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REVIEW

Immune Modulation by Acemannan and fermented Butyrate Focusing on Macrophages and MicroRNA regulation: Case Reports with Aloe Vera Supplement

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ABSTRACT

Based on activation of macrophages and vaccine adjuvant activity by aloe polysaccharide or acemannan, we showed that the importance of butyrate and host-miRNA-microbiota interaction in regulation of gut immunity, and butyrate is a chemical factor capable of facilitating induced pluripotent stem cell generation, an

important step for the establishment of pluripotency. Furthermore we exhibited three case reports on the beneficial roles of aloe vera juice-successive ingestion as nutritional and medicinal uses to influenza virus and norovirus.

Key words: Immune modulation; Acemannan; Fermented butyrate; Regulation of macrophages; MicroRNA; Three case reports; Aloe vera juice successive ingestion

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INTRODUCTION

Macrophages are an important part of the monocyte macrophage system and have a variety of functions in the immune responses. Acemannan (aloe polysaccharide; ACM) in aloe vera gel has a stimulating effect by enhancing the respiratory burst, phagocytosis, and killing *Candida albicans* by murine peritoneal macrophage^[1]. The process of phagocytosis plays an important part of the overall process of immunity. The actual phagocytosis step is really a cleaning up operation after some of the earlier immune processes have taken place. The effect is therefore both protective and cleaning. Macrophages are large phagocytic cells that line blood passage and are found in connective tissue. They have mitochondria and lysosome enzymes that kill bacteria, and can influence every aspect of the immune and inflammatory responses^[2]. Schulthess *et al*^[3] reported that macrophages differentiated in the presence of the bacterial metabolite butyrate display enhanced antimicrobial activity. Butyrate-induced antimicrobial activity was associated with a shift in macrophage metabolism, reduction in mTOR kinase activity, increased LC3-associated host defense and anti-microbial peptide production in the absence of an increased inflammatory cytokine

response. Macrophages maintain gut homeostasis by eliminating invasive pathogens and regulating inflammatory responses. Butyrate imprints potent antimicrobial activity during macrophage differentiation through HDAC3 inhibition function.

Several host microRNAs (miRNAs) present in gut have been found to interact with the intestinal microbial community, these interactions may indirectly affect the neurological system. MiRNAs are small non-coding RNAs that are commonly present in both normal and tumor cells. Aberrant miRNA expression is associated with colorectal cancer (CRC) initiation, progression, and metastasis. Butyrate can modulate cell proliferation, differentiation apoptosis, and miRNA expression in CRC. Furthermore, butyrate regulates oncogenic signaling molecules through miRNAs. The evidence on the effect of butyrate on CRC risk through reducing oncogenic miRNA expression was discussed by Bishop *et al*^[4]. Our earlier report^[5,6,7] summarized that aloe vera fermented with endophytic microbiota produces butyric acid and the extract stimulates anti-inflammatory activity via the suppression of reactive oxygen species as well as both Cox 1 and 2 enzymes, and discussed aloe vera fermentation as a tumor-suppressive process generating microbial-derived butyrate.

In the present article we will discuss immune modulation by ACM and fermented butyrate focusing on macrophages and miRNA regulation, and case reports to improve immune-stimulating properties by aloe vera juice supplement.

ACTIVATION OF MACROPHAGES CELL LINE BY ACEMANNAN

Stuart *et al*^[8] investigated upregulation of phagocytosis and candidal activity of macrophages exposed to the immune-stimulant ACM. Thirty eight percent of *Candida albicans* died after 10 min of exposure to ACM, compared with 0.5% in the control group. When mice peritoneal macrophages were incubated with ACM for 60 min, 96% of the yeast was killed, compared with 0% in the control group. Moreover, a mechanism of action for ACM in activating RAW 264.7 cells has been proposed by Zhang and Tizard^[9]. In RAW 264.7 cells from a mouse macrophage cell line, ACM stimulates the production of macrophage cytokine, nitric oxide release, surface molecule expression and cell morphology. IL-6 and tumor necrosis factor- α were produced in a dose-dependent manner, and morphological changes of cells were observed. The increasing of surface antigen expression is stimulated by an increase in the mixture of INF- γ -ACM. Furthermore ACM induced apoptosis in RAW 264.7 cells in the presence of IFN- γ . These cells exhibited chromatin condensation, DNA fragmentation, and laddering characteristic of apoptosis. The induction of apoptosis by ACM and IFN- γ does not seem to be mediated by nitric oxide. ACM in the presence of IFN- γ also inhibited the expression of bcl-2. These results suggest that ACM in the presence of IFN- γ induces apoptosis in RAW 264.7 cells through a mechanism involving the inhibition of bcl-2 expression^[10].

