A Non Invasive Technique to Assess Mucosal Immunity in Healthy Population by Measuring Immunoglobulin Receptor Expression on Viable Colonocytes

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Abstract

Human gut plays a vital role in the regulation of immune function, mucosal defense and homeostasis. Gut epithelial cells function as an immune cell and express receptors for microbial-associated molecular patterns. The gut epithelium undergoes constant and rapid renewal and some of these cells are exfoliated into the fecal stream. These cells are an important source of macromolecules, which provides a patho-physiological profile of the colonic epithelium. Most of the methods to harvest colonic epithelial cells are highly invasive and involve endoscopy and biopsy. Researchers suggest that studies of gastrointestinal pathophysiology are not feasible by biopsies in neonates and paediatric population. Therefore, isolation of these exfoliated viable colonocytes from human stool is a non-invasive as well as a highly convenient approach that can be used for diagnostic and research purposes. A very few studies are available across the globe and no study is available from India of using this non-invasive technique to recover viable colonocytes in healthy population. For the first time we are reporting the results of the study on healthy Indian population where we recovered viable colonocytes from the stool samples using this non-invasive approach (Cell Sampling Recovery Method) and assessed immunoglobulins (IgA & IgG) receptors expression by Flowcytometry using specific fluorochrom conjugated antibodies. No study is available which provides the normal reference range of IgA and IgG receptor concentration on viable colonocytes in healthy Indian population. In this study we recruited 25 healthy children and 25 healthy adults from North India and provided the range of IgA and IgG receptor concentration on viable colonocytes for both groups. Results indicated that the difference in the mean IgA and IgG receptor concentration was statistically significant in both groups.

Keywords: Microbial Associated Molecular Patterns (MAMPs); Gut immunity; IgA; IgG; Flowcytometry

Introduction

Gastrointestinal (GI) mucosal surface acts as the primary interface between the host and the physical environment [1,2]. The mucosal surface of the human GI tract is about 200–300 m² and is colonized by 1013–14 bacteria of different species and subspecies [3,4]. These bacteria which are necessary for health also have potential to contribute to the development of diseases by a variety of mechanisms [2,5,6]. Mucosal immune responses help to confine the microbiota to the gut, avoiding a damaging systemic inflammatory response to the microorganisms present in the healthy gut [4,7].

Mucosal immune system is a highly integrated and regulated system. This sophisticated system may have evolved as a major defense mechanism against mucosal encountered infectious agents. Mucosal surface requires a considerable outflow of lymphoid cells and effector molecules for immunity. Secondary lymphoid tissues in the GI tract facilitate antigen uptake, processing and presentation for induction of mucosal immune responses. These tissues are also known as inductive sites. Gut-associated lymphoreticular tissues (GALT) are major inductive sites. It consists of the Peyer’s patches, which are units of mostly terminally differentiated colonic epithelial cells. Studies have suggested that these exfoliated colonocytes can be used in diagnosis and researches. A study examined the utility of live colonocytes in stool in studying cellular markers during early neonatal life [13]. In this study Expression of IgA, IgG, cluster of differentiation-45 cells (CD45), and toll-like receptors-2 and 4 (TLR2 and TLR4) were analysed. Colonocyte RNA extracts were used in quantitative real-time PCR (qRT-PCR) to examine the expression of cytokeratin-19, ribosomal protein-24, and tight-junction (TJ) protein zonula occuldens-1 (ZO-1) [13]. A study suggested that the expression (endoscopy, and biopsy) and continued clinical management of Inflammatory Bowel Disease (IBD) [14] remain highly invasive, expensive, and inconvenient for the paediatric patient. Nair et al conducted a study to see if colonocytes obtained from stools of subjects with IBD and normal controls would

Stool is a heterogeneous mixture consists of undigested food residues, microflora, endogenous secretions, and circular cellular components exfoliated from walls of GI tract [12]. These circular components are rich source of viable intact cells. The circular components are made up of mostly terminally differentiated colonic epithelial cells. Studies have suggested that these exfoliated colonocytes can be used in diagnosis and researches. A very few studies are available across the globe and no study is available from India of using this non-invasive technique to recover viable colonocytes in healthy population. For the first time we are reporting the results of the study on healthy Indian population where we recovered viable colonocytes from the stool samples using this non-invasive approach (Cell Sampling Recovery Method) and assessed immunoglobulins (IgA & IgG) receptors expression by Flowcytometry using specific fluorochrom conjugated antibodies. No study is available which provides the normal reference range of IgA and IgG receptor concentration on viable colonocytes in healthy Indian population. In this study we recruited 25 healthy children and 25 healthy adults from North India and provided the range of IgA and IgG receptor concentration on viable colonocytes for both groups. Results indicated that the difference in the mean IgA and IgG receptor concentration was statistically significant in both groups.

