Scientific Research Publishing

Open Journal of Preventive Medicine, 2021, 11, 383-390
https://www.scirp.org/journal/ojpm
ISSN Online: 2162-2485
ISSN Print: 2162-2477

Germline Deletion of the Expression of a Human Bispecific Mucosal Immunoglobulin: Genetic Predisposition to Cancer and Communicable Diseases Predominantly among African-Americans*

Padmanabhan Nair[1,2]

[1]NonInvasive Technologies LLC, Elkridge, MD, USA
[2]Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
Email: PPN@noninvasivetech.com

How to cite this paper: Nair, P. (2021) Germline Deletion of the Expression of a Human Bispecific Mucosal Immunoglobulin: Genetic Predisposition to Cancer and Communicable Diseases Predominantly among African-Americans. *Open Journal of Preventive Medicine*, **11**, 383-390.
https://doi.org/10.4236/ojpm.2021.1110030

Received: October 3, 2021
Accepted: October 23, 2021
Published: October 26, 2021

Abstract

In a previous report we had reported on the discovery of a novel bispecific immunoglobulin expressed by colonic epithelial cells as they transform into immunomimetic cells during exfoliation (Albaugh *et al.* (2020) *Open Journal of Preventive Medicine*, **10**, **126-150**). Colonic cells isolated from 0.5 gm aliquots of fresh stools (SCSR-10, Fecal Cell Isolation Kit, NonInvasive Technologies, Elkridge, MD) preserved at room temperature for up to one week, with viability of >85% were used to determine the number of cells expressing this novel bispecific immunoglobulin. Over the course of this period (18 years) we recognized that these cells opened the opportunity to investigate the expression of cell membrane biomarkers. As the applications grew, we introduced a new terminology, termed COPROCYTOBIOLOGY*. In this study, we surveyed a cohort of 58 free-living adults for the expression of the newly discovered bi-specific chimeric antibody. Almost all of the subjects showed a strong signal during flow-cytometric evaluation of their stool samples; averaging around 65%. However, two subjects exhibited a total loss of this signal and both these individuals were of African-American lineage (one male and one female). These cells upon culturing *in vitro* remained defective in contrast to the rest of the group where their progeny continued to generate the antibody. We propose that this signals the existence of a germ-line deletion of the gene for which a novel test (MEDISHIELD†) is suggested. This syndrome

*This publication is in commemoration of the author's ninetieth birthday.
†Trade Name by use by Non-Invasive Technologies LLC.

may be associated with a lack of response to prophylactic vaccines involving m-RNA.

Keywords

Colon Epithelial Cells, IgA/IgG, Bi-Specific Immunoglobulin, Oncolysis, Anti-Infective Firewall, Heterodimer, Gastrointestinal Progenitor Cells (GIP-C†)

1. Introduction

The gastrointestinal tract is unique among all other organs in the human body. It is systematically exposed to components in the diet, visited by various micro-organisms present in the environment, bathed by secretions from the stomach, liver and pancreas in addition to its own cell-adhesion molecules in the form of mucins along with hormones and immunoglobulins (generated by cells in the lamina propria). Conventional wisdom taught us that the cells lining the GI tract are shed at an enormous rate (approximately 10^{12} cells per day). However, it was thought that these cells undergo apoptosis and are lost following exfoliation. In addition, international investigators [1] had estimated that the mucosal surface is replaced every 5 days.

In 1991, Nair and associates first reported that cells exfoliated from the GI tract do not undergo massive apoptosis (on an average between 9% - 10% undergo apoptosis) and are recoverable in a pristine state from stool samples. This landmark observation opened the avenues for exploring the nature of exfoliated cells, resulting in a number of fundamental discoveries associated with this physiological process. Over a period of nearly a decade and a half this group reported on a number of issues relating to the characterization of exfoliation and the nature of the cells that were isolated from stool samples [2].

Extensive studies on the physicochemical characteristics of these cells (GIP-C† for gastrointestinal progenitor cells) provided us with insights that were never known until then [2]. Optical observations (phase contrast microscopy) and size distribution analyses showed that about 65% - 70% of these cells were in the 2.5 μM - 5.0 μM range while the rest were in the 5.0 μM - 8.0 μM range. Both these cell populations were positive for colon-specific antigen showing their common colonic ontogeny. [3]. Immunocytological studies showed that the larger cells expressed markers identifying them as goblet cells with their characteristic polar bodies [2] while the smaller cells were perhaps derived from exfoliated columnar epithelium [2]. The smaller pyknotic cells microanatomically appeared as globular cells with a nucleus filling almost the entire cell with the cell membrane enclosing a narrow band of cytoplasm. In view of their appearance we determined their mean Stokes radii and sedimentation constants through various media resulting in our determination of a density range of 1.033 - 1.33 [3].

