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Differential ratios of fish/corn oil ameliorated the colon carcinoma in rat by altering intestinal intraepithelial CD8$^+$ T lymphocytes, dendritic cells population and modulating the intracellular cytokines

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ABSTRACT

Intraepithelial lymphocytes (IELs) impart a crucial role in maintaining intestinal homeostasis, yet their role in colon cancer pathogenesis remains unknown. Here, we posited that the modulation of intestinal immune response via dietary interventions might be an implacable strategy in restraining colon carcinoma. In the above context, we studied the effect of differential ratios of fish oil (FO) and corn oil (CO) on the gut immune response in experimentally induced colon cancer. Male Wistar rats were divided into six groups: Group I obtained purified diet while Groups II and III were fed on the diet supplemented with differential ratios of FO and CO i.e. 1:1 and 2.5:1, respectively. The groups were further subdivided into control and carcinogenic group, treated with ethylenediaminetetraacetic acid (EDTA) or *N,N'*-dimethylhydrazine dihydrochloride (DMH), respectively. Initiation phase comprised the animals sacrificed 48 h after the last injection whereas, the post -initiation phase was constituted by animals sacrificed 12 weeks after the treatment regimen. CD8$^+$ T cells, CD8/αβ TCR cells, dendritic cells increased significantly on treatment with DMH as compared to control. However, on treatment with differential ratios of FO and CO these cells decreased significantly. The intracellular cytokine i.e. interferon gamma (IFN-γ) and cytotoxic granules component i.e Perforin and Granzyme decreased significantly in the initiation phase but in the post-initiation phase IFN-γ and Perforin increased considerably on carcinogen treatment as compared to the control group. On treatment with FO and CO in the initiation phase the IFN-γ, Perforin and Granzyme expression increased significantly. However, in the post-initiation phase treatment with differential ratios of FO and CO led to a significant decrease in the IFN-γ, Perforin and increase in Granzyme was observed in these groups. Altogether, FO supplementation appeared to activate the immune response that may further attenuate the process of carcinogenesis, in a dose and time-dependent manner.

1. Introduction

Intestinal intraepithelial lymphocytes (IELs) play a crucial role in maintaining intestinal homeostasis and are found to be conserved throughout the vertebrates. However, the role of IELs in colon cancer pathogenesis is still to be explored. Given that in solid tumors, the intrinsic inflammation creates a favorable microenvironment [1], that triggers an alteration in the expression of different immune mediators and modulators which in conjunction with other cell types orchestrate the process of tumorigenesis [2]. Although, the impact of tumor lymphocytic infiltration and underlying T cell response in tumor pathogenesis is still not clear. This interrelation is a determining factor that influences the tumor immune system and thereby affecting its pathogenesis [3]. Thus, a better understanding of dynamic roles and pathogenesis of immune cells, especially T cells is essential for the formulation of newer strategies against cancer. Previous studies have inferred that lymphocytes residing in tumor microenvironment rather than circulating lymphocytes predict the clinical outcomes.

Earlier studies have reflected that cytotoxic T lymphocytes (CTLs) or CD8$^+$ T cells in the tumor milieu might play an important role in anti-cancer immunity and for better prognosis in several types of cancer [4]. CTLs have the ability to promote apoptosis of target cells by using a combination of granules (perforin/granzyme), and non-lytic effector i.e., interferon gamma (IFN-γ) [5] and thus, make CTLs an attractive target for anti-tumor immunity [6]. Despite ample evidence pertaining to the role of IFN-γ in cancer immune surveillance, several studies have also reported its inverse role in cancer pathogenesis i.e pro-tumorigenic [7,8]. Hence, IFN-γ in tumor microenvironment operates/acts in a dual manner, and its pro or anti-tumorigenic activity lies in the cellular microenvironment and molecular context [9]. Dendritic cells (DCs) are

