Journal of Vaccines & Vaccination

Kumar et al., J Vaccines Vaccin 2014, 5:3
DOI: 10.4172/2157-7560.1000225

Review Article Open Access

"Oral Vaccine Antigen Induced Immune Response Signalling Pathways: Current and Future Perspectives"

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Received date: 12 March 2014; **Accepted date:** 24 April 2014; **Published date:** 26 April 2014

Abstract

Vaccination is the most promising approach to control and prevent the infectious diseases. As most of pathogenic microrganisms gain entry through the mucosal surfaces of host which provide great deal of interest in developing oral vaccines. Despite the success of many vaccines, only little information is available regarding the oral vaccine antigen induced immunogenic signalling pathways. Such information will be helpful to design future vaccines against old and new infectious diseases to reduce the side effects of existing vaccines and increase their efficacy. In this review, the oral vaccine antigen inducing complex signaling pathways of immune system has been discussed. Various strategies to prevent inactivation of oral vaccine by gastric acid and intestinal enzymes have been also included.

Keywords: Immune system; T cell; B cell; Vaccine

Introduction

Immunity is a state of protection mechanism against various kinds of infectious diseases. The immune system is a tightly regulated complex network of various cells (lymphoid, reticular, dendritic and epithelial cells), which are communicating with each other by soluble cytokines mediators. Innate and adaptive immunity works synergistically against the various infectious agents to protect the body. Before an infection, innate immunity is developed in the body which prevents or eliminates the pathogen within hours. Adaptive immunity is another form of immunity which develops in response to infection and adapts to recognize, eliminate, and then remember the invading pathogen [1]. Various chemicals such as deltamethrin, tributyltin have been reported to actively interfere with the activation of complex signaling pathways of the immune system [2,3]. Immune system provides the protection against infectious diseases but in case of strong pathogenic microorganism, immune system is not able to protect the body from infection which leads todiseases.Infectious diseases remain a major problem worldwide [4]. In literature, it has been reported that incidence of infectious diseases has been increased continuously [5]. Antibiotics are commonly used in treatment of infectious diseases but emerging evidence has been shown that increased use of antibiotics leads to microbial resistance [6-8]. So, the next best option to prevent the infectious diseases is vaccines which are designed to generate strong immune responses against the particular antigen delivered through various routes. Mass vaccination programs have been used successfully for total eradication of infectious diseases [9,10]. Despite these achievements, many infectious diseases, especially enteric diseases, remain endemic in large part of the world [11]. These infectious diseases are caused by pathogens that colonise and invade the host at mucosal surfaces. Presently, most of vaccines are administered parenterally because the antigen which are present in vaccines are poorly delivered to the site of specific immunity [12]. The poor delivery of oral vaccine is commonly due to spontaneous or enzymatic breakdown and poor absorption through gastrointestinal tract [13]. Parenteral vaccines are not successful for induction of pathogen-specific mucosal immunity. Therefore, in order to induce a protective immunity against intestinal pathogens, vaccines should be delivered to the intestinal mucosa via oral route. Oral vaccines have lots of advantages over parenteral vaccines, including needle free delivery, increased patient compliance and ease of production due to decrease need to purify bacterial by-products [10]. It can also induce both mucosal and systemic immunity which provide additional protective immune responses [14]. The principle of mucosal immunization has been illustrated by the development of oral vaccine or intranasal vaccines against polio, cholera, typhoid, influenza and rotavirus for human use. Some of these vaccines need further improvement to increase efficacy or to avoid side effects. Many more are in stages of development. However, to date only a few vaccines have become available for mucosal use. These include OPV (Oral polio vaccine), adenovirus, rotavirus, cold-adapted influenza virus, S. *enterica*, and cholera vaccines. Several plant derived vaccines are under research, some are under clinical trials for commercial use.

Recently, Scientists made a concerted effort to find vaccines for complex diseases such as cancer [15], malaria [16], AIDS [17-19] and tuberculosis [20,21]. Unfortunately, these efforts are not become successful due to a limited understanding of how vaccine interacts with the immune system. So, in this review, we try to describe the mechanism of oral vaccine induced immune response signaling pathways based on our own study [22] as well as those reported in recent literature.

