**Cytokines in the management of rotavirus infection: A systematic review of *in vivo* studies**

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**ABSTRACT**

*Objective*: Rotavirus is a leading cause of childhood diarrhoea. Rotavirus vaccines are effective against severe rotavirus gastroenteritis, but have lower efficacy in low income countries in Africa. Anti-rotavirus treatment is not available. This study reviews the literature of animal studies evaluating whether cytokine mediated pathways of immune activation could improve rotavirus therapy.

*Methods*: We performed a systematic review of articles in English published from 2010 to 2016 reporting agents with *in vivo* antirotavirus activity for the management of rotavirus infection. The search was carried in PubMed, EMBASE, Scopus and Web of Science. Animal experiments where cytokines were investigated to assess the outcome of rotavirus therapy were included.

*Results*: A total of 869 publications were identified. Of these, 19 pertained the objectives of the review, and 11 articles described the effect of probiotics/commensals on rotavirus infection and immune responses in animals. Eight further *in vivo* studies evaluated the immunomodulating effects of herbs, secondary metabolites and food-derived productson cytokine responses of rotavirus-infected animals. Studies extensively reported the regulatory roles for T-helper (Th)1 (interferon gamma (IFN-γ), interleukin (IL)-2, IL-12) and Th2 (IL-4, IL-6, IL-10) cytokines responses to rotavirus pathogenesis and immunity, inhibiting rotavirus infection through suppression of inflammation by viral inhibition.

*Conclusion*: Th1 and Th2 cytokines stimulate the immune system, inhibiting rotavirus binding and/or replication in animal models. Th1/Th2 cytokine responses have optimal immunomodulating effects to reduce rotavirus diarrhoea and enhance immune responses in experimental rotavirus infection.

***Keywords***: Cytokines, Diarrhoea, Gastroenteritis, Inflammation, Probiotics, Rotavirus

*Abbreviations*:ELISA, Enzyme-Linked Immunosorbent Assay; ELISPOT, Enzyme-Linked Immunospot Assay; PCR, Polymerase Chain Reaction; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RT-PCR, Reverse transcription-Polymerase Chain Reaction

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**1. Introduction**

Rotavirus is a segmented, double-stranded RNA virus [1] that, causes severe dehydrating diarrhoea in children worldwide [2]. Two oral rotavirus vaccines, RotaTeq(Merck), a pentavalent human-bovine vaccine containing five rotavirus strains, and Rotarix(GlaxoSmithKline), a monovalent human rotavirus vaccine containing a G1P8 strain, are universally recommended to prevent servere diarrhoea in children [3]. Although the vaccines have significantly reduced diarrhoea incidence and diarrhoea related hospitalizations [4], they are less effective preventing rotavirus infections and their efficacy is lower in some countries in Southern Africa [5].

Although synthetic compounds, such as, ribavirin, cimetidine, famotidine, dipyridamole, nifedipine, and isoprinosine and the effect of plants and their natural molecules have been studied using *in vitro* and *in vivo* experimental systems for the treatment of rotavirus gastroenteritis [6-9], but, there are no promising anti-rotavirus drugs to date [9]. The prevention of dehydration, fluid replacement and zinc supplementation therefore continue to be the key interventions to manage severe diarrhoea in childhood [10].

Rotavirus infection triggers an innate immune response of the intestinal epithelial cells, activating proinflammatory signalling pathways with the release of type I and type III interferons (INFs) and other cytokines [11]. These responses are critical to reduce rotavirus replication and to build up protective immunity in later stages of the infection [12,13]. An increasing number of studies have reported the effect of biotherapeutic substances on antiviral immune responses and the underlying cytokine mediated pro- and anti-inflammatory responses for the inhibition of rotavirus replication. This review examines studies reporting the effect of substances with *in vivo* antirotavirus activity for the management of rotavirus infection, highlighting specific cytokines that regulate the immunomodulation on rotavirus infection.

**2. Materials and methods**

This report followed the guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [14].

