CHAPTER I

GENERAL INTRODUCTION
Nutrition and Immune system

The immune system protects the host from infectious agents that exist in the environment including bacteria, viruses, fungi, parasites and from other harmful insults. It relies on two functional branches to accomplish this task namely, the innate and the acquired immune system, both involving a diversity of circulatory factors (complement, antibodies and cytokines) and immune cells (macrophages, polymorphonuclear cells, and lymphocytes). The effective functioning of this defensive system is critically determined by nutrition (Chandra et al., 2002). Without adequate nutrition, the immune system is clearly deprived of the components needed to generate an effective immune response. As vital components in the diet, lipids exert a profound impact in the alteration of the functions of the immune system. The fatty acid composition of the diet alters the fatty acid profile of the lymphocytes and other immune cells. Therefore, a dietary lipid exhibits an immunomodulatory role, which could be directed in the management of some inflammatory diseases such as ulcerative colitis and rheumatoid arthritis, etc, (De Pablo & Alvarez de Cienfuegos 2000).

Dietary fatty acids modulate the immune system through several mechanisms that include reduction of lymphocyte proliferation, pro-inflammatory cytokine synthesis, phagocytic activity and modification of natural killer (NK) cell activity. Such alterations of immune functions may be associated with changes in the cell membrane due to dietary fatty acid manipulation. Fatty acids get incorporated into the plasma membrane after dietary lipid administration and alter the composition of lipids in this cellular structure. However, changes in the phospholipid content of the plasma membrane of these cells depend on many factors including the amount, type and duration of exposure of dietary lipids (Clamp et al., 1997).

The Innate and Adaptive Immune Systems

The immune system comprises of cells, tissues and molecules that are divided into two types, namely innate immunity and adaptive immunity. The innate immune system includes 1) Mucosal epithelial barriers, 2) dendritic cells, 3) phagocytic leukocytes 4) natural killer (NK) cells and 5) circulating plasma proteins.
The adaptive immune system comes into action against pathogens that are able to evade or overcome innate immune defence. The activated cells of adaptive immune system eliminate the microbes by activating, proliferating, and creating potent mechanisms for neutralizing or eliminating these foreign substances. The adaptive immune system includes humoral immunity, mediated by antibodies produced by B lymphocytes, and cell-mediated immunity, mediated by T lymphocytes (Figure 1.1).

**Figure 1.1: Cells of the Innate and Adaptive Immunity. (Adopted from UCSF.edu).**

**Cells of the Immune system**

The elimination of pathogens is accomplished by the multiple interactions and activities of the diverse cell types involved in the immune response. The innate immune response carried out by inflammatory cells such as neutrophils and macrophages, natural killer (NK) cells, and granulocytes. The subsequent complex adaptive immune response is antigen-specific and may take days to develop. The antigen-presenting cells including macrophages and dendritic cells play critical roles in adaptive immunity. The antigen-dependent activation of various cell types including B cells, T cell subsets, and macrophages play critical roles in host defense.
Figure 1.2: Different cells involved in the innate immune response. The mast cells, natural-killer cells, monocytes, macrophages and neutrophils.

Monocytes originate in the bone marrow and have a short span in circulation before entering tissues to replace and replenish aged tissue-specific macrophage populations (i.e. histiocytes, Kupffer cells, osteoclasts and microglia cells). The macrophages are believed to play a critical role as immune effector cells by responding to innate and adaptive immune signals. They have also been suggested to contribute to the process of tissue repair and wound healing (Figure 1.2).

Inflammation: Mechanism and its Effects

Inflammation is a natural phenomenon directed towards microbes and other internal disturbances by the cells of the innate immunity leads to collateral damage to the tissues, which involves the recruitment of plasma proteins, fluid and leukocytes into perturbed tissue. This migration is associated with alterations in the local vasculature that lead to vasodilation, increased vascular permeability and increased blood flow. The objective of inflammation is to eliminate the pathogens, clear damaged tissue and restore tissue homeostasis (Medzhitov et al., 2008, Soehnlein & Lindbon 2010).

The elimination of infection or damage is achieved by the association of pathogen-associated molecular patterns (PAMPs; such as Toll-like receptors (TLRs) etc), which are receptors on the innate immune cells, with the molecules expressed by pathogens, that are essential for their survival. The primary advantage of this mechanism is that mistargeting of host cells and tissues is prevented. In contrast, the adaptive immune system has the ability to distinguish among diverse strains of pathogens (Janeway et al., 1994).
After interaction with ligands, TLRs transduce the signals that culminate the activation and translocation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells; This transcription factor is found in all cell types and remains functionally inactivated state by interacting with inhibitor protein, IκB (Ghosh et al., 1998). Upon transduction of the signal by TLRs, NF-κB dissociates from IκB and translocates to the nucleus, where, the transcription of targeted genes is upregulated through binding to promoter regions (Figure 1.3).
The translocation of NF-κB into the nucleus leads to the inducible expression of proinflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and IL-6, etc. In combination with chemokines and various costimulatory molecules, these inflammatory proteins attract circulatory neutrophils and monocytes to the site of disturbance. Neutrophils create a cytotoxic environment by releasing reactive oxygen and nitrogen species (ROS and RNS) and several proteinases. These substances are effective in the destruction of both pathogens and cells of the host tissue, and the net effect of this process culminates in heat, redness, swelling, pain, and loss of function in the inflammatory area (Figure 1.3).

