Inflammatory Bowel Disease

Chapter 1

Pulling back to the nature: A condition to fight IBD

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1. Introduction

Inflammatory bowel disease (IBD) grouped as autoimmune disease arises due to inflammation of small and large intestine. Based on the target organ IBD is classified under Ulcerative colitis (UC; affects the colon) and Crohn's disease (CD; affects whole intestinal wall but mainly to the ileum) [1]. 1-1.6 million people are suffering from IBD in United States and the main target age group is between 15-30 yrs. The prevalence rate of $201/10^6$ of CD and $238/10^6$ of UC in adults attracts attention to the disease [2]. Abdominal cramping, weight loss, fever, sweats, fatigue, growth retardation, diarrhoea, constipation and abnormal bowel movement are the major symptoms of IBD. It is clearly noticed that IBD is not limited to just inflammation of the digestive tract, but it can lead to other complications like arthritis, thromboembolism, cardiovascular-, pulmonary-&neurological disease affecting the quality of life of an individual. It is clearly seen that IBD runs in the family, and the family members of affected individuals are at the maximum risk of IBD. In last two decades it is shown that environmental factors in the pathogenesis of IBD are playing important role in increasing the incidences of IBD cases in the countries with historically low rates of IBD. In North America and Europe 20-fold increase in IBD cases were found since World War II due to change in dietary habit and environmental factors. The environmental factors also correlates with the similar trends in IBD due to globalization and Westernizing East Asia [3]. Variation in microflora and disregulated immune responses are the key effectors of IBD. In this chapter we have discussed the various factors affecting the pathogenesis of IBD including genetic, environmental and immune factors. We have also discussed that the delay in IBD diagnosis and fail to control it, can result in trans-

formation into the cancer . We have also discussed the role of T cells as well as microflora in pathogenesis of IBD and shed light on its therapeutic aspects.

2. Disregulated Inflammation Predisposes to IBD

Healthy gut contains 10-100 fold more microbes than mammalian cells and intestinal epithelial layer hides this microbial line from intestinal immune system and maintains the peace or non-inflammatory environment. As the name suggests inflammation is the key to inducing IBD and by suppressing inflammation IBD can be ameliorated. In the pathogenesis of IBD, cytokines hold the central position as demonstrated in many genetic and immunological studies **(Table 1)**. Based on their nature of evoking or suppressing the inflammation, they are grouped as pro- and anti-inflammatory cytokines. Both these classes of cytokines are intensively studied for better understanding the IBD biology. Cytokines maintain the normal immune homeostasis by triggering the responses to infections and reverting back inflammation to a basal level after the infection is resolved. But often dysfunction or uncontrolled expression of these cytokines triggers immunological disorders. For example, IL-1 receptor antagonist (IL-1Ra) controls the inflammatory response of IL-1 cytokines, and in steady-state IL-1/IL-1-Ra ration is found to be constant. But an increment in the ratio was observed in case of IBD due to over secretion of IL-1 by monocytes and macrophages. The involvement of IL-1 in IBD pathogenesis makes it a reliable marker for diagnosis of IBD and also make it a suitable target for therapy [4]. Similarly, IL-6, -8, -12, -13, -17, -15 & -21 trigger the inflammation and blocking these cytokines is shown to alleviate IBD [5, 6]. Anti-inflammatory cytokines or cytokine neutralizing Abs are found efficacious for IBD **(Table 1)**. Unlike these pro-inflammatory cytokines, anti-inflammatory cytokines down regulate the inflammatory condition and help in curing the IBD. For examples IL-10, a crucial anti-inflammatory player in CD, controls Th17 cell development and has beneficial effects in IBD. Spontaneous development of IBD observed in IL-10-/- mice [7] support the fact that IL-10 is critical in controlling the IBD. Further, use of IL-10, TGF-β and IL-4 has shown to reverse IBD (Table 1). Genome wide association studies established the importance of STAT-1, -3, -4, CCR6, CCL-2, -13, IL-12R, -23R, JAK2, IL-2,-21, -10, -27 and IFN-γ [8] in induction of IBD.