Oral Vaccine: Need, Challenges and Promising Approaches

Most infectious organisms gain entry into the body through mucosa l surfaces of the host's gastrointestinal, respiratory and urogenital tract s. The capacity to induce local protective immunity within the mucosa

is generally not possible with parenteral vaccination. The next best option is oral vaccine which overcomes the problems which are associated with parenteral vaccine but there are lots of challenges involved to design the oral vaccine. Oral vaccination may fail due to several factors such as inactivation by gastric acid and intestinal enzymes, poor bioavailability and interference from other bacteria and viruses in the gastrointestinal tract. To overcome these problems, now a days formulation scientists using various approaches like administration of antacid solutions prior to vaccination; use of acid resistant polymers, particulate drug delivery systems, oral adjuvants and hybrid vaccines. Recently we also check the immunestimulating potential of antigens in their native and associated form as chitosan microparticles in vitro and observed that increase in immunogenic potential by Cell Envelope Proteins (CEPs) loaded chitosan microparticles in comparison to CEPs as native antigen in the case of cholera [22].

Oral Vaccine Antigen Induced Immune Response Signalling Pathways

The hallmark of vaccination is the stimulation of an antigen specific adaptive immune response which leads to long term protection via the development of memory cells. Activation of immune system for a particular vaccine antigen is a complicated process which requires number of steps. First step is uptake of vaccine antigen by microfold (M) cells, present in the follicle-associated epithelium (FAE) overlying the germinal centres of the Peyer's patches [23-26]. In literature, various studies have been shown that M cells transport macromolecules [27-30], particles [31] and microorganism [32-39] from the gastrointestinal lumen to the underlying lymphoid tissue. These highly specialised M cells have some unique structural and functional features which facilitate endocytosis and transport of macromolecules and bacteria as shown in Figure 1 [40,41].

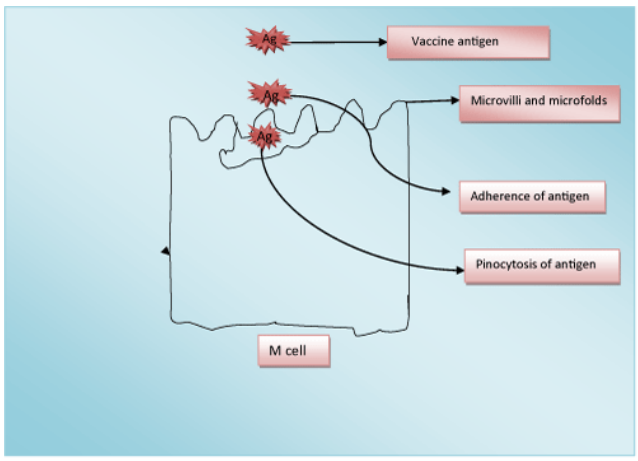

Figure 1: Uptake of vaccine antigen.

Recognition of oral vaccine antigen

Intestinal epithelial cells (IEC) are well known physical barrier which provide protection to the body against pathogens [42,43]. Because of their barrier function, IEC are the first cells which are exposed to the intestinal pathogens and act as immunological sensors detecting the pathogen associated molecular pattern (PAMP) through different classes of pattern recognition receptors (PRRs). List of PRRs and their targets has been summarized in Table 1. An important family of PRRs is the TLRs which have broad specificity for conserved molecular patterns shared by bacteria, viruses and parasites have been summarized in Table 2.

Receptor (PRRs)	Target (PAMPs)
Mannose-binding lectin (MBL)	Microbial cell walls
C-reactive protein (CRP)	Phosphatidylcholine
Toll like receptors (TLRs)	Microbial products
Lipopolysacchride-binding protein (LBP)	Gram –ve bacterial cell walls
Nucleotide binding oligomerization domain (NOD proteins)	Gram +ve bacterial cell walls
Scavenger receptors (SRs)	Gram +ve and –ve bacterial cell walls

Table 1: List of pattern recognition receptors (PRRs) and their targets.

TLRs	Target microbes
TLR1	Mycobacteria
TLR2	Gram +ve bacteria
TLR3	Viruses
TLR4	Gram –ve bacteria
TLR5	Bacteria
TLR6	Mycobacteria,Yeast and Fungi
TLR7	Viruses
TLR8	Viruses
TLR9	Bacterial DNA

Table 2: List of different types of Toll like receptors (TLRs) and their target microbes.

