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Commentary

Harnessing the antibacterial and immunological properties of mucosal-associated invariant T cells in the development of novel oral vaccines against enteric infections

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ABSTRACT

Enteric infections are a major cause of mortality and morbidity with significant social and economic implications worldwide and particularly in developing countries. An attractive approach to minimizing the impact of these diseases is *via* the development of oral vaccination strategies. However, oral vaccination is challenging due to the tolerogenic and hyporesponsive nature of antigen presenting cells resident in the gastrointestinal tract. The inclusion of adjuvants in oral vaccine formulations has the potential to overcome this challenge. To date no oral adjuvants have been licenced for human use and thus oral adjuvant discovery remains a key goal in improving the potential for oral vaccine development. Mucosal-associated invariant T (MAIT) cells are a recently discovered population of unconventional T cells characterized by an evolutionarily conserved αβ T cell receptor (TCR) that recognizes antigens presented by major histocompatibility complex (MHC) class I-related (MR1) molecule. MAIT cells are selected intra-thymically by MR1 expressing double positive thymocytes and enter the circulation with a naïve phenotype. In the circulation they develop a memory phenotype and are programmed to home to mucosal tissues and the liver. Once resident in these tissues, MAIT cells respond to bacterial and yeast infections through the production of chemokines and cytokines that aid in the induction of an adaptive immune response. Their abundance in the gastrointestinal tract and ability to promote adaptive immunity suggests that MAIT cell activators may represent attractive novel adjuvants for use in oral vaccination.

1. Introduction

Enteric infections represent a substantial economic and social burden worldwide. Globally, although mainly in sub-Saharan Africa and south and south-east Asia, diarrheal disease is responsible for one in every ten fatalities in children under the age of five [1]. In African and Asian children the main causes of moderate-to-severe diarrhoea are: rotavirus, *Cryptosporidium*, Enterotoxigenic *Escherichia coli* (ETEC) producing heat-stable toxin, *Shigella*, *Aeromonas*, *Vibrio cholerae* O1, and *Campylobacter jejuni* [2]. Beyond the risk of death as a result of acute symptoms there can also be long term consequences of these infections including: Guillain–Barre syndrome, reactive arthritis and haemolytic uraemic syndrome. Chronic enteric infections due to *Helicobacter pylori* affect up to 50% of the world's population, and increase the risk of ulcers and gastric cancers in adults. Therefore, due to the extensive burden of enteric infections, novel strategies to protect against these enteric pathogens are crucial, especially in developing countries where there is a higher risk of infection.

Vaccination involves the administration of antigenic material to stimulate adaptive immunity, thus conferring lasting protection against infectious diseases. To date the majority of vaccines are administered by injection and this has been successful for a number of pathogens, however there are a number of benefits to developing mucosally administered vaccines (reviewed by Freytag and Clements [3]). Firstly, many pathogens gain access *via* the

Abbreviations: APC, antigen-presenting cell; CT, cholera toxin; CDR, complementarity-determining region; CB, cord blood; DC, dendritic cell; DN, double negative; DP, double positive; ETEC, enterotoxigenic *Escherichia coli*; GIT, gastrointestinal tract; LT, heat-labile enterotoxin of *E. coli*; IgA, immunoglobulin A; ICU, intensive care unit; IFNγ, interferon gamma; IL, interleukin; IEC, intraepithelial cell; IEL, intraepithelial lymphocyte; iNKT, invariant natural killer T; LP, lamina propria; LVS, live vaccine strain; MHC, major histocompatibility complex; MR1, MHC class I-related; MAIT, mucosal associated invariant T; TCR, T cell receptor; Th, T helper; TB, tuberculosis; TNF, tumour necrosis factor; vita-PAMPS, viability-associated PAMPs.

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mucosal surfaces; therefore, directly targeting the entry site is attractive. In general, systemic immunization is ineffective or suboptimal at promoting sustained mucosal immunity. Secondly, a needle free approach is beneficial for a number of reasons including: reduced pain and stress, decreased potential for contracting diseases through contaminated needles and a reduced requirement for medically trained personnel to immunize. Thus making it a more economic and sustainable vaccination method in poorer countries. Thirdly, it has been suggested that optimally formulated mucosal vaccines could have further economic and practical benefits due to improved stability, longer shelf life and decreased requirements for cold storage which would improve the possibility for mass vaccination, especially in developing countries.

Despite these clear benefits and growing interest in developing oral vaccines, progress has been slow. This is partly due to difficulties overcoming the combination of physiological, physical and chemical barriers present in the gastrointestinal tract (GIT) (acidic pH, tight junctions, peristalsis, proteases, bile and mucus). Although these barriers are effective in providing host protection, they also make the development and successful delivery of oral vaccines challenging. Despite these obstacles a handful of oral vaccines are currently licenced for human use, these include; Oral polio vaccine (polio), Vivotif® (typhoid fever), Rotarix®, RotaTeq® (rotavirus), Shanchol™, Dukoral® and mORC-vax™ (cholera). In many cases the effectiveness of whole cell killed and subunit oral vaccines is lower in endemic regions than that seen in trials carried out in developed countries and in travellers. This is due to poorer immunogenicity in local populations. This phenomenon is often referred to as the tropical barrier, and is likely the result of nutritional deficiencies, differences in microflora composition and parasites [4]. Future vaccines against enteric diseases should, therefore, focus on being immunogenic in residents of impoverished countries, and cost effective, to allow for mass immunization in high risk regions.