Among the cytokines TNF-α is a potent activator of inflammatory responses and hence drawn attention of researchers working in the area of IBD. TNF-α is not only involved in pathogenesis of IBD but also plays dominant role in other disorders like rheumatoid arthritis, psoriasis, psoriatic arthritis, and ankylosing spondylitis [8]. Over expression of TNF-α leads to a cascade of inflammatory events which results in auto-immune diseases including CD and UC. It shows pleiotropic effects by increasing the expression of adhesion molecules, stimulating fibroblast proliferation, IL-1β, IL-6, IL-33,ST2 expression [Table 1: Cytokines involvement in IBD 8]. Elevated levels of TNF-α were found in the serum, mucosa and stool of IBD patients. Deletion of TNF-α synthesis regulatory elements is found to evoke IBD in mice

models. Interestingly, models of inducing IBD through chemicals failed in TNF -/- mice [9] showing the importance of TNF-α in IBD. TNF-α induces IBD through several mechanisms, for example, recruitment of neutrophils on inflammation site, activation of coagulation and fibrinolysis, and granuloma formation. TNF-α has two isoforms and it was demonstrated that membrane-bound TNF-α is dominant in evoking IBD than soluble form [8].

Table 1: Cytokines involvement in IBD

Sr. No.	Cytokines	In Immune Homeostasis	In IBD
Pro-inflammatory Cytokines			
1	**IL-1 family:** IL-1α, -1β, -18, -33, -36α, β, γ	1. IL-1Ra controls the activity of IL-1 2. Constant IL-1/IL-1Ra ratio	1. Overproduction by monocytes & macrophage hence increase IL-1/IL-1Ra ratio. 2. Reliable marker of inflammation. 3. Targeting IL-1 has therapeutic values [4].
2	**TNF-α**	Critical player for defence against microorganisms and immune system development.	1. Major source macrophages, monocytes, and Th1 cells. 2. Shows pleiotropic effects, 3. Increase the expression of adhesion molecules, 4. Stimulate fibroblast proliferation, 5. Stimulate IL-1β, IL-6, IL-33, ST2 expression [5]. 6. TNF-α inhibitors are efficacious for IBD.
3	**IL-6 family:** IL-6, IL-11, IL-31	1. IL-6 is an immunoregulatory cytokine 2. Acts as both anti- and pro-inflammatory 3. Signals through IL-6-sIL-6R-receptor gp130 complex. 4. Immune response during infection and after trauma.	1. Over-secretion by mononuclear cells & intestinal epithelial cells. 2. Increased level of sIL-6R was seen in UC and CD patients. 3. IL-6 induces NF-kB activation, STAT-3 and expression of the intercellular adhesion molecule 1. 4. IL-6 helps in Th17 differentiation. 5. Controls anti-apoptotic signals of $CD4^+$ T cells at the site of inflammation. 6. anti-IL-6 receptor antibodies reverse the IBD [10].
4	**IL-8**	Involves in neutrophil activation and migration	1. Higher tissue level was found in active UC. 2. Poor marker of disease activity [11].
5	**IL-12 family:** IL-12, IL-23, IL-27 and IL-35	1. Role in Th17 cell differentiation. 2. Produce in response to TLR stimulation or endogenous signals.	1. Elevated IL-12 levels in the mucosa of UC patients. 2. IL-23 over secretion leads to activation of NK cells, NKT cells, $CD4^+$ T cells and $CD8^+$ T cells. 3. IL-35 involves in T-cell-dependent colitis [5].
6	**IL-13**	1. Allergic inflammation 2. Also show anti-inflammatory activityw 3. Induces IgE secretion.	1. Higher expression was found in UC mucosa. 2. Over secretion by $CD161^+$ NKT cells. 3. Inhibitors of IL-13 signalling pathways showing promising results [5].
7	**IL-17 family:** IL-17A, B, C, D, E (IL-25), F	1. Allergic responses 2. Delayed-type immune reactions by increasing chemokine production and recruiting monocytes and neutrophils to the inflammatory site.	1. Upregulation in IL-17 level is associated with UC. 2. Anti-IL-17 antibodies, IL-17 receptor blocker and IL-17 inhibitors are useful in targeting IBD [5].
8	**IL-5**	Eosinophil differentiation factor	Over expression of mucosal IL-5 by mononuclear cells is seen in active UC [5].