***2.1. Search strategy***

We conducted a comprehensive search for papers in PubMed, EMBASE, Scopus and Web of Science limited to Medical Subjects Headings Index (MeSH/DeCS) using the key words "Rotavirus", "Rotavirus infection", "Cytokine" and "Rotavirus vaccine" and related terms. The databases were searched for publication years 2010 to 2016 inclusive. In addition, we reviewed the references lists of the articles selected to identify further reports not included in the database search.

***2.2. Study selection***

Two independent reviewers (GRG and VKSC) screened the titles and abstracts for relevance. Articles considered to have original material were obtained and assessed in detail. Anti-rotavirus studies that examined immune responses by promoting cytokine dependent signalling events in animal models were included. However, *in vivo* studies in humans, *in vitro* studies, review articles, meta-analyses, abstracts, conference proceedings, editorials/letters, patents and case reports were excluded. In case of disagreement between the two reviewers, a third reviewer (JSSQ) was consulted regarding the non-consensus features.

***2.3. Data extraction***

Data were extracted into a pre-defined standardized form containing, article information (first author, year, study location), methods (substances studied, animal model, strain, randomization, blinding procedures, cytokines assessed, study design, outcomes, biochemical assays, molecular mechanisms) and results.

***2.4. Methodological quality and assessment of the risk of bias***

The risk of bias in the experimental design of studies included was explored using checklists and standard scales [15]. The methodological quality was assessed by whether there was adequate randomization and distribution of animal numbers, blinding of drug administration and, assessment of outcomes, description of animal losses and completeness of outcome data.

**3. Results and discussion**

*3.1. Search results*

The selection steps of the studies are summarized in Fig. 1. We identified 869 citations (PubMed: 149, Scopus: 323, EMBASE: 263 and Web of Science: 134). After elimination of duplicates and screening for eligible titles and abstracts, 25 publications were selected for full-text review. Of these, 19 fulfilled the inclusion criteria and were characterized into (a) studies of probiotics for cytokine responses, (b) studies of plant-derived products and secondary metabolites for cytokine responses and (c) studies of fungal secondary metabolites and food-derived products for cytokine responses.

*3.2. Study description*

The characteristics of the studies selected are described in Tables 1 to 3. Eleven articles investigated the immunoregulatory and immunostimulatory properties of probiotics/commensals supplementation against oral inoculation of live mammalian viral strains such as human (Wa), porcine (RVA K85 and OSU) and murine (EW) rotavirus strains individually infected in neonatal gnotobiotic pig or mice models. Three studies evaluated the immunomodulatory and protective effect of natural phytochemicals and five assessed the effects of dietary supplements and fungal secondary metabolites on rotavirus infectivity. Neonatal gnotobiotic pigs have been used widely as a model of human rotavirus diarrhoea and exhibits a similar intestinal epithelial structure to humans[16]. This model is preferred in experimental studies, since it furnishes significant details of the intestinal immunological homeostasis after rotavirus infection [17].

Several methods have been described to study changes in rotavirus infectivity, including the faecal consistency score, faecal virus shedding, intestinal lesions score, histomorphology, analysis of non-structural protein-4 (NSP4) mRNA expression, analysis of intestinal epithelial barrier function, levels of innate, T-helper (Th)1, Th2, Th17, T regulatory, pro- and anti-inflammatory cytokine concentrations, levels of immunoregulatory and immunostimulatory dependent signalling molecules and transcription factors. Among the methods examined for rotavirus inhibition, faecal consistency, faecal virus shedding, histology of small intestine and levels of Th1/Th2 cytokines were used in a larger number of studies.