The tolerogenic and hypo-responsive environment of the GIT is also a difficult challenge to overcome and increases the need for adjuvants in whole cell killed and subunit oral vaccine formulations. The primary role of adjuvants in vaccine formulations is to activate the innate immune system. This in turn triggers the induction of adaptive immunity, which is essential for protection against infectious diseases. Experimental oral adjuvants such as ADP-ribosylating enterotoxins (cholera toxin (CT) and the heat-labile enterotoxin of *E. coli* (LT)), have shown very promising results in animal models [5,6]. It has also been reported that synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG ODN), and monophosphoryl lipid A (MPL) have mucosal adjuvant properties [3]. CT has been the gold standard experimental oral adjuvant for many decades and it has been a very useful tool in demonstrating the protective potential of oral vaccination. Unfortunately, its toxicity precludes it from human use in its native form. Attempts to reduce the toxicity of CT and LT while maintaining their adjuvanticity have shown some promise and these derivatives remain among the leading potential oral adjuvant candidates [4,7]. One such candidate is double mutant LT (dmLT); in recent clinical trials it was found that doses up to 100 μg of this adjuvant can safely be administered orally without toxic side effects [8]. While it is hoped that such molecules can be applied to oral vaccines in humans, there is a pressing need for a panel of mucosal adjuvants with the potential to promote humoral and/or cellular immunity against viral and bacterial pathogens. Since no oral adjuvants are currently included in licensed vaccines for humans, the search for alternative, safe and effective, mucosal adjuvant candidates remains an important goal for mucosal vaccine development.

A significant body of adjuvant research has focused on the discovery and development of immunostimulatory compounds which act directly on antigen-presenting cells (APCs) (*e.g.* toll like receptor agonists), thus enhancing activation of the adaptive immune system. However, targeting populations of unconventional T cells may represent a highly productive strategy to improve the efficacy of oral vaccines at mucosal surfaces. Mucosal associated invariant T (MAIT) cells are a recently discovered innate populations of unconventional T cells [9,10]. These cells accumulate at mucosal sites, home to mucosal tissue when activated, rapidly produce immunomodulatory cytokines in response to bacterial stimuli, can bridge innate and adaptive immunity, and share common features with the more widely studied invariant Natural Killer T (iNKT) cells, agonists of which have previously been shown to have vaccine adjuvant properties. Together, these characteristics suggest that MAIT cells may be harnessed in the development of oral vaccines.

2. Unconventional T cells: promising targets for future vaccine development

Conventional T cells allow the host to recognize pathogens *via* the generation of a highly diverse repertoire of receptors specific for antigens presented on major histocompatibility complex (MHC) molecules. These T cell receptors (TCRs) are composed of two peptide chains, with variable (V) and constant (C) domains. The V domain contains three complementarity-determining regions (CDRs) which together form the antigen binding site in the TCR. Conventional T cells expressing an αβ TCR recognize their specific cognate peptide antigen only when presented by MHC molecules. In contrast to the tremendous diversity in conventional TCRs, a subset of unconventional T cells, characterized by limited T cell diversity exists. These unconventional T cells include γδ T cells, iNKT cells and MAIT cells [11]. They usually exhibit tissue-specific localization and have "natural memory" phenotype and functions, such as the ability to mount rapid responses following a pathogenic challenge [12]. The most extensively studied unconventional T cells are the iNKT cells and the γδ T cells [13], both of which have been suggested to have potential immunotherapeutic applications [14–16].

2.1. iNKT cells

NKT cells are characterized by their expression of cell surface markers associated with natural killer (NK) cells and by a TCR, characteristic of T cells. Human iNKT cells have a highly conserved invariant αβ TCR composed of a Vα24-Jα18/Vβ11. They are selected intra-thymically *via* ligand presentation by the MHC class 1 related CD1d molecule on double positive (DP) hematopoietic cells ($CD4^+$ and $CD8^+$). NKT cells reside in the intestines of both mice and humans and it is estimated that 1% of intraepithelial lymphocytes (IEL) are iNKT cells in both humans and mice [17], although mice have higher numbers of intestinal iNKTs [18]. iNKT cells express CD161 with variable expression of NK1.1 [19]. A high level of sequence similarity exists between the TCR CDR3α and CDR2β loops in human and mouse iNKTs [20]. Expression of a variety of other T cell markers including CD4, but not CD8, has also been reported on iNKTs.

While conventional T cells respond to peptide antigens, the iNKT cells have been shown to respond to glycolipids presented by CD1d [21]. MHC class 1 and CD1 molecules share a common evolutionary origin, however, CD1 molecules are mostly non-polymorphic and have evolved very different antigen-binding grooves which enable them to present hydrophobic lipid molecules [22]. The antigen binding groove of CD1 molecules is defined by two α-helices, which are located above an 8 stranded antiparallel β sheet [23]. Five isotypes of the CD1 molecule family have been identified and these are divided into 2 families, group 1