VACCINE ADJUVANT ACTIVITY OF ACEMANNAN FOR CHICKENS, CAT AND DOGS

Adjuvants are crucial components of vaccine. They significantly improve vaccine efficacy by modulating, enhancing, or extending the immune response and at the same time reducing the amount of antigen needed. ACM was able efficiently and durably to increase the activation capacity of macrophages from systemic immune compartment (in particular from the blood and spleen after an

intramuscular injection) in chickens, especially for NO production. These findings provide a better understanding of the adjuvant activity of ACM for viral and tumor diseases^[11]. ACM was approved by the United State Department Agriculture for use as a vaccine adjuvant in chickens, cat and dogs. In an earlier paper aloe high molecular fraction (AHM) containing ACM and glycoprotein (verectin) shows immunomodulatory and inflammatory activities^[12,13]. AHM could be used as biodegradable safe and non-toxic adjuvants, and may function, at least in part, through macrophage activation^[14].

ALOE POLYSACCHARIDE CAN SERVE AS THE EFFICIENT IMMUNOLOGIC POTENTATE AND ADJUVANT

Sun *et al*^[15] examined effect of aloe polysaccharide (APS) on chicken immunity indexes; 40 mg/ml of APS has excellent effect of immunity enhancement. APS can serve as an efficient immunologic potentate and adjuvant. Furthermore, the authors investigated the in vitro activity of APS could inhibit the replication of a H1N1 subtype influenza virus, and the most obvious inhibitory effect was observed in the viral adsorption period. Experiments on H1N1 virus infection in mice demonstrated that APS considerably ameliorated the clinical symptoms and the lung damage of the infected mice, and significantly reduced the virus loads and mobility. The direct interaction between APS and influenza A virus is a likely mechanism of inhibiting viral infection^[16].

THE IMPORTANCE OF BUTYRATE, A MICROBIAL-DERIVED METABOLITE, IN REGULATION OF GUT IMMUNITY

The short chain fatty acid butyrate is produced by fermentation of dietary fiber by the intestinal microbiota. Fernando *et al*^[17] hypothesized that butyrate would reinforce an macrophages differentiated with IL-4[M (IL-4)s] can suppress colitis, the authors showed that in the presence of butyrate M (IL-4) s display reduced expression of their hallmark markers Ardl and Ym1 and significantly suppressed LPS-induced nitric oxide, IL-12p40, and IL-10 production. Butyrate treatment likely altered the M (IL-4) phenotype via inhibition of histone deacetylation. Functionally, M (IL-4) s treated with butyrate showed increased phagocytosis and killing of bacteria, compared with M (IL-4) s and this was not accompanied by enhanced pro-inflammatory cytokine production.

The demonstration that butyrate promotes phagocytosis in M (IL-4) s that can limit T-cell production of IL-17A reveals novel aspects of bacterial-host interaction in the regulation of intestinal homeostasis.

HOST-MIRNA-MICROBIOTA INTERACTIONS IN COLORECTAL CANCER

Targeting miRNA-dependent p21 gene expression in human colon cancer by the microbe-derived butyrate

Several microRNAs (miRNAs) have shown different expression levels in response to histone deacetylase inhibition (HDACi) treatment. Some of them can potentiate the anti-tumor responses or on the contrary, decrease it. Since tumor cells release miRNAs through exosomes that can be detected in all body fluids, such as plasma, urine or saline, analysis of circulating miRNAs in patients liquid biopsies, provides promising biomarkers to monitor drugs in patients.