*3.3. Cytokine responses to probiotics in rotavirus infection*

Studies reported that probiotic strains, such as; *Lactobacillus acidophilus* NCFM™plus *Lactobacillus reuteri* ATCC 23272*, Lactobacillus rhamnosus* GG ATCC 53103 plus *Bifidobacterium lactis* Bb12, *Escherichia coli* Nissle plus *L. rhamnosus* GG and *L. rhamnosus* GG plus *E. coli* Nissle 1917 synergistically colonize the gut and reduce the duration and severity of rotavirus diarrhoea, enhancing virus-specific immune responses and preventing viral replication in the gnotobiotic pig [18-22] (Table 1). *L. rhamnosus* GG, *Lactobacillus ruminis* SPM 0211, *Bifidobacterium* longum SPM 1205 and SPM 1206 individually had antiviral activity against rotavirus infection, alleviating diarrhea, protecting ileal epithelium, improving intestinal microbiota, promoting the intestinal immunoregulatory properties and reducing the levels of virus shedding in the gnotobiotic pig and neonatal mice models [23-27] (Table 1). Innate (interferon alpha (IFN-α), interferon beta (IFN-β)), Th1 (interferon gamma (IFN-γ), interleukin (IL)-2, IL-12), Th2 (IL-4, IL-6, IL-10), pro-inflammatory (tumor necrosis factor alpha (TNF-α), IL-1, IL-8), Th17 (IL-17), anti-inflammatory (IFN-γ) and T regulatory (transforming growth factor beta (TGF-β)) cytokines were measured to describe immune response patterns and its modulations by commensal colonizations.

The most defined therapeutic immunomodulating mechanisms for probiotics strains was to enhance the induction of Th cells including Th1, Th2 and Th17 to promote responses against rotavirus and maintain immunological homeostasis by controlling the production of T regulatory (TGF-β) cytokines. The initial release of Th1/Th2 cytokines such as IFN-γ, IL-4 and IL-12 inhibits significant viral replication by promoting cell-mediated immunity [28]. In addition, IFN-γ production is a major contributing factor against infections caused by a diverse group of cytopathic viruses [29], and it plays a vital role in the defence against viral infections. Furthermore, IL-6 and IL-10 play potential roles in the pathogenicity and immunity against rotavirus infection [30], the latter influencingthe modulation of immune responses, ion transport in the small intestine and anti-inflammatory effects against infectious diseases [31].

Probiotic strains belonging to *Lactobacilli* (*Lactobacillaceae*) individually or jointly with *Bifidobacteria* (*Bifidobacteriaceae*) reduced the levels of rotavirus infectivity by blocking or inhibiting the viral replication and modulation of the immune response through promoting Th1/Th2 cytokines, such as IFN-γ, IL-2, IL-4, IL-10, IL-12, which act as key regulators to enhance immune response against human rotavirus infection [32]. The review also included a comprehensive investigation of other commensal species from *Lactobacilli* and *Bifidobacteria,* which could enable studies of novel anti-rotavirus agents to enhance Th1/Th2 cytokines modulators and immunomodulating effects to control human rotavirus.

The early colonization of probiotic species can play a role enhancing the human rotavirus vaccine efficacy [33]. Among the bacteriotherapies, a considerable number of studies using probiotics such as *L. rhamnosus* GG and *B. lactis* Bb12 reported to regulate innate immune responses, with enhanced innate and Th1 cytokines (IFN-α, IFN-β and IL-12) responses to the vaccines [19,20,27] and reduced the severity of rotavirus diarrhoea in gnotobiotic pigs vaccinated with the human rotavirus vaccines. These probiotics are described as vaccine adjuvants, with significant impact on vaccine immunogenicity and efficacy upregulating Th1 cytokine production. Many of these probiotic species claim functional properties and health benefits and may enhance vaccine efficacy due to their enhanced induction of innate immune responses.

In the gnotobiotic pig model, the use of dietary rice bran in combination with probiotic *L. rhamnosus GG*  and *E. coli* Nissle species reduced human rotavirus induced diarrhoea severity and the interval of episodes and strengthened probiotic growth, gut epithelial health and innate immune responses [34]. In addition, the combination enhanced intestinal IFN-γ concentrations, resulting in Th1 dependent immune responses. These have led to investigators hypothesizing this combination could lead to anti-inflammatory and intestinal epithelial cure for rotavirus infections due to its ability to promote intestinal innate immune system responses.