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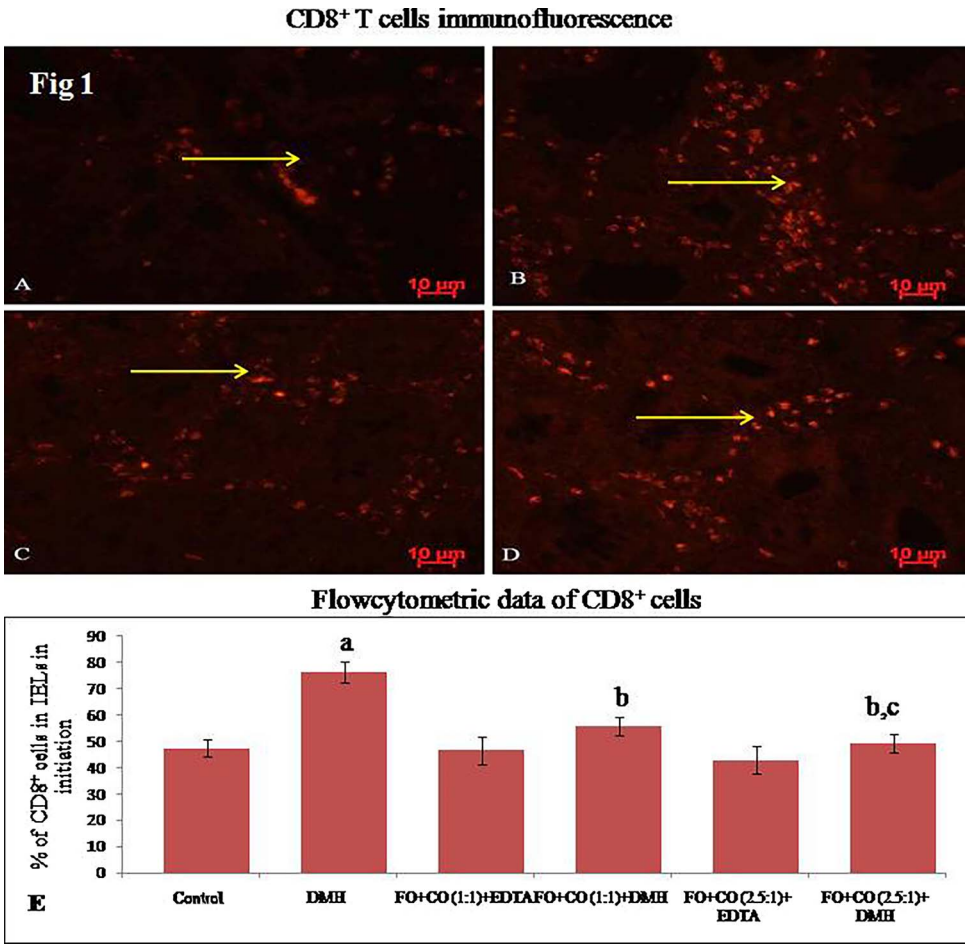

Fig. 1. Effect of supplementation of differential ratio of FO/CO on $CD8^+$ T cells (40X). A–D represents immunofluorescent staining of $CD8^+$ cells in colon tissue A) Control group. B) DMH treated group. C) FO + CO(1:1) + DMH group. D) FO + CO (2.5:1) + DMH (Yellow arrow shows the $CD8^+$ T cells F. Graphical representation of flow cytometric analysis of $CD8^+$ T cell percentage. Values were represented as mean ± SD (n = 5-8). $^a p < 0.001$ with respect to control group, $^b p < 0.01$ with respect to DMH, $^c p < 0.05$ with respect to FO + CO (1:1) + DMH.

the key regulator of adaptive immune response in the intestine, which imparts an important role in the generation and regulation of immune response against antigens. DCs are immensely involved in the presentation of various intracellular and tumors antigens to different T cell subsets i.e $CD4^+$ and $CD8^+$ T cells and thereby instigate the immune response. The ability of DCs to activate the anti-cancer immune response has been affirmed by several researchers and hypothesized as an attractive target for therapeutic manipulation [10,11].

Several factors viz. nutritional status, gut microenvironment and external environmental factors are known to influence the intestinal immune system of an individual [12–15]. Therefore, efforts are going on to identify the natural bioactive compounds from food and microbes for the formulation of functional foods [16–18]. Among dietary elements the composition and concentration of dietary fats play a substantial role in the immune modulation [19]. The n-3 polyunsaturated fatty acids (PUFAs) and n-6 PUFAs are crucial fatty acids and have inverse role in the process of carcinogenesis, therefore a balanced ratio of n-3/n-6 PUFAs is essential rather than their absolute intake [12]. Experimental studies suggested that PUFAs might do all this by interfering with the activation of T cells, cytokines synthesis, and antigen presentation [19–21]. Earlier, we investigated that administration of different ratios of fish oil (FO; n-3 PUFAs) and corn oil (CO; n-6 PUFAs) to colorectal cancer (CRC) animals led to a significant modulation in oxidative stress, apoptosis, DNA damage, cell proliferation and alteration in mitochondrial structure and functions [22,23]. Out of various combinations analyzed the 2.5:1 and 1:1 ratios of FO and CO showed the significant chemopreventive effect, thus the same combinations were opted for the present study. To date, no report is available concerning the implication of differential ratios of FO/CO on the different immune cells in colonic IELs. This missing link in the knowledge has envisaged us to design the present study wherein, the implication of differential ratios of FO/CO on the $CD8^+$ T lymphocyte, their cytokines, and DCs in the IELs in experimentally induced colon carcinoma.

2. Material and methods

2.1. Chemicals

The chemicals used to induce colorectal cancer, for isolation of IELs, the composition of n-3/n-6 PUFAs and mineral mixture were in accordance with our previous publication [12]. Monoclonal antibodies utilized for Phycoerythrin (PE) conjugated CD8, granzyme, dendritic cell (OX-62 monoclonal antibody), and αβTCR were from BD Biosciences (San Jose, USA) while Perforin, PE-labeled IgG_1, secondary antibodies were obtained from Santa Cruz Biosciences (Santa Clara, USA). FITC-IFN-γ and FITC labeled IgG_1 were ordered from Biolegend (San Diego, CA, USA). All other chemicals otherwise stated were of analytical grade.

2.2. Animals and diet

Male Wistar rats (100–150 g) were obtained from Central Animal House of Panjab University, Chandigarh, India after getting Institutional Ethics Committee (AEC/158–169) approval. After a week's acclimatization, the animals were randomly divided into different groups and fed experimental diet up to four weeks. The diets were formulated as per AIN-76A composition in such as way that animals of all groups would consume the same amount of calories.