9	**IL-21**	1. Th1 mediated inflammation 2. Inducer of IFN-γ production	1. Uncontrolled secretion of IL-21 by $CD4^+$ lamina proprial T cells leads to IFN-γ production result in IBD. 2. IL-21 inhibits Treg differentiation which ultimately results in inflammatory responses [12].
Anti-inflammatory Cytokines			
1	**IL-10 family:** IL-19, IL-20, IL-22, IL-24 and IL-26	1. Regulate diverse host defense mechanisms from epithelial layer during various infections 2. Important for maintenance of the integrity and homeostasis of tissue epithelial layers 3. Down-regulate inflammatory responses and controls tissue disruptions caused by inflammation.	1. Crucial anti-inflammatory player in CD. 2. IL-10 controls Th17 cell development by inducing IL-1 secretion. 2. Blocking of IL-10 leads to IL-12 and IFN-γ production. 3. Lower expression of IL-10 was seen in inflamed mucosa and granulomas of CD patients [13,14]. 4. IL-22 is elevated in CD mucosa and serum.
2	**TGF-β**	1. Acts as an inhibitory cytokine 2. Regulate the immunological homeostasis and inflammatory responses	1. TGF-β induces IL-13 expression and which promotes the expression of cell invasion proteins. Both can be targeted for CD. 2. Down-regulated expression in CD patients, but interestingly up-regulated in UC [15]. 3. Some reports suggest the up-regulation of TGF-β1 in both UC and CD, but inhibited by Smad7. 4. Inhibiting Smad7 restores TGF- β1 activity which suppresses inflammation and helps in recovery [16].
3	**IL-4**	1. Stimulator of B and T cells 2. Immunosuppressive activity in the intestine	1. Use of anti-IL-4 antibody decreases Th2-type cytokine production and increases IFN-γ production, suggesting role of IL-4 in IBD pathogenesis. 2. Administration of IL-4 resulted in down-regulation of VEGF in active CD and UC patients [5,17].

3. Uncontrolled IBD May Lead To Cancer

The link between inflammation and cancer is well accepted [18]. The direct relation of IBD with cancer, particularly for colorectal cancer (CRC) was shown by Burrill Crohn in 1925 [19]. Due to chronic intestinal inflammation, the IBD patients are found to have an increased risk of developing CRC and the risk increases with the progression of IBD [20]. The risk of CRC was calculated for UC and it was found that patients with UC after 10 years, 20 years and 30 years of starting of disease has risk by 2%, 8% and 18% respectively [21]. Similarly, the relative risk was found to be 2.59 for CRC and 28.4 for small bowel carcinoma in CD cases [22].

During IBD, inflammation is the major cause for the development of CRC [23]. A significant increase in the level of inflammatory mediators like cyclooxygenase-2, nitric oxide synthase-2 and interferon-inducible gene *1–8U* was found in IBD patients leading to CRC [24]. TLR4 induced Cox-2 and EGFR signalling are also involved in the promotion of CRC [25]. In active IBD elevated level of claudin-1, -2 and Beta-catenin was also observed which

is correlated with disease transformation [26]. Similarly, in IBD patients, IL-6, -23, NF-κB and TNF-α are also shown to involve in the development of CRC [27-31]. Also the role of oxidative stress in CRC development was explored and found that nitric oxide has contributing role in the same [32]. Researchers are making attempts to reduce the risk of CRC in IBD by the early diagnosis of CRC, treatment by colonoscopic surveillance and by chemoprevention [33] including 5-aminosalicylic acid (5-ASA) i.e., Mesalazine and Sulfasalazine [34-36] have shown very positive results.