A series of flow cytometric studies revealed significant numbers of cells expressing IgA and IgG along with about 65% co-expressing both immunoglobulins. Tissue culture studies showed these cells to thrive in stem cell medium and could be maintained for several generations. In addition these smaller cells also expressed Musashi-1 and Lgr-5, biomarkers for gastrointestinal progenitors that are normally seen only in the lower third of the colonic crypt where proliferating cells are sequestered. We generally concluded that during exfoliation some of the cells undergo a remodeling into immunomimetic stem cell-like bodies [4].

Further mapping of cell surface markers revealed that these cells are truly representative of the entire colon based on the fact that approximately 35% of the cells expressed blood group antigens. It is known that in the adult human, cells lining the proximal third of the colon spanning the segment between the ileo-cecal junction and the hepatic flexure express blood group antigens [4].

During the course of the subsequent decade, having convinced ourselves of the value of this technology for a wide variety of investigations, we coined the terminology, COPROCYTOBIOLOGY† to encompass all of the information that may accrue as a result of this approach [2] [5].

Our attention now turned to investigate whether we could distinguish colon cancer cells from normal nonmalignant cells. In this regard we made the observation that colon cancer cells expressed insulin-like receptors while the normal counterparts were negative [6]. Cloning studies revealed that in normal cells the translational activity of the messenger RNA is silenced, a phenomenon now considered to be abrogated during the transition to a malignant phenotype. This was hailed as a major breakthrough in our armamentarium for early colon cancer detection [7].

During the course of our work with these exfoliated cells we noticed a significant number of cells expressed immunoglobulins [8]. This group of immunomimetic cells was thought to bear a significant influence on the nature of mucosal immunity. In view of this observation we planned the current study involving a cohort of free living subjects and their status on mucosal immunity.

2. Materials and Methods

Collection of Stools—Freshly passed stools were collected from anonymous free-living donors, providing only age, sex, and race as identifiers. Subjects were free of any obvious ailments. No other clinical data were collected. Aliquots of the samples (1.0 gm - 1.5 gm) were placed in a specially formulated transport medium (SCSR-010, NonInvasive Technologies LLC, Elkridge, Maryland) (termed as *Acqua sominifera*) to preserve the viability of the exfoliated cells at room temperature for several days. Cells were isolated as described earlier, counted in a cell counter and aliquots were stored at −75˚C in freezing medium.

Isolation of cells—Cells were isolated as described earlier within 9 days of collection [6]. The transport medium preserved the cells such that they could be sent via courier at room temperature. The medium underwent rigorous field

tests in India before adopting it for our work. The isolation procedures were conducted in carefully segregated laboratories using dedicated biosafety cabinets. Operators were trained and certified for these procedures and received frequent supervisory audits. Personnel conducting these isolations were additionally protected by the transport medium that contained a non-toxic component that inhibited pathogenic viruses, bacteria and amoebae.

Flow cytometry—Isolated exfoliated colonic cells consisted of two distinct populations. About 70% of the cells were in the size range of 2.5 μ - 5.0 μ, morphologically globular in appearance and the rest mostly 5.0 μ - 8.0 μ, recognized by antibodies to antigens on goblet cells. A standardized procedure for detecting cell surface antigenic markers was developed and described in an earlier report [8].

The exfoliated cells isolated from stools (GIP-C cells) readily replicated in culture and expressed Lgr-5 and Musashi-1, markers of GI stem cells [6]. Immunomimetic cells were enumerated using anti-human IgA FITC (alpha chain specific, Sigma Cat. F2879) and anti-human IgG PE (Sigma P8047). Test runs also included flow cytometric studies for the co-expression of both IgA and IgG in a sub-population of cells that we identified earlier.

Development of a unique transport medium—The success of our SCSR technology was singularly possible because of our ability to formulate the transport medium with predetermined characteristics as shown here.

Anticipated hurdles: Most eukaryotic cells are in environments that prevent isolation as single-cell suspensions. In addition, they are labile and sensitive to degrading enzymes or toxic agents and are not generally well preserved (usually fixed in fixatives) and thereby non-viable. The presence of cell adhesion molecules (such as the mucins) adds to the complexity of the problem. In many instances the biological material is placed in liquid nitrogen and transported to the diagnostic lab, a limitation when samples are collected in the field far away from the labs where the ultimate analysis is carried out. In tropical countries, such as India and Africa this poses a major hurdle.

1) A liquid medium that is non-toxic (absence of heavy metals, aldehydes, mercurials etc.

Our product is made up of natural compounds considered generally safe.

2) The medium should retain the viability of the cells in suspension at ambient temperature for at least 9 days following transit from collection center to lab. Should release single cell suspensions.

Fecal cell isolates were performed on 310 *stool samples collected in Oddisa and mailed to Trivandrum in India without refrigeration. All samples at* 8 *days post collection gave* <85% *viability.*

Our product retains viability for 10 *days. Viability* > 85% *of single cell suspensions.*

3) The product should be stable for at least six months.