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Viable colonocytes from stool were isolated by Somatic Cell Sampling Recovery, (SCSR) method (Non-invasive Technology, USA). This is a globally most accepted commercial available kit method for the isolation of viable colonocytes isolation from stool samples. In this study aliquots of stool samples (0.5-1.0 gm) were collected in a collection vial containing nontoxic preservative solution (SCSR transport medium, provided in the kit) and processed further to isolate viable colonocytes and immuno-phenotyping.

**Materials and Methods**

**Collection of sample**

The study conducted at All India Institute of Medical Sciences, New Delhi, India. Ethical approval for the study was obtained from the 'Institute Ethics Committee' of All India Institute of Medical Sciences, New Delhi, India. Before enrollment the detail participant information sheet of the study was provided to the adult participants and parents of the child participants. Objective and expected outcomes of the study were also explained. After receiving written participant information consent stool samples were collected from 50 subjects including 25 children and 25 adults. For the collection of fresh stool sample a sterile stool collection vial was provided to the participants. Parents of the child participant were explained and instructed for proper collection of sample to maintain maximum sterile condition. It was ensured that the collected stool sample to be transported to the lab in the minimum possible duration in ice cooled box to maintain 4-8°C. After receiving in laboratory the aliquots of stool samples (0.5-1.0 gm) were transferred in a collection vial containing nontoxic preservative solution (SCSR transport medium, provided in the kit) and processed further to isolate viable colonocytes and immuno-phenotyping.

**Isolation of viable colonocytes**

Viable colonocytes from stool were isolated by Somatic Cell Sampling Recovery, (SCSR) method (Non-invasive Technology, USA). This is a globally most accepted commercial available kit method for the isolation of viable colonocytes isolation from stool samples. In this study aliquots of stool samples (0.5-1.0 gm) were collected in a collection vial containing nontoxic preservative solution (SCSR transport medium, provided in the kit). Samples were adequately mixed by vortex for 15-30 sec. After proper mixing, the dispersed sample was passed through a 330 µm nylon mesh to remove large particles. For further removal of undesired large particles, the filtrate was again passed through a second filter cap (40 µm, BD). The final filtrate was collected in a fresh falcon tube (50 ml). The final suspension under laid with 10 ml of cushion solution (SCSR cushion medium, provided in kit). After adding cushion medium the sample was centrifuged at 200 X g for 10 minutes in refrigerated centrifuge. After centrifugation the light cells moved to interface, and heavy cells migrate into the cushion. Each fraction was collected separately. The light cells were aspirated from the interface and the heavy cells were recovered from the pellet. The collected fraction containing cell population was washed three times with phosphate buffer saline.

**Sample preparation, immunoglobulin receptor staining, acquisition in flowcytometer and analysis**

After adequate washing cell pellets were suspended in 200 µl PBS. A wet preparation of the final cell suspension when viewed under a phase-contrast microscope shows a large numbers of cells. Cells were stained with trypan blue (0.4%) to distinguish viable cells from necrotic cells and observed in phase contrast microscope (PH 2, Nikon, Japan). Simultaneously the cells were counted in a Coulter counter (ZV Coulter Particle Count and Size Analyzer, Beckman Coulter) to give size distribution and calculate cell numbers. The SCSR procedure generally yields 20-40 million cells per gram of stool and size distribution histograms show the existence of 2 distinct populations, one between 2-5 µ and another between 5-8 µ [10,16]. In our all assay a total count of 3 x 10^6 cells were taken in each tube for further staining with florochrome conjugate antibody. To measure the IgA & IgG receptor concentration on viable colonocytes we used IgA-FITC conjugated (Sigma) & IgG-PF conjugated (Sigma). The cells were incubated with specific antibody using a standard protocol and acquired in Flowcytometer (CyAn ADP, Beckman Coulter). Acquired data were analyzed with specific software (Summit Software-Beckman Coulter).