4. T cells and IBD

Gut is the prime entry site for pathogens coming through food and orally administered substances. GALT (gut associated lymphoid tissue) directs tissue restricted and localized immune response to the intestinal antigens. T cells primed against invading pathogen migrate from GALT to the site of infection and protect the gut. After resolution of infection these antigen-experienced T cells migrate to the LP (lamina propria) and epithelial compartments where they reside as long-lived effector memory T cells and accumulate over time. Upon re-encounter with the pathogen these cells respond swiftly and vigorously to eliminate the pathogen, preventing systemic infection [18, 19]. These effector memory T cells proliferate slowly, produce low amount of inflammatory cytokines that help maintain organ integrity [20]. LP is also enriched with the Treg cells required for preventing the excessive responses against the gut microbes, thereby maintaining the gut integrity [21].

In gut there is presence of self specific T cells expressing high levels of both effector and regulatory molecules. They are usually in quiescent stage and upon strong stimulation they become highly cytolytic like conventional T cells. They restrict microbial invasion by killing infected or damaged IECs [37, 38]. TCRγδ $^{+}$ T cells produces pro-inflammatory cytokines and antimicrobial factors which set the basal mucosal inflammatory tone and limit enteric bacterial translocation [39]. Interestingly, TCRγδ $^{+}$ T cells secrete epidermal growth factor (EGF) which also helps repair the epithelium insults [40]. Though these cells act like first-line defence, their activation is fine-tuned and highly regulated to avoid uncontrolled immune responses and tissue destruction.

Varieties of microbial species coexist and establish commensal relationship in the gut of the humans. In return of the supply of food from the host, microbes help in metabolism and development of the immune system. Breaching of this relation leads to the IBD due to aberrant and persistent immune responses. As describe above GALT is involved in neutralizing the potentially harmful pathogens [41]. Controlling the overwhelming immune responses after pathogen clearance is required to avoid response against the self or harmless antigens. Perturbation in immune regulatory mechanisms may predispose the host to the pathogenesis of IBD [42]. Different cell types of the intestinal lymphoid tissue are involved in maintaining the

immune tolerance to the luminal antigens and protection against the pathogens. Infiltration of cells mediating innate immunity (neutrophils, macrophages, and dendritic cells) and adaptive immunity (T and B cells) is associated with the active IBD. $CD4^+$T cells (Th1, Th2, Th17, and T follicular helper TFH) play important role in defence against pathogens. Regulatory T cells (nTreg, iTreg; Tr1 and Th3) are required for controlling the effector responses [41].

T cells accumulate in the inflamed gut of IBD patients as a result of enhanced recruitment, increased cell cycling and resistant to apoptotic signals. Presence of $CD4^+$ T cells into inflamed mucosa suggests the involvement of these cells in the pathogenesis of IBD. The key role of $CD4^+$ T cells in IBD is corroborated by the effectiveness of anti-CD4 antibody therapies and suppression of IBD in HIV patients [41]. In the animal models of experimental colitis, the adoptive naive T cell transfer into a lymphopenic host is shown to induce colitis [41]. On the other hand, blocking the $CD4^+$T cell function is found to improve the experimental colitis. Role of $CD8^+$ T cells in IBD has been demonstrated in experimental animal models and patients. Elevated numbers of $CD8^+$ T cells is also reported in IBD patients [37]. Recently, it was found that apoptosis machinery of T cells has intrinsic defects in IBD patients, thus the defective pathway in mitochondria has important implications for the treatment of IBD. The effectiveness of TNF neutralising drugs for IBD treatment have correlated well with their capacity to induce T cell apoptosis. Attempts have been made to use the inhibitors of T cell activation, proliferation, differentiation or homing to control IBD.