Oral vaccine antigen induced signal transduction pathways

Signal transduction pathways mediate the sensing and processing of stimuli. Vaccine antigen activate intracellular signalling pathway that l ead to the expression of proinflammatory mediators such as cytokoine s and chemokines. These proinflammatory mediators attract monocyt es, granulocytes and natural killer cells to inflammatory sites where monocytes differentiate into macrophages and immature dendritic cells (DCs) are converted to mature DCs. The mature DCs present the antigens to naive T lymphocytes.

Mechanism of presentation of processed oral vaccine antigen to the adaptive immune system

Dendritic cells act as a bridge between the innate and adaptive imm une system. Two signals are required for activation of naive T cells: on e signal triggered by MHC vaccine peptide complex, and another from the costimulatory molecules. Costimulatory molecules provide the sig nals necessary for lymphocyte activation as shown in Figure 2. There are many families of co-stimulatory molecules which play a

crucial role in T-cell activation, the B7 family members are the first to be identified. In literature, the most studied B7 family members are CD80 and CD86 and their interaction with CD28 and CTLA-4 (cytotoxic T-lymphocyte-associated antigen-4) on T cells. CD28 is expressed constitutively on human and murine T cells which deliver a positive co stimulatory signal upon interaction with CD80 or CD86 [44-46]. These co stimulatory signals causes' dendritic cells to up regulate the expression of B7 co-stimulatory molecules on their surface as shown in Figure 2. These activated dendritic cells migrate to the local draining lymph node, where they present antigen to T cells. Before it is presented by major histocompatibility complex (MHC) molecules, antigen is processed into short peptides by proteolytic enzyme [47]. The activated naive CD4+ T cells are differentiating into T-helper (Th) subsets for acquire effector functions. These subsets are distinguished as Th1, Th2 and Thr (Regulatory T cells) which are characterized by their varying ability to produce cytokines [48]. It can take several steps of activation for T cells to differentiate terminally to Th1, Th2 and Thr as shown in Figure 3 [49], which suggest that T cells can be activated and expanded in a non-polarized manner. Th1 cells produce IFN-γ (Interferon- γ) and help the induction of CD8+ cytotoxic T cells, which kill the cells infected with the intracellular pathogens whereas Th2 cells produce cytokines IL-4, IL-5 and IL-10 which induce IgE and eosionophil-mediated destruction of the pathogens [50,51]. Furthermore, T regulatory cells suppress the proliferation and differentiation of T- helper or cytotoxic T cells serve to limit the potential immunopathology that might be caused by an over expression of immune response [38].

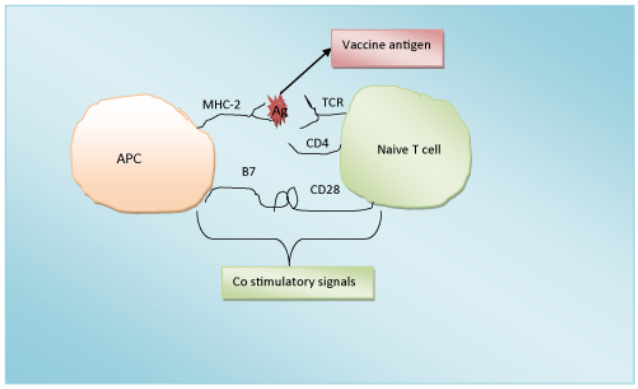

Figure 2: Presentation of vaccine antigen to naive T cell.

Oral vaccine antigen induced T cell signalling pathways

Antigenic peptide which is bound to either a helper T cell or cytotoxic T cell activates cell signalling pathways. In a resting T cell, p56 lck, a protein tyrosine kinase (essential for the initiation of TCR signalling), is sequestered from the TCR complex and become activated. This activated complex phosphorylates the immunoreceptor tyrosine based activation motifs (ITAMs) of the CD3 component polypeptides [52] Phosphorylated tyrosines in the ITAMs of the zeta chain provide docking sites to which another protein tyrosine kinase called ZAP-70 attaches and become active. This event catalyzes a series of intracellular events beginning at the inner surface of the cell membrane and culminating in the nucleus, resulting in the transcription of genes that drive cell cycle and differentiation of the T cell as shown in Figure 3. Upon encounter with the antigens, naive T-cells undergo maturation to create memory cells that recognise the antigen on subsequent encounters, there by creating the basis of antibody independent vaccination as shown in Figure 4.