Colonic microbiota ferment non-absorbed dietary fiber to produce prodigious amounts of short chain fatty acids (SCFAs) that benefit the host through a myriad of metabolic, trophic and chemo-preventative effects. The chemo-preventative effects of butyrate are, in part, mediated through induction of p21 gene expression.

Hu *et al*^[18] concluded that microbe-derived SCFAs, butyrate, regulate host gene expression involved in intestinal homeostasis as well as carcinogenesis through modulation of miRNAs. Butyrate also regulates the expression of miRNA to inhibit colorectal cancer (CRC) cell proliferation. Han *et al*^[19] investigated whether butyrate regulates miRNA-203 to inhibit CRC growth and explore the promising target gene of miRNA 203 in CRC cells.

Humphreys *et al*^[20] identified competing roles for miR-17-92 cluster members, in the context of a histone deacetylase inhibitor (HDI)-induced changes in CRC cells. The dysregulated microRNA expression observed in HT29 and HC116 CRC cells could be epigenetically altered by HDIs; butyrate, suberoylanilide hydroxamic acid and trichostatin A. These HDIs decreased expression of miR-17-92 cluster miRNAs, with a corresponding increase in miR-17-92 target genes, including PTEN, BCL2L11, and CDKN1A. The growth effects of HDIs may be mediated by changes in miRNA activity, with down-regulation of the miR-17-92 cluster a plausible mechanism to explain some of the chemo-protective effects of HDIs. Designed to target HDAC, these drugs can modify the expression pattern of numerous genes including those coding for miRNA from a tumor can be investigated in the tumor itself, as well as in patient body fluids. Autin *et al*^[21] summarized current knowledge about HDAC and HDACi in several cancers, and described their impact on miRNA expression.

Several miRNAs participate in signaling networks through the intervention of the gut microbiota. The interaction between the gut microbiota and miRNA plays a crucial role in vascular dysfunction and hepatocellular carcinoma. Feng *et al*^[22] reported the crosstalk between the gut microbiota and physical organs and how the gut microbiota and miRNAs regulate each other while influencing the host via genes, proteins, or metabolites.

Butyrate regulates oncogenic signaling molecules through miRNA and methylation

Butyrate, as a signaling molecule, has effects on multiple signaling pathways. The most studied effect is inhibition on HDAC, which leads to alterations of several important oncogenic signaling pathways such as JAK2/STAT3, VEGF. Butyrate can interfere with both mitochondrial apoptotic and extrinsic apoptotic pathways. Furthermore, butyrate regulates oncogenic signaling molecules through miRNAs and methylation. Therefore, butyrate has the potential to be incorporated into cancer prevention and treatment regimens. Chen *et al*^[23] summarize recent progress in butyrate research as an anti-cancer agent with emphasis on its effects on oncogenic signaling pathways.

Multiple sclerosis (MS) is an autoimmune disease that affects the functioning of the central nervous system. Recent studies on MS and its animal model, experimental autoimmune encephalomyelitis (EAE), have shown that the composition and abundance of microbes in the intestinal microbiota are an environmental risk factor for the development of MS and EAE. Metabolites including SCFAs, such as butyrate, are the primary signaling molecules produced by intestinal microbiota that interact with the host immune system, suggesting an association between MS pathophysiology and gut microbiota. In addition, several host miRNAs present in the gut have been found to interact with the intestinal microbial community, these interactions

may indirectly affect the neurological system. Fan *et al*^[24] reviewed the use of diet to alter the gut microbiota and its application in the treatment and prevention of MS and suggested that MS/EAE development is significantly affected by the interactions between certain miRNA and gut microbiota.

The role of miRNA in macrophages to tumor immunity

Macrophages are essentially present in cell tissues, and they are crucial effects of wound healing, homeostasis, cancer, and immune responses. Diversity and plasticity are the foremost characteristics of cells derived from a monocyte-macrophages linkage, especially for tumor-associated macrophages. The tumor microenvironment (TME) is the primary area where tumor cells and host immune system interact. Bidirectional communication between tumor cells and the associated stromal cell types within the TME influences disease initiation and progression, as well as tumor immunity. Macrophages and natural killer (NK) cell are crucial components of the stromal compartment and display either pro- or anti-tumor properties, depending on the expression of key regulators. MiRNAs are emerging as such regulators. They affect several immune cell functions closely related to tumor evasion of the immune system. Xu *et al*^[25] discussed the role of miRNAs in the differentiation, maturation, and activation of immune cells as well as tumor immunity, focusing particularly on macrophages and NK cells.

