UNIVERSITI TEKNOLOGI MARA

EXPRESSION OF MICROFOLD (M) CELLS IN 3D CO-CULTURE MODELS TOWARDS PROPAGATION OF HUMAN NOROVIRUS

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Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Medicine)

Faculty of Medicine

April 2023

ABSTRACT

Microfold (M) cells are specialised cells of the intestinal follicular associated epithelium (FAE) in Peyer's patches. The M cells play crucial role in initiating immune responses within the mucosa-associated lymphoid tissue and transcytosis of particles such as antigen and bacteria from the apical side into the dome-like structure of lymphocytes in the basolateral region of the intestinal epithelium. As an enteric virus, human norovirus (HuNoV) has been proposed to invade host cells by exploiting the formation of M cells as it breaches the human intestinal epithelium. Although HuNoV infections cause an estimated 200,000 deaths per year worldwide, HuNoV remains poorly characterised due to the lack of a reliable and reproducible cell culture system for its replication. Therefore, this study aims to develop three-dimensional (3D) culture models expressing the M cells to accommodate HuNoV replication. The 3D culture model comprised of colon carcinoma cells and lymphocytes, co-cultivated on two separate systems; the Transmembrane well plate and alginate hydrogel beads (Alcart©). The integrity and permeability of the cell monolayer were measured as transepithelial electric resistance (TEER) whereby the highly polarised and dense Caco-2 monolayer showed high TEER value that decreased after co-culture with Raji B cells. Scanning electron microscopy (SEM) of the Caco-2 monoculture showed a dense brush border structure, indicating well-distributed microvilli, while both 3D co-culture models showed a sparse and truncated cell structure with clear appearance of a reduced apical surface in the hydrogel beads-entrapping model. Expression of the M cells was further confirmed by immunocytochemistry and Western blot analysis, whereby the expression of alpha-5 protein was highest in the Transmembrane co-culture compared with the Alcart© bead co-culture. On the other hand, beta-1 protein expression was reduced in both 3D co-culture when compared with the monoculture. Interestingly, sialyl Lewis A antigen (CA19-9) was not expressed in either model. Meanwhile, due to lack of HuNoV fresh isolates, this study used a molecular cloning technique to construct a synthetic virus (sHuNoV), to be propagated in both M cells 3D co-culture models. The gene recombinant strategy comprised of digestion and ligation of cDNA fragments into several plasmid vectors. However, various problems hindered the insertion of the fragments into the vectors, resulting in the unsuccessful recombinant DNA plasmid. Consequently, work on *in vitro* transcription and translation of sHuNoV could not proceed. Nevertheless, this study concludes that 3D co-culture models support the formation of M cells, which have high potential for further development in HuNoV propagation.

ACKNOWLEDGEMENT

Firstly, I wish to thank God for giving me the opportunity to embark on my PhD and for completing this long and challenging journey successfully. Alhamdulillah for giving me such ideas, way out of trouble, keeping me insane, positive and protecting me from any disgraceful events.

My gratitude and thanks go to my supervisors Assoc Prof Dr Mudiana Muhamad, Assoc Prof Dr Sharaniza Ab. Rahim and Assoc Prof Dr Lakshmi Selvaratnam for their assistance.

This thesis is dedicated to my family, especially Mak and Abah for being such a supportive parent mentally and financially. Every word that came out from their heart became a booster for me to keep going on this path, facing the truth and challenges in life.

Special thanks to my wife, Nurliyana Mohamad for always be by my side. Always understanding, distracting me whenever needed, trust and keep holding and believing in me. Also, to my little kid, Nuqman bin Mizanurfakhri. You are my outmost motivation to finish my study.

Not forgotten, my colleagues in SYBER, friends, staffs of IMMB, staffs of Faculty of Medicine Kampus Sungai Buloh, staffs of UiTM, staffs of UPM, staffs of MKAK, suppliers company and whoever did help me with this project either directly or indirectly.

Finally, I would like to acknowledge this loving memory to myself. Proud of yourself, be kind to others, keep positive thinking, stay humble. A lot of things you have learnt, a lot more to come. Afterall, you did it man. Alhamdulilah.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Research background

Noroviruses of the Caliciviridae family are important pathogens that cause foodborne illness, especially human norovirus (HuNoV). The virus is the leading cause of nonbacterial acute gastroenteritis (Subahir et al., 2019) in all age groups presenting symptoms including nausea, vomiting, diarrhea and low-grade fever. Infection by HuNoV was responsible for the 685 million cases reported worldwide with approximately 200000 deaths, attributable to its rapid transmission through foecal-oral route (Kirk et al., 2015; Farahmand et al., 2021). The non-enveloped virus consists of a single-stranded positive-sense RNA genome and has been classified into ten genogroups, of which genogroups I and II (GI and GII) were reported to cause infections in humans (Jin et al., 2020), with genotype GII.4 being predominant in several outbreaks (Tohma et al., 2019; Grytdal et al., 2020).

Despite the significant economic and public health impact, HuNoV remains as one of the most poorly characterized RNA viruses, mainly due to lack of a reliable and reproducible cell culture system for its propagation. This major setback also limits studies of virus-host interactions and virus pathogenesis. It has been suggested that HuNoV binds to glycans known as histo-blood group antigens (HBGA) upon entry into host cells (Almand et al., 2017a). Other studies reported the role of HBGA in modulating HuNoV pathogenesis in the human host (Singh, Leuthold and Hansman, 2016). In addition, HuNoV has also been reported to bind to the appropriate receptors expressed on the surface of the human gastrointestinal epithelium, most likely the microfold (M) cells (Straub et al., 2013; Mabbott et al., 2013; Karst and Wobus, 2015). As an enteric virus, HuNoV has been proposed to enter host cells by exploiting the formation of M cells as they penetrate the human intestinal epithelium (Jones et al., 2014; Gonzalez-Hernandez et al., 2014). M cells are defined as specialized epithelial cells found in the follicle-associated epithelia of Peyer's patches (Kernéis et al., 1997). The role of M cells involves the induction of immune responses within the mucosa-associated lymphoid tissue and transcytosis; a process in which small