The last phase of inflammation is its resolution which is crucial for limiting collateral damage to the host tissue. Resolution of inflammation is a coordinated program executed by tissue-resident and recruited macrophages. During acute inflammation, these cells produce inflammatory prostaglandins and leukotrienes, but rapidly switch to lipoxins, which favour enhanced infiltration of monocytes and block further neutrophil recruitment, which is important for wound healing (Serhan & Savill 2005). The progress or resolution of inflammation is thus a coordinated process among different effector cells namely neutrophils, macrophages and monocytes. The loss of regulation in this complex process leads to pathological abnormalities which are associated with inflammatory disorders namely; ulcerative colitis, rheumatoid arthritis, etc (Figure 1.3).

**Fatty acids: Structure and Nomenclature**

Fatty acids are carbon chains with a methyl group at one end of the molecule (designated as omega, ω) and a carboxyl group at the other end (Figure 1.4). The carbon atom next to the carboxyl group is called the α carbon, and the subsequent one the β carbon.

$$\text{CH}_3 - (\text{CH}_2)_n \text{CH}_2 - \text{CH}_2 - \text{COOH}$$

**Figure 1.4: The structure of a fatty acid**

Fatty acids are classified into two categories namely saturated and unsaturated fatty acids

**Saturated fatty acids**

Saturated fatty acids are saturated straight chain even numbered hydrocarbons which are found universally in all the animals, plants and microorganisms (example; Palmitic acid;
16:0, Stearic acid; 18:0, etc.). Short-chain saturated acids with 8–10 carbon atoms are found in coconut triacylglycerols and milk.

**Unsaturated fatty acids**

The fatty acids which contain one or more double bonds in their structure are called unsaturated fatty acids. The fatty acids with one double bond are called monounsaturated fatty acids (MUFA). The most common monoenoates have a chain length of 16–22 and a double bond with the cis orientation. This implies that the hydrogen atoms on either side of the double bond are oriented in the same direction. Trans isomers may be produced in the gastrointestinal tract of ruminants and also during industrial processing (hydrogenation) of unsaturated oils. The melting points of cis fatty acids are lower compared to trans fatty acids or saturated counterparts. Oleic acid (18:1 ω-9) is the most abundant MUFA in plants and animals. It is also found in microorganisms. The fatty acids with two or more double bonds are called polyunsaturated fatty acids (PUFA). Linoleic acid (18:2 ω-6) is a common fatty acid found in plant lipids. If the first double bond is found between the third and the fourth carbon atom from the ω carbon of polyunsaturated fatty acids; these are called ω-3 fatty acids. If the first double bond is between the sixth and seventh carbon atom, then they are called ω-6 fatty acids. The double bonds in PUFA are separated from each other by a methylene grouping (Figure 1.5).

![Figure 1.5: The structure of different fatty acids with a methyl end and a carboxyl (acidic) end (Rustan, et al., 2005, John Wiley & Sons).](image-url)

<table>
<thead>
<tr>
<th>ω-characteristics</th>
<th>Methyl end</th>
<th>Carboxyl end</th>
<th>Saturation</th>
<th>Δ-characteristics</th>
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<tbody>
<tr>
<td>Stearic 18:0</td>
<td></td>
<td>COOH</td>
<td>Saturate</td>
<td>18:0</td>
</tr>
<tr>
<td>Oleic 18:1, ω-9</td>
<td></td>
<td>COOH</td>
<td>Monoene</td>
<td>18:1 Δ9</td>
</tr>
<tr>
<td>Linoleic 18:2, ω-6</td>
<td>6</td>
<td>9</td>
<td>Polyene</td>
<td>18:2 Δ9,12</td>
</tr>
<tr>
<td>α-Linolenic 18:3, ω-3</td>
<td>3</td>
<td>15</td>
<td>Polyene</td>
<td>18:3 Δ9,12,15</td>
</tr>
<tr>
<td>EPA 20:5, ω-3</td>
<td>3</td>
<td>17</td>
<td>Polyene</td>
<td>20:5 Δ5,8,11,14,17</td>
</tr>
<tr>
<td>DHA 22:6, ω-3</td>
<td>3</td>
<td>19</td>
<td>Polyene</td>
<td>20:6 Δ4,7,10,13,16,19</td>
</tr>
</tbody>
</table>
Arachidonic acid (20:4 ω-6) is a major component of membrane phospholipids in the animal kingdom, and is found very little in the diet. α- Linolenic acid (18:3 ω-3) is found in higher plants (rape seed oil and soybean oil) and algae. Eicosapentaenoic acid (EPA; 20:5ω-3) and docosahexaenoic acid (DHA; 22:6ω-3) are major fatty acids found in fatty fish and marine algae and fish oils. DHA is found in high concentrations especially in phospholipids of the brain, testes and retina.

**Fate of Essential Fatty acids in the body**

The PUFA, which are produced only by plants and algae, are essential to all higher organisms, including mammals and fish. Though, the humans are not capable of synthesizing PUFA; they are able to further elongate them to higher chain fatty acids by elongations and desaturation. The metabolism of two families of essential fatty acids is shown in the Figure 1.6.

![Figure 1.6: Synthesis of long chain ω-3 and ω-6 polyunsaturated fatty acids (Rustan, et al., 2005, John Wiley & Sons).](image-url)

The intake of oils rich in LA is increased in recent times in Western countries and also in India has resulted in significant increase in the ratio of n-6 to n-3 PUFA in the diet.
Although LA and ALA cannot be synthesized by humans, they can be metabolized to other fatty acids (Fig. 2). The LA can be converted via γ-linolenic acid (18:3 n-6; GLA) and Dihomo-gamma-linolenic acid (20:3 n-6; DGLA) to Arachidonic acid (20:4 n-6; ARA) (Fig. 2). The same set of enzymes converts ALA to EPA and DHA. Retroconversion, e.g. DHA to EPA also takes place in the body (Figure 1.6).