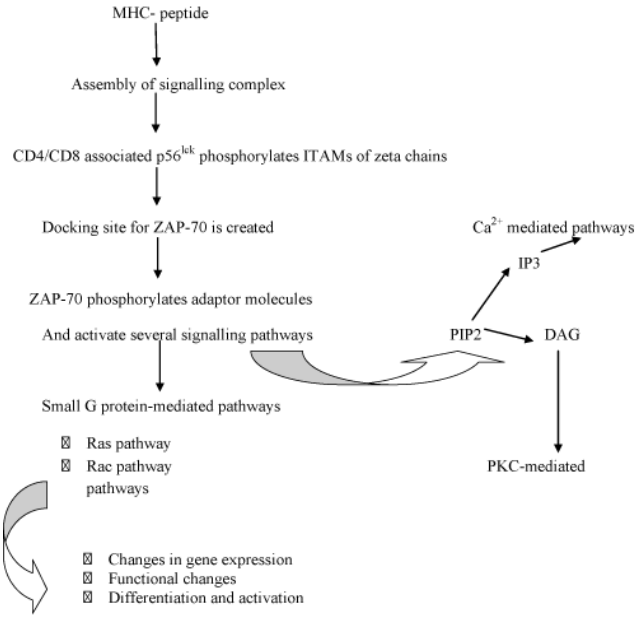

Figure 3: Oral vaccine antigen induced T cell signalling pathways.

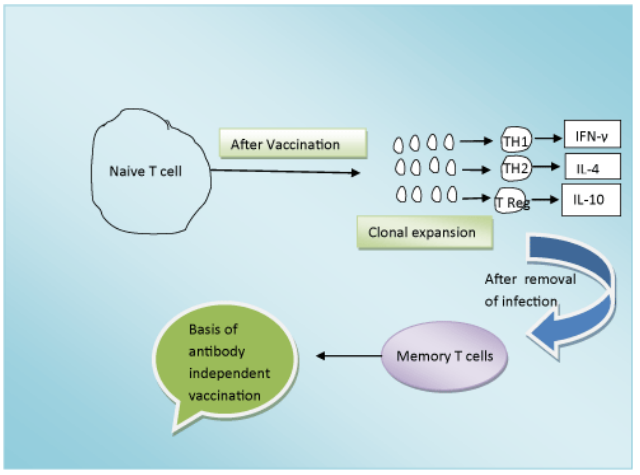

Figure 4: Basis of antibody independent vaccination.

Oral vaccine antigen induced B cell signalling pathways

B cell signalling pathways is activated by the T_H cells and B-cell receptor (BCR). Naive TH cell recognizes and interacts with an antigen-MHC class 2 molecule complexes which undergoes metabolic transformation and begins to secrete various cytokines. The secreted cytokines play an important role in activation of B cells, TC cells, macrophages and various other cells that participate in the immune response. B cells interact with antigen and then differentiate into antibody-secreting plasma cells as shown in Figure 5. The secreted

antibody binds to the antigen and facilitates its clearance from the body. The major antibody isotype in external secretions are secretory immunoglobulin A which provides specific protection against many respiratory, enteric, and genital infections.

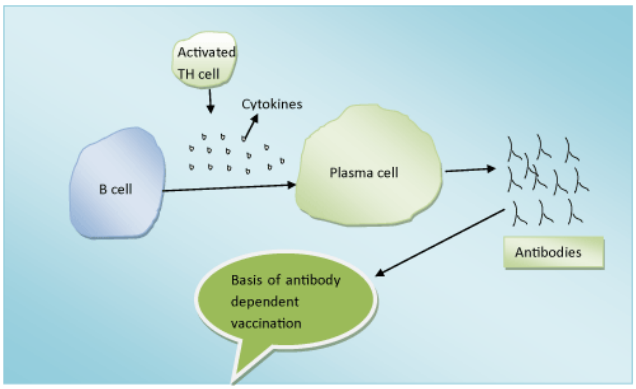

Figure 5: Basis of antibody dependent vaccination.

