestive of its relevance in imparting protection against brucellosis in mice. Singhaaet al. have evaluated prophylactic prospective of scheriosome based DNA vaccine co-expressing Cu–Zn superoxide dismutase (SOD) along with interleukin-18 (IL-18) against experimental murine brucellosis in mice. The immunization schedule involves liposome-mediated delivery of pVsod (encoding SOD of Brucellaabortus) and pVIL18-sod (encoding IL-18 of mouse and SOD of B. abortus) DNA constructs. The co-expression of SOD along with IL-18 ensued in higher lymphoproliferative response and IFN-γ production in comparison to the group of animals that were immunized with free form of SOD-DNA. Antibody response developed upon immunization with both DNA vaccines was of IgG2a type mainly. The results of the present study show that co-expression of IL-18 along with SOD polarized the antigen specific immune responses toward Th-1 direction, a desirable feature to control intracellular pathogens [16]. Ahmed et al. have checked fusogenic non-PC liposomes in elicitation of protective immune response against experimental murine salmonellosis [17]. The degradation of the protein by the cytosolic proteolytic system forms a cardinal step for the induction of cytotoxic T lymphocytes (CTLs). Potent primary CTL response against a soluble protein, ovalbumin, can be induced in mice by encapsulating it in the liposomes comprised of Escherichia coli membrane lipids has been achieved and very well demonstrated by Ahmed

and co-workers [14]. These lipids were shown to induce strong membrane–membrane fusion as evident from resonance energy transfer and content mixing assays. Furthermore, the fusion of these liposomes with living cells (J774 A1) was demonstrated to result in effective transfer of a fluorescent lipid probe to the plasma membrane of the cells. Moreover, ricin A, a protein synthesis inhibitor that does not cross plasma membrane, was demonstrated to gain access to the cytosol when it was encapsulated in these liposomes. Finally, the liposomes were demonstrated to behave like efficient vehicles for the in vivo delivery of the antigens to the target cells resulting in the elicitation of antigen reactive CD8+ T cell responses.

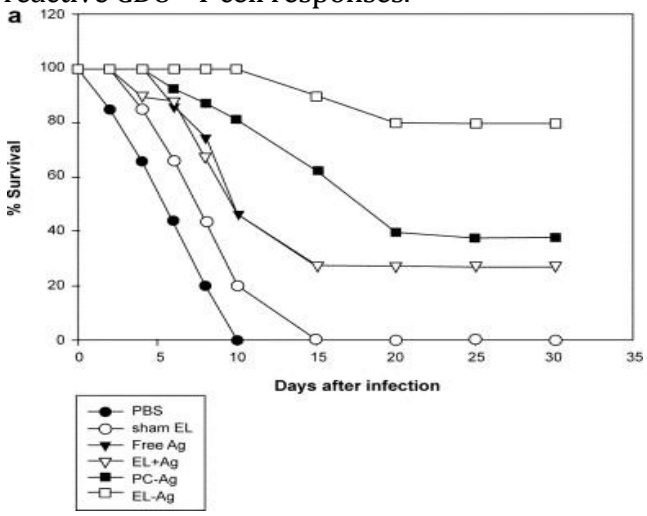

Figure 1. Prophylactic potential of escheriosomes in terms of survival rate of animals that were challenged with S. typhimurium infection after immunization with various preparation of vaccine [17].

Aluminum hydroxide (alum) is the only adjuvant currently approved for use in human vaccines. While subunit vaccines represent an attractive strategy for potentially improving the effectiveness of vaccines, effective implementation requires adjuvants that are more potent than alum and are nontoxic. Using a hamster model for leptospirosis, Cornell researchers have demonstrated that total polar lipids from Mycobacterium smegmatis, designated as smegmosomes, Leptospirabiflexaserovarpotac, designated as leptosomes, and non-pathogenic Escherichia coli, designated as escheriosomes were each more effective as an adjuvant than alum or conventional liposomes [18]. Anticancer efficacy of a novel propofol–linoleic acid-loaded escheriosomal formulation against murine hepatocellular carcinoma has successfully been demonstrated and evaluated [19]. In veterinary vaccination, improvement of the existing vaccines and/or vaccination schedule and development of new generation vaccines including subunit, geneticallyengineered, edible, and synthetic and combination vaccines with concurrent development and improvement in vaccine delivery systems through use of escheriosomes have been captivated by Indian Veterinary Research Institute [20].

Future prospects

Present technologies have come to provide more promising experimental approaches to understand the origin of life. These can be used to ascertain the molecular mechanism in the future. Vaccines based on liposomes formulated with lipid A and hydrogel have been shown to be potent and safe in humans. Escheriosomes may function as a novel immunoadjuvants and emerge as an effective tool for generating protective immunity. It proves to be attractive niche for the scientist in the delivery of vaccine. Future developments in adjuvants are likely to include the development of more site-specific delivery systems for both mucosal and systemic administration. In addition, the identification of specific receptors on APCs is likely to allow targeting of adjuvants for the optimal induction of potent and specific immune responses. However, further developments in novel adjuvants will likely be driven by a better understanding of the mechanism of action of

currently available adjuvants and this is an area of research that requires additional work [1].

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