BUTYRATE PROMOTES INDUCED-PLURIPOTENT STEM CELL GENERATION

Recent studies have demonstrated that embryonic stem cell-like induced pluripotent stem (iPS) cells can be generated by enforced expression of defined transcription factors. Liang *et al*^[26] reported that butyrate significantly increases the efficacy of mouse iPS cell generation using the transcription factors Oct4, Sox2, Klf4, and c-Myc. Furthermore, butyrate not only changes the reprogramming dynamics, but also increases the ratio of iPS cell colonies to total colonies by reducing the frequency of partially reprogrammed cells and transformed cells. Demonstration that butyrate is capable of facilitating iPS cell generation suggests that alteration of epigenetic status is an important step for the establishment of pluripotency. The study identifies butyrate as a chemical factor capable of promoting iPS cell generation. Mali *et al*^[27] investigated that butyrate greatly enhances the efficacy of iPS cells derivation from human adult or fetal fibroblasts. To gain insights into the underlying mechanisms of butyrate stimulation, the authors conducted genome-wide gene expression and promoter DNA methylation microarrays and other epigenetic analysis on established iPS cells and cells from intermediate stages of the reprogramming process. By days 6 to 12 during reprogramming, butyrate treatment enhanced histone H3 acetylation, promoter DNA demethylation, and the expression partially substitutes for butyrate stimulation. Butyrate stimulation provides an efficient method for reprogramming various human adult somatic cells, including cells from patients that are more refractory to reprogramming.

Small molecules (SM) can greatly enhance the efficacy of iPS cell generation, but the mechanisms by which they act have not been fully explored. Zhang *et al*^[28] investigated that SM cocktail (sodium butyrate, PD0325901, and SB431542) significantly promotes iPS cell generation from human fibroblasts, and sodium butyrate (NB) is more potent than the other two common HDAC inhibitors (valproic acid and trichostatin A) in promoting cellular reprogramming. Furthermore, SM cocktail substantially upregulates the miR302/367

cluster expression by increasing the stability and transcriptional level of this miRNA cluster in a manner dependent on the four defined transcription factors (TFs). NB alone can enhance the TFs-dependent upregulation of the miR302/367 cluster. Collectively, the findings suggest that the SM cocktail promotes reprogramming at least partly through the induction of the miR302/367 cluster expression. MiR-302/367 is the most abundant miRNA cluster in human embryonic stem cells (hESCs) and can promote somatic cell reprogramming. Zhang *et al.*^[29] show that butyrate upregulates miR-302/367 cluster expression and butyrate alleviates hESCs expression from apoptosis induced by knockdown of miR-302/367 cluster.

BUTYRATE CAN REDUCE THE COLORECTAL CANCER FORMATION THROUGH REGULATING PLAC8 EXPRESSION

Tumor metastasis or recurrence often occurs in patients with curative resection of colorectal cancer (CRC). Placental-specific 8 (PLAC8), which is a protein that in human is encoded by the PLAC8 gene and has increased expression in stool, may be associated with CRC recurrence. Huang *et al.*^[30] found that the tumorigenic and invasive effects of PLAC8 on CRC cells were confirmed in a xenograft mouse model. The authors identified reduced levels of two butyrate-producing organisms, *Buyricoccus* and *Prevotella* spp., in stools from CRC patients, suggesting that butyrate downregulated PLAC8 expression and induced apoptosis in PLAC8-overexpressing cells. The data suggest that PLAC8 gene and protein expression and dysbiosis of gut microbiota, especially in butyrate-producing microorganisms, may be indicators of CRC progression.

TARGETING THE MICROBIOME FOR HUMAN IMMUNODEFICIENCY VIRUS-INFECTED SUBJECTS

The components, metabolites of short chain fatty acids, of human gut microbiome have been found to influence a broad array of pathologic conditions ranging from hearts disease to diabetes, cognitive impairment and even to cancer. Human immunodeficiency virus (HIV) infection upsets the delicate balance in the normal host-microbe interaction both through alterations in the taxonomic composition of gut microbial communications as well as through disruption of the normal host response mechanisms. The mechanism of how gut microbiota is involved in the host inflammatory processes that influence HIV replication and disease progression in HIV was elucidated in our earlier report^[31].