**Inflammatory Bowel Disease**

Inflammatory Bowel Disease is a relapsing and remitting disease constitutes both Crohn’s Disease (CD) and Ulcerative Colitis (UC). In Crohn’s disease, inflammation occurs in any part of lower GIT. In contrast, the symptoms limit to the colon in UC. Ulcerative colitis is clinically characterized by diarrhea, abdominal pain and rectal bleeding. The histopathological observations of the colon sections display crypt distortion, edema, crypt abscess and infiltration of inflammatory leukocytes. The erosion of mucous producing goblet cells in the luminal mucosal layer is an important feature of the disease (Figure 1.7). The normal function of water absorption is disrupted that results in diarrhea along with blood in the stools.

![Figure 1.7: Hematoxylin and eosin stain of a colonic biopsy showing goblet cell erosion, crypt abscess and distortion, which are classical features of ulcerative colitis.](image)
Incidence of IBD

In geographical areas including North America, Northern Europe, and the United Kingdom are traditionally associated with IBD and the rates of UC and CD incidence have increased dramatically since 1960. As of 2004, 1.4 million individuals in the United States and 2.2 million persons in Europe suffered from IBD. In North America, the mean age at diagnosis is 33–45 years for CD, and 5–10 years later for UC. The increasing incidence of these conditions amongst children is of great concern. Epidemiological studies have revealed the alarming rate of incidence of childhood CD in the United Kingdom and Western Canada. Therefore, recognizing the causes of this increase in childhood IBD cases is an important quest.

Leicester city in the United Kingdom has a large population of migrants from South Asia with a low incidence of IBD. However, a prospective study on the incidence of UC in Leicester city between 1991 and 1994 found extensive incidence colitis in second generation South Asian migrants than in the first generation. It is even more interesting that the rate of incidence of colitis in second generation migrants was equivalent to those observed in the European community. Data from a study conducted in Western Canada substantiate these findings where the incidence of IBD among children of East Indian decent was three-fold higher than the local paediatric community. These results suggest that the Indian sub-continent people have a higher predisposition to IBD than Europeans, but this susceptibility does not perceptible until they are exposed to a Western “pro-IBD” diet (Natalie et al., 2012)

A population-based study of incidence and prevalence of ulcerative colitis in Punjab, North India, has revealed that there was a prevalence rate of 44.3 per 1, 00, 000 inhabitants (95% CI 29.4–66.6) in Punjab state. This data highlight that there is an alarming rate of incidence of IBD in India. This may be due to change in life style, food habits, and the environment. Thus, environmental factors are the primary suspect for its increased incidence, since genetic predisposition to IBD was found to be higher in some populations than others. The data from various sources point out that dietary fat, especially polyunsaturated fatty acid (PUFA) content, is a potential contributor of pathogenesis of IBD (Sood et al., 2003).
Factors contribute to the pathogenesis of IBD

Genetic factors

The convincing evidence for genetic factors has been reported contributing to IBD susceptibility. IBD is found to be caused by the complex interactions among various host susceptibility genes (CARD4/NOD1, CARD15/NOD2, TLR4, HLA, DLG5, NF-κB1), environmental factors including colon microbiota and food antigens, and abnormal immune balance (Timmer et al., 2005, Shanahan 2001). Accumulating data suggests that dysregulation of the mucosal immune system is a major factor contributing to the pathogenesis of IBD (Xavier 2007). These immune responses may be induced by increased intestinal permeability, defects in the epithelial barrier, adherence of bacteria, and decreased expression of defensins (Fiocchi 1998). These factors drive the gut immune system to produce excessive cytokines, reactive oxygen metabolites, growth factors and adhesion molecules, resulting in tissue injury.

Cytokines

There is an altered profile in the production of many cytokines have been described in patients with active IBD (Rogler et al., 1998).

![Diagram of T-cell activation and differentiation](image)

Figure 1.8: A primer of T-cell activation and differentiation in the presence of multitude of cytokines.

The naive T-cells undergo differentiation into Th1, Th2 and Treg subsets followed by the activation. IL-10 and TGF-β transform naive T-cells into Tregs, whereas, INF-γ influence them to undergo Th1 subtype. IL-4 stimulates them to undergo Th2
polarisation. In the presence of IL-6; TGF-β signals the T-cells to undergo Th17 type differentiation (Figure 1.8).

The controversy has been continued to ascertain whether these changes really represent a dysregulated immune function or a secondary consequence of immune activation (Podolsky 2000). There is an imbalance in regulatory and effector T cells in active IBD, where effector T cells (Th1, Th2) predominate over regulatory T cells (Th3, Tr). In ulcerative colitis, there is a modified Th2 response associated with cytokines such as IL-15 and IL-10 (Brown et al., 2007, Schreiber et al., 1995, Yen et al., 2006). Among these cytokines, Interleukin-10 is a regulatory cytokine which plays a crucial role in the regulation of the mucosal immune system. Intriguingly, IL-10 and TGF-β promotes physiological activation and prevents the pathological inflammation in IBD (Schreiber et al., 1995) (Figure 1.8). However, this pathophysiological concept for IBD is changing as a result of recent description of another type of effector immunologic response called interleukin-23/interleukin-17 axis.