*3.4. Cytokine responses to plant-derived products and secondary metabolites in rotavirus infection*

Three studies reported the use of phytotherapeutics in the regulation of immune responses in rotavirus-inoculated mice and piglets including Qiwei Baizhu powder (QWBZP), a herbal product from China, *Glycyrrhiza uralensis* Fisch (Fabaceae) and 18β-Glycyrrhetinic acid. These substances are reported to improve small intestinal pathological changes, shorten the duration of diaarhoea and reduce the levels of virus shedding [35-37] (Table 2). Phytochemicals effects on mRNA concetrations of pro-inflammatory, Th1 and Th2 cytokines were investigated to describe their regulatory roles in rotavirus-induced reactive oxygen species formation [35,36]. IFN-γ, IL-5, INF-α and IL-12 secreted from intra-epithelial lymphocytes exhibited strong anti-infection properties and immune tolerance protecting the small intestinal mucosa epithelial cells [38-40]. Plant extracts and natural molecules improved the mRNA expression of IL-2, IL-4, IL-8, and IL-10, IFN-γ, IFN-β and TNF-α and related signalling molecules in small intestinal mucosa epithelial cells**.** These cytokines have special characteristics, interfering rotavirus-induced reactive oxygen species formation and protecting intestinal epithelial cells [41]. These studies highlight the potential of Th1/Th2 cytokine components as anti-rotavirus drug targets as novel antivirals for rotavirus gastroenteritis.

*3.5. Cytokine responses to fungal secondary metabolite and food-derived products in rotavirus infection*

Table 3 describes the role of fungal secondary metabolitesand food-derived productsfor the management of rotavirus gastroenteritis and their immunomodulatory effect on virulent rotavirus infection [42-46]. Human milk oligosaccharides and vitamin D significantly inhibits rotavirus infectivity and enhances cytokines-mediated antiviral responses against experimental rotavirus diarrhoea [42,45,46]. Dietary supplements are useful as functional foods with physiological benefits for immunocompromised children [47]. Human milk is likely to contain anti-rotavirus funtional components and breast-fed infants have a lower incidence of diarrhoeal diseases than formula-fed children [48,49].

Cyclosporin A, a secondary metabolite of *Tolypocladium inflatum* which has been used as treatment of autoimmune disorders [50]. Its role on immunoregulatory, anti-infective and immune responses to rotavirus infection include the enhancement of mucosal Th1/Th2 cytokine responses, modulating mucosal immunity and inhibiting rotavirus binding and/or replication [43,44]. Intestinal mucosa Th1/Th2 cytokine concentrations replicate the immunomodulatory action of therapeutic agents on rotavirus-induced acute gastroenteritis [46] and may reflect the curative effects of the agents in animals challenged with rotavirus and the development of intestinal immune responses through customized Th1/Th2 cytokines quantities. A balanced Th1/Th2 cytokine expression is essential in the initiation and coordination of the immune activities againts viral infections [28]. These dietary supplements and secondary metabolites may constitute a novel nutritional approach to protect against rotavirus infection in infants and animals.

*3.6. Methodological quality/risk of bias*

Studies lacking well designed randomization and inadequate outcome blinding of outcome measurements have a high risk of bias [51]. We found that most experimental studies carried a high risk of bias in relation to measurements to assess precision, directness and applicability of the interventions or exposures. Eighteen of the 19 studies allocated the experimental animals randomly, but only one reported the blinding procedures. The protocol details for the blind assessment of the experimental drugs on animal groups or the investigator awareness of the group allocation and results was not clearly described. The methodological quality of the studies is displayed in Figures 2 and 3.

**4. Conclusion**

This systematic review combined scattered information on the impact of therapeutic substances in the immune function and cytokine responses against rotavirus infection. The review may further our understanding of the antivirals mechanisms enhancing mucosal Th1/Th2 cytokine responses, the stimulation of the immune system and inhibition of rotavirus binding and/or replication in animal models. This report highlights the immunomodulating effects that reduce rotavirus diarrhoea and enhance immune responses during rotavirus infection.

**Conflicts of interest**  
 The authors declare they have no conflicts of interest for this publication.  
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**Figures legends**

**Fig. 1.** Flow chart of study selection process.

**Fig. 2.** Methodological quality of studies. Green bars represent the proportion of studies for which the item was applicable; red bars represent the proportion of studies for which the item was not applicable; yellow bars represent the proportion of studies for which the item was unclear.

**Fig. 3.** Risk level and type of bias in thestudies. Green: Low risk; Yellow: Unclear risk; Red: High risk.