Apart from the increase in the number of cells, defective regulatory T cell responses are also considered as risk factors for IBD. Natural Treg (nTreg) has the ability to recognize both self and foreign antigens, and plays an important role in the maintenance of self-tolerance and prevention of chronic immune stimulation in IBD. TCR ligation with Ag in the presence of TGF-ß induces FoxP3 on $CD4^+$ T cells, converts them into Tregs and suppresses the immune system [38]. Higher frequencies of $CD25^{bright}$ and $FoxP3^+$ Tregs cells were seen in lamina propria of active IBD patients and increased as disease progresses [38]. Interestingly, colonic biopsies demonstrated less number of Tregs in IBD patients compared to non-IBD inflammatory diseases [38]. Current IBD therapeutics may have an effect on the frequency and function of Tregs. Manipulation of regulatory pathways would have therapeutic impact against IBD.

It is found that the administration of Tregs in IBD patients have beneficial effects. Apart from administration of exogenous Tregs, attempts have been made to expand the endogenous Treg population, converting naive T cells into Tregs by insertion of FoxP3 genes, modulating $CD4^+$ T cells in the presence of TGF-ß and improving the survival of Tregs via stabilized ß-catenin & upregulation of Bcl-xL [38]. Induced Treg (iTreg) cells predominantly secrete TGF- β and IL-10 to maintain mucosal homeostasis and protect from experimental colitis, respectively [41]. Immuno-suppressors with ability to induce Treg are useful, but increasing the number of may also down-regulate the desired immune response, therefore, critical analysis is required before

targeting the pathway of Tregs.

5. Gut microbiota and IBD

Host microbial interactions and importance of intestinal microbiota for metabolism and host defence are well established. Humans evolve with these microbes and it is known that around 1,800 genera with 15,000 - 36,000 bacterial species co-exist in the intestine [43]. It is interesting to know that the bacterial load (i.e., 10^{14} bacteria) in human gut is 100 times more than the human cells (i.e., 10^{12} cells). Mucosal immune cells can target these microbes and evoke immune responses. The epithelial layers covering the bowel wall control the entry of gut flora and minimize the interaction of microbes with the immune cells [44]. A large body of studies is focussed on immune responses as well as maintenance of immune tolerance to the intestinal microbes. The immune responses are largely provoked by recognition of PAMPs (pathogen associated molecular patterns) by toll-like receptors (TLRs). Apart from microbes, the metabolites from microbes are shown to play important role in inducing inflammation. Sometimes microbes penetrate the bowel wall and provoke immune responses [45]. There are many reports suggesting the role of enteric flora in the pathogenesis of UC and CD [46-48]. It is observed that the area with highest bacterial load shows highest degree of inflammation. Lowering the bacterial load by antibiotics has been shown to abrogate the progression of IBD. It is worth noting that inoculation of bacteria successfully induces IBD in experimental mice [45]. Genome-wide analysis of healthy and IBD patients provides the clue of microbial connection with IBD and it is found that >160 SNPs are identified as markers of risk for IBD. Interestingly, many of these genes are critically involved in host responses to the microflora. Mutations in NOD2, ATG16L1, IRGM, CARD9 and IL23R appear to be critical in inducing IBD [1].

To understand the involvement of bacteria in Crohn's disease a large study involving 668 children was conducted. The result shows the decrease in numbers of beneficial bacteria and increase in numbers of harmful bacteria in the gut of patients [49]. Firmicutes to Bacteroidetes ratio was found to be decreased in IBD (both CD and UC) [50, 51]. Apart from the involvement of a bacterial shift in IBD, role of fungus is also reported. By suppressing the fungal flora by probiotic Schreiber et al. successfully treated the UC suggesting the requirement of not only bacterial diversity but also fungal and bacterial ratio. IBD can also develop due to infections of *Mycobacteria, Shigella, Salmonella, Yersinia, Clostridium difficile* and *Bacteroides vulgates* [52]. The reduction in Bifidobacteria load and increment in *E. coli* and *clostridia* load in rectal mucosa was observed in UC patients and a 250-fold change in the balance was observed [53]. Surprisingly, in CD cases the *E. coli* load was found increased but no change was observed in Bifidobacteria load [53].