**Mucosal epithelial barrier function**

The epithelial cells act as a tight barrier between the high concentrations of antigens (dietary, bacterial) at the luminal surfaces in the mucosa, and immune cells at the basolateral surface (Baumgart et al., 2002). Intestinal epithelial cells (IECs) are linked by tight junctions, which are tightly regulated in response to cytokines. However, the junctional complexes are down-regulated in human IBD (E-cadherin and β-catenin) (Gassler et al., 2001). The constant communication of IECs with luminal microbiota and the underlying innate and adaptive immune cells results in constitutive expression of costimulatory molecules (Toy et al., 1997), Toll-like receptors (TLR) (Cario et al., 2000), constituents of the human major histocompatibility complex (MHC) (Hershberg et al., 1997), inflammatory cytokines (Yang et al., 1997), NOD proteins (Kobayashi et al., 2005), as well as antimicrobial peptides (Canny et al., 2005). The leaky and porous epithelial barrier in IBD leads to increased permeability that allows the proliferation of non-pathologic organisms (normal microflora) in juxtaposition to components of the mucosal immune system. Also, mucosal epithelial cells have a different pattern of TLR expression in IBD (Cario et al., 2000).
Reactive Oxygen Species (ROS)

The intestinal mucosa is prone to oxidative stress from the free radicals generated by the luminal contents such as bacterial metabolites, oxidized food debris, transition metals such as iron and copper, bile acids and salivary oxidants (Rezaie et al., 2007). Under pathological conditions, a balance between oxidant and antioxidant systems is impaired. Oxidant-mediated injury plays an important role in the pathophysiology of IBD (Keshavarzian et al., 1990). The inefficient regulation of activation of tissue macrophages as well as the recruitment and activation of circulatory phagocytic leukocytes (PMNs, eosinophils, monocytes) will result in a dramatic increase in the production of ROS in the tissues. Normally, most of the tissues possess sufficient amounts of protective enzymatic (SOD, catalase, GSH peroxidase) and non-enzymatic (thiols, ascorbate, Tocopherol) antioxidants that decompose most of the noxious oxidants that spill into the surrounding environment thereby limiting “bystander” tissue damage. However, the uncontrolled overproduction of ROS could easily overcome these protective mechanisms resulting in extreme damage to cells and tissue. In fact, several studies suggest that chronic inflammation in the colon tissue is subjected to significant oxidative stress (Conner et al., 1996, Grisham et al., 1994; Harris et al., 1992).

Regulation of Inflammatory Gene Expression in Colitis

It is well established that Th1 and macrophage-derived cytokines (e.g., TNF-α, lymphotoxin-α IFN-γ, or IL-1β) induce inflammation in vivo. The mechanisms by which these proinflammatory agents promote inflammation have not been completely understood. However, an accumulating body of data suggests that cytokine-receptor interactions may activate the proinflammatory nuclear transcription factor-kappa B (NF-κB). NF-κB is a pleiotropic regulator of numerous inflammatory and immune responses (Baeuerle 1996). This heterodimeric protein is composed of the p50 and p65 subunits primarily, and under basal conditions is sequestered in the cytosol as an inactive ternary complex bound to its inhibitor protein IκB. A diversity of stimuli, including inflammatory cytokines, bacterial products, and oxidants, activate this transcription factor. Once activated, NF-κB translocates to the nucleus of the cell where it binds to consensus sequences on the promoter-enhancer region of different genes, thereby activating the transcription of genes known to be important in the immune and inflammatory responses.
For example, NF-κB regulates the transcription of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, IL-12, TNF-a), endothelial cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1), as well as NOS2 (iNOS) and the inducible isoform of cyclooxygenase (COX-2). The mechanisms by which oxidants may activate NF-κB have been poorly defined. Intracellular oxidative stress is thought to activate several intermediate reactions, one or more redox-sensitive kinases that specifically phosphorylate IκB. Once phosphorylated, IκB is selectively ubiquitinated and then degraded by 26S proteolytic complex, thereby allowing the transcriptionally active p50/p65 heterodimer to translocate to the nucleus. Thus, the 26S proteasome represents an important step in the activation of NF-κB and has become an attractive target for pharmacological interventions. In addition, it has been demonstrated that antisense oligonucleotides specific for the p65 subunit of NF-κB are effective in inhibiting the increased cytokine expression and colonic inflammation observed in experimental models of colitis (Neurath et al., 1996). This suggests that NF-κB activation and subsequent expression of a multitude of inflammatory genes is crucial for the development of chronic gut inflammation.

Animal models of IBD

In the recent years, more than 20 experimental models with a similar range of clinical manifestations to those observed in human IBD have been developed. These models contributed to significant advances in our current understanding of the pathogenesis as well as treatment of IBD. However, the precise etiology of IBD remains unclear, although genetic, environmental, and immunologic influences may all contribute to the disease process.

The various animal models of IBD are categorized into five broad classes:

(i) Gene knockout (KO) models
(ii) Transgenic mouse and rat models
(iii) Spontaneous colitis models
(iv) Inducible colitis models
(v) Adoptive transfer models

Of them, chemically inducible colitic models are broadly discussed here.
Inducible colitis models

Acetic acid induced colitis

Mucosal necrosis and transient inflammation can be induced by luminal instillation of dilute acetic acid in a dose-responsive fashion (Elson et al., 1995). In the original description of the model (MacPherson 1978), the diluted acetic acid (0.5 ml of 10–50% acetic acid) was instilled into the rectum of Sprague–Dawley rats. After 10 seconds of surface contact, the acidic solution was withdrawn, and the lumen was flushed three times with 0.5 ml saline. The initial injury in this model was due to epithelial necrosis and edema that variably extended into the lamina propria, submucosa, or external muscle layers, depending on the concentrations and length of exposure of acetic acid. Epithelial injuries are relatively specific reaction to organic acids because HCl at similar pH did not induce a similar injury (Yamada 1991). Mucosa and submucosal inflammation followed initial injury and was associated with activation of arachidonic acid pathways ((Elson et al., 1995). Acetic-acid induced colitis is an easily inducible model of IBD, and the similarity of the inflammatory mediator profile to IBD, suggest that the inflammatory phase bears little resemblance to acute human intestinal inflammation (Elson et al., 1995). Hence, this model has not been popular due to necrotic nature, rather than involvement of inflammation in the intestinal injury.