Deactivation of Signalling Pathways

Activation of the signal transduction pathways is critical for mounting an aggressive immune response to eliminate invading pathogens, but deactivation of these signalling pathways is also very much important to prevent self-destruction. Variety of negative regulators operates at multiple steps of signal transduction pathways to deactivate these signals. These negative regulators modulate the strength and duration of the transduced signals and control the production of inflammatory mediators [53]. For example, in response to LPS (lipopolysaccharides), TLR-4 was shown to be transiently suppressed [54]. A number of anti-inflammatory proteins such as IL-1 receptor-associated kinase (IRAK) [55], the suppressor of cytokine-signalling (SOCS)-1 [56], the NF-κB inhibitor (IκB), and anti-inflammatory cytokines such as IL-10 [57] are also induced for deactivation of these pathways. Through these inhibitory proteins, cells terminate the signalling cascade at the cell surface as well as switch off downstream mediators which results in the silencing of signalling pathways and also stopping the production of pro-inflammatory cytokines.

Current Challenges and Future Perspectives

Oral vaccine has great potential and many benefits over parenteral vaccines. However, despite many efforts, vaccinologists still struggle to develop highly efficient oral vaccine due to many problems posed by the gastrointestinal tract. To overcome these problems, numerous delivery systems such as polymeric nanoparticles, M cell targeting methods has been developed. Despite the proven power of these tools, vaccines are still unavailable for many of the infectious diseases due to complexicity of the immune system. In recent years, the introduction of genetic engineering has fuelled rapid advances in vaccine technology and is now leading to the entry of new products in the marketplace. These advances in biotechnology and molecular biology have opened new ways like DNA vaccination, cell based vaccination and use of chitosan and nanoparticles for the delivery of vaccines antigens [58-60]. Other methods include plant molecular farming in which genetic manipulations in plants are carried out to make a range of recombinant proteins which can be used as oral vaccine components [61-66]. DNA vaccines are considered the best approach to induce both humoral and cellular responses and also have the ability to provoke immune responses against the wide range of pathogenic strains [67]. For further benefits from DNA vaccines, immunomodulators such as cytokines and other co-stimulatory molecules can be utilised for the development of much safer, effective and low cost DNA vaccines. DNA vaccines have proven their efficiency against a number of pathogenic targets including influenza, Chikungunya (CHIKV) disease and infectious bursal disease (IBD). In literature, it has been observed that DNA vaccines provide a promising platform for the immunisation against many viral diseases, including Severe Acute Respiratory Syndrome (SARS), influenza and Simian immunodeficiency virus (SIV) [68]. The immune responses generated by DNA vaccines are highly specific and sustained. Mainly vaccination produces antibody dependent immune response and saving millions of lives every year. However, there is very little information regarding antibody independent vaccination. Recent advances in T cell biology open up new approaches for vaccine development especially in field of antibody independent vaccination. Dendritic cells connect innate and adaptive immune system, so dendritic cell targeting strategies is also most promising approach for induction of antigen specific immunity. In future more studies should be conducted to understand the mechanism of vaccine efficacy.

Conclusion

The mucosal immune system is a complex system that generates lar ge amounts IgA as well as cell mediated immunity at mucosal surfaces to kill pathogens. Mucosal vaccines are attractive strategy to provide protection against various infectious diseases. Our current understanding of vaccine antigen induced immune signalling pathways is something still very much developing. Emerging technologies will continue to provide more understanding of these pathways. By understanding these pathways, we may able to develop highly effective vaccine for complex diseases such as malaria, AIDS and tuberculosis. Today, research and development must continue to progress in the development of oral vaccines to abolish the diseases which are without suitable treatment options. Research also requires the need of continuous improvement for available vaccines to elicit fewer adverse effects and to develop formulations that can be made less costly and more widely available. New or improved vaccines for the malaria, cancer, AIDS and tuberculosis are currently under development.

Acknowledgement

Author gratefully acknowledges the DST, New Delhi, India for providing financial assistance in the form of a DST-INSPIRE fellowship.

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