**Results**

In this study, we recruited 25 healthy children and 25 healthy adults. The mean age of the children group was 13.6 months (range from 01 month to 48 months). Among children male: female ratio was 12: 13 (male 48%; female 52%). The mean age of adult group was 34.12 year (range from 18 year to 58 year). Among adults male: female ratio was 13: 12 (male 52%; female 48%).

The range of IgA receptor on viable colonocytes isolated from stool of children varied from 64.2% - 86.5% with the mean concentration of 71.30 ± 0.97% and median value of 70.2%. The range of IgG receptor on viable colonocytes isolated from stool of children varied from 56.5% - 76.6% with the mean concentration of 65.73 ± 1.13% and median value of 66.5% (results summarized in Table 1 and Figure 1). The difference in the mean IgA and IgG receptor concentration was statistically significant in this group (P, 0.0005). All the statistical analysis was carried out by using Microsoft excel 2007 and Graph Pad Prism Software.

The range of IgA receptor on viable colonocytes isolated from stool of adults varied from 50.5% - 78.2% with the mean concentration of 66.82 ± 1.26% and median value of 66.8%. The range of IgG receptor on viable colonocytes isolated from stool of adults varied from 45.5% - 70.4% with the mean concentration of 62.71 ± 1.32% and median value of 66.5% (results summarized in Table 1 and Figure 1). The difference in the mean IgA and IgG receptor concentration was statistically significant in this group (P, 0.0292). Further when the IgA receptor concentrations were compared between children and adult groups it showed the difference was statistically significant (P, 0.0072). And when the IgG receptor concentrations were compared between children and adult groups it showed the difference was not statistically significant (P, 0.0894).

**Discussion**

Human stool is a heterogeneous mixture of undigested food residues,
null
In addition, the stool miR-92a level demonstrated a higher sensitivity for distal CRC than proximal CRC (p<0.05), and a higher sensitivity for advanced adenoma than minor polyps (p<0.05) [24].

We applied this non-invasive approach to assess gut immune system in healthy pediatric and adult population. We recovered significant number of viable colonocytes from fresh stool samples and measured IgA and IgG receptor expression on theses viable colonocytes. The range of IgA receptor on viable colonocytes isolated from stool of children varied from 64.2% - 86.5% with the mean concentration of 71.30 ± 0.97%. The range of IgG receptor on viable colonocytes isolated from stool of children varied from 56.5% - 76.6% with the mean concentration of 65.73 ± 1.13%. The range of IgA receptor on viable colonocytes isolated from stool of adults varied from 50.3% - 78.2% with the mean concentration of 66.82 ± 1.26%. The range of IgG receptor on viable colonocytes isolated from stool of adults varied from 45.5% - 70.4%.

Chandel et al. [13] reported colonocyte yield varied between 5x10⁴ - 2x10⁵ cells/g of stool. Meconium samples yielded a highly enriched population of viable cells. Although low, all samples showed CD45-positive cells during the initial weeks of life. Starting as early as d 2, IgA expression was observed in 69% of the cells. Low to moderate expression of IgG was observed with a linear increase as the infants grew. There was an almost total lack of TLR2 staining; however, >55% of the colonocytes showed TLR4 expression. Although high levels of IgA in gut cells may serve as a natural protectant during neonatal period, increased TLR4 may provide a niche for lipopolysaccharide (LPS)-mediated epithelial damage.

The major human antibody is secretory IgA (slgA), which comprises approximately 80% of all human immunoglobulin and is present in all exocrine secretions. It prevents microbes from reaching mucosal membranes and causing infections [25,26]. After exposure with microbes the lymphoid cells migrate from Peyer’s patches to mucosal membranes and cause infections [25,26]. In the present study, for the first time, we have reported the use of a non-invasive approach to recover viable colonocytes from stool and application of these viable colonocytes for the measurement of immunoglobulin (IgA & IgG) receptors expression using specific fluorochrome conjugate antibodies and flow cytometry. This is a pioneer study in the subject area and it will also encourage further intensive study with diverse parameters and components using this non-invasive approach in different demographic segments. This non-invasive technique can be exploited further for the detection, evaluation and management of different GI diseases including IBD and Colon Cancer.

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**References**


Inflammation and Lineage on Exfoliated Colonic Cells In Pediatric Inflammatory Bowel Disease. J Gastrointest Dig Syst 8: 1-6.