Dextran Sulfate Sodium induced colitis

The administration of dextran sulfate sodium (DSS) dissolved in water to rats or mice or causes body weight loss, diarrhea, mucosal ulcers, shortening of the intestine, and infiltration of neutrophils. Acute colitis was induced by innate immunity, and acquired immunity has less effect on the disease because it also occurs in severe combined immunodeficiency (SCID) mice. However, chronic colitis is caused by lymphocytes that are activated by the secreted cytokines from the activated macrophages (Okayasu et al., 1990). It was shown that used the T cells are also involved in the development of DSS-induced colitis (Shintani et al., 1998). DSS induced colitis is associated with increased activation of NF-κB. The cell adhesion molecules (CAM) such as intercellular adhesion molecule 1 (ICAM-1), mucosal addressing cell adhesion molecule 1 (MAdCAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are immunoglobulin super family which play a critical role in homing of vascular inflammatory cells in colitis. The clinical and inflammatory markers in DSS induced colitis closely resemble that of human ulcerative
colitis. Hence, this model has been extensively used to study the pathogenesis as well as to assess the therapeutics in IBD.

**Trinitrobenzene sulphonic acid (TNBS) induced colitis**

Colitis can be induced in rats and mice by treatment with a TNBS enema that destructs the mucosal barrier (Hibi et al., 2002). Administration of TNBS enema causes infiltration of inflammatory cells in all the layers of the colon intestine. The isolated macrophages produce excess amounts of IL-12. Similarly, isolated lymphocytes produce large amounts of IFN-γ and IL-2. This suggests that the TNBS-colitis is characterized by a dominant Th type-1 response, (Neurath et al., 1995). The absorption of water is clearly diminished in the inflamed mucosa. This effect would contribute to diarrhoea that resembles clinical symptoms of human IBD. The TNBS model has been served in clinical investigations for the testing and development of therapeutic molecules with large potential for the management of the human disease.

**Modulatory effects of PUFA on immune cell functions**

Dietary PUFA can modulate immune functions in different ways which can be categorized here under.

- Proliferation of Lymphocytes
- Modulation of cytokine production
- The phagocytic activity of the inflammatory cells
- Apoptosis of immune cells

**Influence of fatty acids on Lymphocyte proliferation**

Several studies in animals and humans have been consistently demonstrated the ability of unsaturated fatty acids to reduce the proliferation of T lymphocytes. Therefore, diets containing polyunsaturated fatty acids such as EPA and DHA suppress the proliferation of lymphocytes to a larger extent than diets rich in saturated fatty acids (Calder 1995). However, oleic acid also plays an important role in this process (de Pablo et al., 1998). In fact, its modulatory effect on immune functions is lower than that of n-3 PUFA (Calder 1995). Nevertheless, a study on human volunteers has reported that DHA does not inhibit many of the lymphocyte functions modified by fish oil consumption (Kelley et al.,
This indicates that fish oil enriched diet modulates the immune response by the action of EPA rather than DHA. Hence, there is a need to ascertain that which lipid component is responsible for modulation of the immune system from diets containing different fatty acids.

**PUFA can modulate cytokine production**

Fatty acids can regulate cytokine production, and this might be responsible for the inhibition of lymphocyte proliferation in both humans and animals. This affirmation is based on the study which suggests that diet rich in n-3 polyunsaturated fatty acids inhibits the expression of the CD25 molecule, which is a part of the IL-2 receptor (Soyland et al., 1994). Cytokines such as IL-1 and TNF-α are important in inflammation and dietary fatty acids have been demonstrated that they reduce the pro-inflammatory response induced by IL-1 and TNF-α (Endres et al., 1996; de Pablo et al., 1998; Endres et al., 1989). Many studies have reported conflicting effects of dietary fatty acids on inflammatory cytokine production and these inconsistencies may be associated with the different polyunsaturated fatty acid actions on cell populations of various species (Blok et al., 1996). The effects of dietary fatty acids on cytokine production of macrophages depends on various factors such as dose, duration of feeding, the type and state of activation of macrophages, the species of experimental animal, and so on (Billiar et al., 1988; Lokesh et al., 1990, Chang et al., 1992; Tappia et al., 1994). Furthermore, the inhibition of other cytokines by dietary fatty acids is also crucial. In fact, IL-10 inhibits LPS-induced production of inflammatory cytokines. In this way, it was demonstrated that mice fed a diet containing coconut oil has low TNF levels, whereas, IL-10 production is significantly increased (Sadeghi et al., 1999).

**Effect of PUFA on Phagocytosis of inflammatory cells**

Phagocytosis is an important function of inflammatory cells for the elimination of microbes or foreign particles, which is mediated by production of endocytic vesicles. Hence, membrane fluidity plays a pivotal role in this process. Dietary lipids can alter the lipid environment of plasma membranes of macrophages and other phagocytic cells. In fact, several studies suggest that unsaturated fatty acids increase phagocytosis. Peritoneal macrophages stimulated with zymosan particles have shown a significant increase of phagocytosis in cells isolated from mice fed diets enriched in olive oil and polyunsaturated fatty acids (Calder 1990). This effect may be due to the increase of
hydrogen peroxide production, which enhances superoxide release (Badwey et al., 1984). In monocytes, an increase of EPA and DHA content and a reduction of stearic and arachidonic acid and has been correlated with a reduction in superoxide production (Fisher et al., 1990).