Our product is stable for over 6 *months* (*estimated at about* 13 *months or*

more)

4) Tests of functional capacity of these cells should be robust.

Using blood lymphocytes we were able to see stimulation with mitogens at 9 - 10 *days after blood was transported at ambient temperature* [9].

5) Transport medium should have a color indicator that changes color when medium has deteriorated.

We have a crimson colored transport medium that changes to yellow on deterioration.

6) The medium should inactivate proteases and peptidases.

Yes, the medium inactivates most degrading enzymes.

7) The medium disables pathogenic viruses and bacteria.

Yes, it does, protecting lab personnel from infections

8) The medium could be ideal for transporting pathological exudates, pancreatic aspirates, ascites fluids, duodenal aspirates, pleural effusion etc. without refrigeration.

We have not tested this in the field.

9) We should have formulations that would lyse cells releasing intracellular organelles as well as any pathogens.

We have formulations that lyse cells.

3. Statistical Analyses

Data were analyzed by using the SPSS program.

4. Results

Table 1 summarizes the results of flow cytometric analyses of stool samples from a cohort of 54 subjects.

The results showed that in this cohort of free-living individuals almost all of them expressed IgA and IgG in significant numbers. There were no significant differences between male and female subjects. Among African-Americans there was a statistically significant lower value for IgG as well as for the expression of the bi-specific

Table 1. Expression of IgA, IgG and Co-expression of IgA/IgG.

Sample ID	IgA	IgG	IgA/IgG	N
Total sample	90.9 ± 1.3	68.4 ± 3.7	61.0 ± 4.2	54
Male	88.1 ± 2.6	67.9 ± 5.3	59.3 ± 6.9	23
Female	93.1 ± 1.2	68.7 ± 5.2	62.4 ± 5.2	31
	(−0.06)	(−0.92)	(−0.72)	
Caucasian	91.2 ± 1.9	73.6 ± 6.1	66.9 ± 6.1	16
African-American	92.1 ± 1.2	67.4 ± 4.5	61.0 ± 4.8	36
	(−0.001)	(−0.034)	(−0.001)	
African-American			**0.0**	**2**

Values are means ± SEM, values within parentheses are *p* values.

IgA/IgG heterodimer. In this regard, the most noteworthy observation was the discovery of two African-Americans (one male and one female) with no evidence of the chimeric IgA/IgG heterodimer. When their cells were grown in culture they continued to be negative for this heterodimer showing that the absence of this antibody is likely the result of a germline deletion or silencing thereby raising the possibility of a genetic condition associated with the loss of expression of this gene. Normal exfoliated colonic epithelial cells when grown in stem cell medium continued to express the bi-specific IgA/igG heterodimer showing the gene associated expression of this antibody.

5. Discussion

In this study we were able to show that exfoliated colonic epithelial cells isolated from stools are a valuable source of biological material obtained in a noninvasive manner. We demonstrated that during exfoliation there is dynamic transition to immunomimetic cells expressing not only IgA and IgG but also a chimeric heterodimer consisting of IgA/IgG, the expression of which is variable. In a cohort of free-living subjects, two of them did not express the chimera. Both these subjects were of African-American lineage indicating the existence of a genetic abnormality associated with the absence of this dimeric antibody. Since epidemiological studies have indicated the greater susceptibility to malignancies in the African-American segment of the US population, could this be the proximate organic defect that is contributory to this risk?

In an extension of these observations we believe that this syndrome, "agipsitumab" may also have an overlap with infections like COVID-19. Conventional wisdom passed down the ages has bestowed humans with a natural firewall against contracting communicable diseases and or malignancies. These concepts largely propagated through the utterances of sages from prehistorical times have not been corroborated by modern scientific methodology although they have received conceptual acceptance inferentially. This recent discovery of the existence of a genetic defect associated with the loss of expression of a protective broad spectrum bi-specific antibody (GIPSITUMAB®, NonInvasive Technologies LLC, Elkridge, Maryland, USA) [8], among some ethnic communities (African-American, Asian) has opened the possibility of explaining how some individuals may not respond to prophylactic vaccines based on messenger RNA (blue print of viral protein).

In order to clarify the implications of this finding we have to provide a historical background of the concepts behind these observations. With the advent of the COVID-19 pandemic there was a global surge in the quest for an effective preventive vaccine to control the spread of this infection. This resulted in practically hundreds of scientists getting involved in this effort [10]. To put it in simple language, the idea was to provide a piece of genetic material (e.g.: messenger RNA coding for the spike protein of COVID-19) that is unique to the invading microbe to the human host who will then be alerted to a possible invasion by a

potential aggressor and then respond by countermeasures involving the generation of protective antibodies. By inference it was assumed that all human beings had the same capacity to respond to this RNA vaccine by incorporating it into the body's antibody producing cells to generate the protection. The discovery of the new genetic defect makes it impossible for the body to "cook up" the protective antibody, thereby making the subject a silent carrier of the virus without generating any symptoms of disease. Further corroboration of this hypothesis will have to wait for additional studies in the future.