CASE REPORTS

Case 1: A 7-years school child had body temperature at 39.8°C, sore throat, cough and fatigue on Feb. 2014, and was diagnosed as influenza. She ingested aloe vera juice (AVJ) 500ml/day and next day she had vomiting and severe diarrhea. While she had positive to influenza virus test, she continued to ingest AVJ for two days. She ameliorated clinical syndrome showing 37°C body temperature on next day and spent a well-school life on the day after next day. It is an important case description that she suffered only one day from high body temperature and restored to influenza syndrome with AVJ ingestion.

Case 2: A 10-years school child had positive reaction to urine protein and high blood sugar, and a sore throat on Feb. 2010. She was

diagnosed as streptococcus infection judging from antigen-antibody reaction. She ingested AVJ 200ml/day for one day in the morning. Then she spent a well-school day without any side effect. It seems to be cured with her self-healing power.

Case 3: About 40-years three ladies living in Toshima-ku, Ikebukuro, Tokyo, were infected with norovirus on Dec 3, 2006. Norovirus is a very contagious virus that causes vomiting, stomach pain and diarrhea. One of them having an eruption was treated with Orengekuto (Kampo-oriental drug) and the other who ingested AVJ 200ml/day every day, have no sever syndrome. They were all recovered from norovirus infection with kampo-oriental drug and AVJ ingestion without an eruption and diarrhea. An announcement of norovirus outbreak in Toshima-ku Ikebukuro was reported by Toshima-ku Health center, on Dec. 5, 2006.

DISCUSSION

Biogenics is defined by T. Mitsuoka^[32] as food ingredients which beneficially affect the host by directly immune-stimulating, suppressing mutagenesis, tumorigenesis, peroxidation, hypercholesterolemia, or intestinal putrefaction: the candidates are bioactive peptides including immune- potentiates, flavonoids, vitamin A, C, E, (biological response modifiers) *etc.* Metabolites produced during the fermentation by endophytic microbiota in aloe vera leaves, such as short chain fatty acids, and vitamins as well as amino acids and oligosaccharides in aloe vera gel promote beneficial effects to the host^[5]. This metabolic production from the microbiota is considered biogenics. Short chain fatty acids, acetate, propionate and butyrate help improve the balance of the microflora by keeping the environment in the intestine controlling the growth of harmful bacteria.

As one of a pivotal function for homeostasis in the host are macrophages. Macrophages maintain gut homeostasis by eliminating invasive pathogenic bacteria and toxin, and regulating inflammatory responses as biological response modifiers. Several host micRNA which are small non-coding RNA that are commonly present in normal cells, have been found to interact with the intestinal microbial community. Microbe-derived SCFAs, butyrate, regulate host gene expression involved in intestinal homeostasis as well as carcinogenesis through modulation of micRNAs^[17]. Demonstration that butyrate is capable of facilitating induced-pluripotent stem cell generation suggests that alteration of epigenetic status is an important step for establishment of pluripotency^[27].

In vitro and vivo tests revealed that aloe polysaccharides could inhibit the replication of a H1N1 subtype influenza virus, and reduced the virus loads and mortality^[15,16]. In three case reports we demonstrated that aloe vera juice successive ingestion ameliorated the clinical symptoms and reduced the virus loads without any side effects.

SUMMARY

Aloe polysaccharides (APS) and aloe vera juice exhibited efficacy to influenza virus and norovirus infection in case reports. In vitro and vivo tests revealed that aloe polysaccharides could inhibit the replication of a H1N1 subtype influenza virus, and reduced the virus loads and mortality^[15,16].

Activation of macrophages and vaccine adjuvant by APS or ACM may originate in butyrate, a microbial-derived gut metabolite or aloe vera gel fermented by endophytic bacteria.

In case reports it is suggested that successive ingestion of AVJ could improve natural immune healing power to norovirus infection.

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