**Fatty acids induce apoptosis of immune cells**

Several studies suggest that fatty acids induce apoptosis in cultured cells. Palmitic acid induces cell death by dissipation of the mitochondrial transmembrane potential, the process that precedes nuclear apoptosis. In fact, a cell-free system assay has shown palmitate induce apoptosis via a direct effect on mitochondria culminate with the release of soluble factors which are capable of inducing the apoptosis of isolated nuclei (de Pablo et al., 1999). Another study has shown that stearic and palmitic acid induces apoptosis by *de novo* synthesis of ceramide (Paumen et al., 1997). Although some discrepancies have been observed, it is probable that palmitate-induced apoptosis occurs by a direct effect on mitochondria (not mediated via ceramide) (de Pablo et al., 1999). EPA has been studied as an apoptosis inducer which promotes apoptosis in monocytic U937-1 cells (Finstad et al., 1998). In contrast, DHA reduces the apoptotic effects induced by sphingosine (an important second messenger). In this case, the inhibition of cytosolic phospholipase A$_2$ could be involved in the inhibitory activity of this fatty acid (Kishida et al., 1998). Extension of the results of *in vitro* cultures, dietary fatty acids may induce apoptosis *in vivo*. Splenic cells from mice fed high-fat diets containing saturated fatty acids were more susceptible to death by apoptosis than mice fed unsaturated fatty acids (Carratelli et al., 1999). Therefore, dietary fatty acids alter immune function via modulation of the phagocytic activity of inflammatory cells.

**Mechanisms by which PUFA modulate the functions of the cells of the immune system**

Various mechanisms by which PUFA modulate immune cell functions is listed below.

- Membrane fluidity
- Lipid Peroxidation
- Eicosanoid production
- Inflammatory gene expression.
Membrane Fluidity

Figure 1.9: Model for modulation of the plasma membrane fluidity by polyunsaturated fatty acid (PUFA) based on bilayer experiments.

Biophysical measurements reveal that steric incompatibility between rigid cholesterol molecules and PUFA acyl chains causes phase segregation of PUFA-containing phospholipids from raft microdomains. The model predicts that dietary PUFA incorporation into membrane phospholipids push proteins from their resident raft-rich environment into raft-poor PUFA-rich phases or vice versa, which may alter cellular activity. Relative sizes of lipid molecules and proteins are not drawn to scale. As a result, the binding of cytokines to their perturbed receptors on the cell membrane surface may be altered (Stubbs et al., 1994). In fact, high unsaturated fatty acid incorporation is associated with an increase in the phagocytosis of zymosan particles (Calder 1990). Expression of adhesion and major histocompatibility molecules from human monocytes are altered by EPA (Grimble et al., 1995) (Figure 1.9).

Lipid peroxidation

Lipid peroxides are harmful to cells, in fact, PUFA are incorporated into the cell membrane and increase the demands for antioxidants such as tocopherol, which protect membrane PUFA from lipid peroxidation (Virella et al., 1989). However, several reports suggest that the lipid peroxidation is not mechanism involved in the fatty acids on lymphocyte proliferation since the addition of antioxidants does not avoid the
suppression of mitogen stimulated lymphocyte proliferation (Soyland et al., 1993; Calder et al., 1993). Further, PUFA are more sensitive to lipid peroxidation than MUFA or saturated fatty acids. This enhanced lipid peroxidation by PUFA affects the expression of surface molecules such as HLA-DR (Gruner et al., 1986). This is confirmed by the fact that diet enriched with β-carotenes increases the expression of cell adhesion and histocompatibility molecules in human peripheral blood monocytes (Hughes et al., 1997) (Figure 1.10).

Eicosanoids are the link between PUFA and inflammation.

Classical studies have demonstrated that several members of eicosanoids derived from arachidonic acid participate in inflammatory processes, which are related to immunomodulatory effects (Goodwin et al., 1983). Fatty acids are released from the phospholipids via the action of phospholipases and undergo enzymatic synthesis to produce eicosanoids, which are lipid mediators. For instance, prostaglandin E₂ (PGE₂) or Leukotriene B₄ (LTB₄) (which are derived from arachidonic acid) perform a wide variety of functions including cytokine production (Rappaport et al., 1981; Rola-Pleszczynski et al., 1982), modulation of lymphocyte proliferation (Shapiro et al., 1993), and cytotoxicity (Bray et al., 1986). However, dietary n-3 PUFA decreases the levels of leukotrienes and prostaglandins through reduction of arachidonic acid (Figure 1.10).

The products derived from n-3 fatty acids display different biological properties in comparison with those derived from arachidonic acid. Prostaglandin E₃, which is derived from EPA, inhibits in vitro mitogen-stimulated lymphocyte proliferation (Shapiro et al., 1993). Similarly, leukotriene B₅ is also derived from EPA has been assumed that its biological activities are lower than leukotrienes derived from arachidonic acid. Regardless of these studies, eicosanoids regulate a great number of inflammatory effects, such as induction of fever, vasodilatation and production of macrophage and lymphocyte-derived cytokines (Rola-Pleszczynski et al., 1982).
Figure 1.10: Schematic representation of different mechanisms of regulation of the immune system by dietary fatty acids (de Pablo et al., 2000).

Regulation of gene expression

The effect of fatty acids on gene expression is not completely understood. Nevertheless, the fatty acids released from membrane phospholipids can act as secondary messengers or substitute for the classical second messengers, such as cyclic AMP or inositide phospholipid signal transduction pathways (Graber et al., 1994). Several studies have shown that fish oil feeding in the diet of autoimmune disease-prone mice increases the mRNA levels of various cytokines including IL-4, IL-2 and transforming growth factor (TGF)-1β (Fernandes et al., 1994). Similarly, fish oil reduces mRNA production of IL-1, IL-6 and TNF-α (Chandrasekar et al., 1994). These findings support the important role of fatty acids as substances capable of influencing signal transduction pathways (Figure 1.10).