NonInvasive Technologies (NIT) has developed a test (Medishield®) to identify those who are carriers of this genetic defect so that we can then provide them with information on their increased risk of contracting diseases and also assist them with fully formed antibodies (NIT has developed active antibodies) that do not require the body to make. The aftermath of this discovery is the potential of leaving a significant segment of the population with a silent infection, that can continue to spread in the community.

Researchers from the Pasteur Institute, Paris, working in Africa have shown that approximately 80% of those who are infected with COVID-19 are asymptomatic and are carriers of the infection. By extension, we believe that countries in the Middle East, India and East Asia may have the same problem and this can be assessed by running screening MEDISHIELD® among the general public to ascertain the degree of infectivity among their populations.

Acknowledgements

The author is deeply indebted to members of his research team who generated data for segments of this study. These investigators came from all corners of the globe and over a period of two decades generated an unprecedented amount of transformative knowledge.

Conflicts of Interest

The author is the founder CEO of NonInvasive Technologies LLC.

References

[1] Lipkin, M., Sherlock, P. and Bell, B. (1963) Cell Proliferation Kinetics in the Gastrointestinal Tract of Man. II. Cell Renewal in Stomach, Ileum, Colon, and Rectum. *Gastroenterology*, **45**, 721-729. https://doi.org/10.1016/S0016-5085(19)34805-X

[2] Nair, P.P., Lagerholm, S., Dutta, S., Shami, S., Davis, K., Ma, S. and Malayeri, M. (2003) Coprocytobiology: On the Nature of Cellular Elements from Stools in the Pathophysiology of Colonic Disease. *Journal of Clinical Gastroenterology*, **36**: S84-S93. https://doi.org/10.1016/S0016-5085(19)34805-X

[3] Albaugh, G.P., Iyengar, V., Lohani, A., Malayeri, M., Bala, S. and Nair, P.P. (1992) Isolation of Exfoliated Colonic Epithelial Cells: A Novel Non-Invasive Approach to the Study of Cellular Markers. *International Journal of Cancer*, **52**, 347-350. https://doi.org/10.1002/ijc.2910520303

[4] Kamra, A., Kessie, G., Chen, J.-H., Kalavapudi, S., Shores, R., McElroy, I., Gireesh, T., Sudhakaran, P.R., Dutta, S.K. and Nair, P.P. (2005) Exfoliated Colonic Epithelial

Cells: Surrogate Targets for Evaluation of Bioactive Food Components in Cancer Prevention. *Journal of Nutrition*, **135**, 2719-2722.
https://doi.org/10.1093/jn/135.11.2719

[5] Ryan, L., Wong, Y.T., Dwyer, K.M., Clarke, D., Kyprian, L. and Craig, J.M. (2021) Coprocytobiology: A Technical Review of Cytological Colorectal Cancer Screening of Fecal Samples. *SLAS Technology*, 1-14.
https://doi.org/10.1177/24726303211024562

[6] Malayeri, M., Albaugh, G., Davis, D., Bhathena, S.J. and Nair, P.P. (1997) Exfoliated Human Colonic Epithelial Cells: Insulin Binding and Molecular Cloning of the Insulin Receptor. *Medical Science Research*, **25**, 801-803.

[7] Dutta, S.K, and Nair, P.P. (1998) Noninvasive Detection of Colorectal Cancer by Molecular Tools: Coming of Age. *Gastroenterology*, **114**, 333-1335. (Editorial)
https://doi.org/10.1177/24726303211024562

[8] Albaugh,G.P., Dutta, S.K., Iyengar, V., *et al.* (2020) Identification of a Native Novel Oncolytic Immunoglobulin on Exfoliated Colon Epithelial Cells: A Bispecific Heterodimeric Chimera of IgA/IgG. *Open Journal of Preventive Medicine*, **10**, 126-150.
https://doi.org/10.4236/ojpm.2020.106010

[9] Laurin, E.L., McKenna, S.L.B., Sanchez, J., Bach, H., Rodriguez-Lecompte, J.C., Chaffer, M. and Keefe, G.P. (2015) Novel Cell Preservation Technique to Extend Bovine *In Vitro* White Blood Cell Viability. *PLoS ONE*, **10**, e0140046.
https://doi.org/10.1371/journal.pone.0140046

[10] Dolgin, E. (2021) The Tangled History of RNA Vaccines *Nature*, **597**, 318-324.
https://doi.org/10.1038/d41586-021-02483-w