Links between adipose tissue, lipid profile and IBD

The adipose tissue associated with the pericardium, heart and large blood vessels was found metabolically active as opposed to being merely structural. This has led to the investigation of adipose tissue deposits at other sites (Soltis et al., 1991). In mammals adipose tissue is compartmentalized into a few large depots and many disseminated
smaller ones. Many of the lymph nodes are surrounded by adipose tissue depots in which large lymph nodes and their ducts are embedded in adipose tissue. It is interesting that the leanest mammals contain adipose deposits around lymph nodes and these lipid reservoirs seem to be preferentially released during starvation. These interpretations led to the hypothesis that perinodal adipose tissue could be involving in the immune response.

Growing evidence suggest that perinodal adipose tissue had special properties, that enable it to influence the local lymphoid tissues (Pond, 1999). Perinodal adipose tissue undergoes rapid lipolysis when co-cultured with isolated lymphoid cells surrounding it, compared to similarly treated samples from adipose deposits elsewhere (Pond et al., 1995). In addition, adipocytes around omental, popliteal and mesenteric lymph nodes have lower rates of spontaneous lipolysis in the presence of combinations of noradrenaline, TNF-α and interleukins compared to adipocytes from sites in the same depot more distant from the nodes (Pond et al., 1998). Analysis of triacylglycerols content in all perinodal adipose tissues revealed that it contains more polyunsaturated fats and fewer saturated fatty acids compared to adipose tissue remote from nodes (Mattacks et al., 1997).

These observations were in agreement with the hypothesis that perinodal adipose offers energy source for lymphoid cells, and therefore plays a role in the association between dietary lipids and immune activity by protecting fatty acids for the exclusive use of the immune system. The dendritic cells in lymph nodes and ducts, which play a major role in antigen presentation, may be facilitating this interaction, as they are noted to obtain large lipid droplets (Maroof et al., 2005). This close relationship between the immune system and the adipose tissue might be related to redistribution of adipose tissue occur during chronic inflammation. Corroborating these findings, abnormalities of fat in the mesentery have been noted on surgical specimens of patients of IBD, which is a characteristic feature of the disease. The cytokine TNF-α may be involved in the abnormal behavior of the mesenteric adipose tissue (Desreumaux et al., 1999). Intriguingly, in HIV, which is an immune related disease, there are also notable changes in the morphology of the adipose tissue with significant enlargement in those areas connected with lymphoid tissue and reduction in those that are not. Retroviral infection
is known to alter the cytokine secretion of dendritic cells and modulate the response of perinodal adipocytes (Kelleher et al., 1999; Dhurandhar et al., 1997).

**Effect of PUFA in the Animal models of colitis**

Animal models have demonstrated that marine n-3 PUFAs decrease chemically-induced colonic damage and inflammation. The effects on disease severity were, in all cases, associated with a reduction in production of arachidonic acid-derived eicosanoids (Calder 2008). A recent study investigated chemically-induced colitis in fat-1 mice (Hudert et al., 2006). The mice showed much less colonic damage and inflammation than wild type mice, and this was associated with a marked change in the pattern of inflammatory mediators present in the colonic tissue. A study in IL-10 knock-out mice that spontaneously develop colitis demonstrated significantly reduced colonic inflammation if the mice fed fish oil (Chapkin et al., 2007).

Investigation on the therapeutic efficacy of n-3PUFAs in trinitrobenzene sulphonylic acid (TNBS)-induced colitis in rats has shown that an elemental diet (ED) enriched with 2% n-3 rich perilla oil has lower levels of LTB$_4$ and the ulcer index was significantly decreased, compared to an ED enriched with 2% n-6 rich safflower oil. In this study, the enrichment of the ED with ALA was therapeutically superior compared to enrichment with EPA/DHA (Shoda et al., 1995). Similarly, the suppression of PGE$_2$ was correlated with lower colonic lesions in dextran sulphate sodium (DSS)-induced rat model of UC (Hirata et al., 2001). The further evidence for a protective effect of n-3 PUFAs was provided using a rat model of 4% acetic acid-induced mild colitis (Empey et al., 1991). In a comparative study, rats were fed a diet enriched with either n-3 or n-6 fatty acid for 2 weeks before enteritis was induced by TNBS enema. Histologically, the mucosal damage was significantly more severe in the rats fed the n-6 diet compared to those that had fed the n-3 diet. This was associated with higher serum IL-6 levels in the n-6 group compared to the n-3 group implicating that a plausible mechanism for suppression of mucosal inflammation could be inhibition of mucosal IL-6 secretion. Intriguingly, there were no considerable differences in TNF-α levels between these two groups (Andoh et al., 2003). Despite the promising therapeutic efficiency of n-3 PUFAs in various animal models of colitis, authors have contested the wisdom of extrapolating results from animal studies directly to humans, given the potential pathological and physiological differences that exist between human form and the animal models of the disease.
Effect of dietary lipids on IBD- Clinical trials

Previous studies have examined fatty acid profiles in patients with IBD and shown deficiencies in n-3 fatty acids (Kuroki et al., 1997; Holman et al., 1986). Moreover, evidence suggests that essential fatty acid deficiency in IBD patients can contribute to the pathology of IBD (Siguel et al., 1996). At the cellular level, IBD is characterized by elevated concentrations of IL-1 and proinflammatory leukotriene B4. Immunomodulatory mechanisms proposed for n-3 fatty acids in IBD include altering cell membrane fluidity, cell signal transduction, eicosanoid synthesis, gene expression and intraluminal bacterial content (Teitelbaum et al., 2001). Therefore, several clinical trials have conducted to evaluate the effects of n-3 fatty acids on clinical outcomes in IBD. However, these studies have reported mixed results which are outlined here in the Figure 1.11.

Some of these clinical trials indicated the benefits of fish oil including improved gut mucosal histology, clinical score, a lower rate of relapse, sigmoidoscopic score and decreased use of corticosteroids. However, the available data are not sufficiently met the requirements to draw conclusions about the effects of n-3 fatty acids for immunosuppressive therapy in IBD. This may be due to methodological pit falls, and are not followed a regular clinical score to assess the severity of the disease (Figure 1.11).

Epidemiological observations suggest that significant amount of fat in the formulation of elemental diets seen to induce remission in IBD. This led to a number of clinical trials to study the seemingly anti-inflammatory effects of n-3 PUFAs as an alternative treatment in IBD. The altered plasma phospholipid fatty acid profile found in the patients of IBD strengthened the rationale for using n-3PUFAs as a treatment. In addition, the ARA derived eicosanoids including LTB4 and TXA2 are elevated in inflamed intestinal mucosa (Sharon et al., 1984, Rampton et al., 1993) (Figure 1.12). The conflicting results from the trials have been attributed to various reasons, the foremost being patient selection, patient compliance, choice of placebo, influence of concomitant therapy and differences in study design. In general, the new formulations of fish oils that have less unpleasant odour offer potentially therapeutic benefit for the management of IBD. A therapeutic approach that prevents relapse would be of particular benefit, because follow-up of patients who have had undergone ileal resections in IBD show a postoperative reappearance rate of 75% within 1 year and 84% within 3 years (Rutgeerts et al., 1990).
### Characteristics of identified studies of the effects of n-3 fatty acids in inflammatory bowel disease

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Study design</th>
<th>Study duration</th>
<th>Sample size</th>
<th>Source and dose of A: n-3 fatty acid and B: control</th>
<th>Jadad score</th>
<th>Concealment of allocation</th>
</tr>
</thead>
</table>
| Almallah et al (19), 1998 | RCT | 6            | 18          | A) Fish oil, 15 mL/d  
B) Sunflower oil, 15 mL/d | 2           | Yes          |
| Aslan and Triadafilopoulou (20), 1992 | RXT | 3            | 11          | A) Max EPA (fish oil), 15 capsules/d  
B) Oleic, palmitic, and linoleic acids, 15 capsules/d | 5           | NR           |
| Belluzzi et al (21), 1996 | RCT | 12           | 78          | A) Fish oil, enteric coated, 15 g/d  
B) Miglyol 812, 15 g/d | 5           | NR           |
| Greenfield et al (22), 1993 | RCT | 9            | 43          | A) Max EPA (fish oil), 12 g/d followed by 6 g/d  
B) Olive oil, 12 g/d followed by 6 g/d  
B) Super evening primrose oil, 3 g/d followed by 1.5 g/d | 2           | NR           |
| Hawthorne et al (17), 1992, and Hawthorne et al (18), 1990 | RCT | 12           | 96          | A) Hi EPA, 20 mL/d  
B) Olive oil, 20 mL/d | 3           | Yes          |
| Loeselke et al (23), 1996 | RCT | 24           | 64          | A) Fish oil, 5.1 g/d  
B) Corn oil, 5.1 g/d | 5           | NR           |
| Lorentz et al (24), 1989 | RXT | 7            | 39          | A) Max EPA, 11 mL/d  
B) Olive oil, 11 mL/d | 5           | Yes          |
| Lorentz-Meyer et al (25), 1996 | RCT | 12           | 204         | A) Fish oil, 6 g/d  
B) Corn oil, 6 g/d | 3           | NR           |
| Mantzaris et al (26), 1996 | RCT | 12           | 50          | A) Max EPA, 20 mL/d  
B) Olive oil, 20 mL/d | 2           | NR           |
| Middleton et al (27), 2002 | RCT | 12           | 63          | A) GLA + EPA + DHA, 6 capsules/d  
B) Sunflower oil, 6 capsules/d | 3           | NR           |
| Stenson et al (28), 1992 | RXT | 4            | 24          | A) Max EPA, 18 capsules/d  
B) Vegetable oil, 18 capsules/d | 2           | NR           |
| Varghese and Coomansingh (29), 2000 | RCT | 6            | 51          | A) n-3 EFAs, 6 mg/d  
B) Sunflower oil, dose not reported | 2           | NR           |

Note: A Jadad score ≥ 3 indicates high methodologic quality. DHA, docosahexaenoic acid; EFA, essential fatty acids; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; NR, not reported; RCT, randomized controlled trial; RXT, randomized controlled crossover trial. Max EPA (RP Scherer, Clearwater, FL); Miglyol 812 (Dynamit Nobel Chemicals, Witten, Germany); Hi EPA (Scotia Pharmaceuticals, Surrey, UK).

Figure 1.11: Overview of the results of n-3 PUFA supplementation in IBD (MacLean et al., 2005)
In addition, the differences in dose, source, and type of n-3 fatty acid may affect clinical outcomes. The baseline dietary intake of n-3 and n-6 fatty acids may affect the outcome of supplementation with n-3 fatty acids. Another limitation that has been observed in these studies is most of the studies reviewed substituted n-6 fatty acids with n-3 fatty acids. Therefore, the observed effects could be the result of reducing absolute n-6 fatty acid intake rather than increasing n-3 fatty acid consumption.

Therefore, there is no conclusive evidence across studies to suggest that n-3 fatty acids are beneficial in the treatment of IBD. More importantly, these studies have provided little information on many variables including the dose, duration, type of n-3 fatty acids and also type of control groups used to understand the effects of n-3 fatty acids in IBD. This study was envisaged to understand the possible role of different oils on ulcerative colitis in an animal model.