6. Microflora and T cells shape each other: a check point for IBD

Gut microbiota is postulated to direct and modulate the T cell activation and differentiation. It is evident from the fact that germ free (GF) rodents do not develop intestinal inflammation or immune activation and they have reduced numbers of $CD4^+$ and $CD8^+$ T cells that get restored following recolonization with microbiome [54]. T cell clones specific to *Enterobacteriaceae, Bacteroides*, and *Bifidobacterium* were isolated from the gut [55]. The immune system is trained to develop responses not only against the pathogens, but also to the commensal trying to escape the homeostasis mechanisms. When intestinal parasite *Toxoplasma gondii* infection occurs, $CD4^+$ T cells develop responses against the pathogen and also against the translocating commensal breaching the tolerance to commensal [41]. These microbes also modulate Th-17 cell responses as well as IL-17 secretion; and it was observed that GF mice or mice treated with antibiotics to eliminate the commensal bacteria have comparatively lower numbers of Th-17 cells. Segmented Filamentous Bacteria (SFB), a group of gut microbe have been shown to play important role in the differentiation of the Th17 T cell lineage as well as IL-22-producing $CD4^+$ T cells in the small intestine and the colon, and also mediate protection against enteric infection by *Citrobacter rodentium* [41]. Disproportionate SFB frequency has direct association with IBD. Commensal bacteria also modulate the balance between regulatory and effector T cells via pattern recognition receptors, including TLRs. Dendritic cell stimulation by TLR5 leads to an increase in IL-23, which further promotes the Th17 effector cell pathway to the detriment of $Foxp3^+$ Treg [41].

It is established that regulatory T cells (Tregs) also play a critical role in the maintenance of gut homeostasis. Interestingly it is observed that the generation and function of Tregs are influenced by the colon flora like *Clostridia* and *Bacteroides fragilis* but not by the microbes in small intestine. Clostridia clusters (*Clostridium leptum* and *Clostridium coccoides*) are found to induce proliferation and differentiation of peripheral Treg and help accumulate $CD4^+$ $CD25^+$ $Foxp3^+$ Treg in colon [41]. By reconstituting GF mice with a mix of 46 Clostridial strains it was shown that a significant number of Tregs were accumulated, in the cecum and the colon, to levels similar to that of SPF mice but similar accumulation was not possible by 16 Bacteroides species, SFB, and 3 Lactobacillus species. The protective effect of Clostridia species may be mediated to a large extent by SCFA (short chain fatty acids) which support the expansion of the existing pool of colonic Tregs [41].

It is amply clear from the above findings that microflora critically shapes the T cell responses. On the other hand, interestingly, T cells appear to shape the Gut Microbiome. There is emerging evidence which demonstrates this reciprocal relationship between T cells and their ability to shape the composition of the gut microbiota. The difference in microbiota diversity and load was seen in Rag1−/− mice vs. normal mice. The immunodeficient mice have increased load of Akkermansia muciniphila but decreased load of Lactobacillales and Enterobacteriales [41] compared to that of normal mice strongly supports the idea that T cell plays

important role in shaping the Gut Microbiome.

7. Targeting IBD

Currently IBD is treated by the wait and watch approach with the help of drugs which alleviate the symptoms by regulating the inflammation. Aminosalicylates and Corticosteroids are used to control the inflammations [56, 57]. Antibiotics like Metronidazole and Ciprofloxacin are being used to reduce the bacterial load. Apart from these traditional methods, many biologics are now being tried to for IBD. The antibodies that control the load of fungus, *Saccharomyces cerevisiae* [3] are in use target the pro-inflammatory cytokines, induce anti-inflammatory cytokines and also to block the intravascular adhesion molecules. TNF-α inhibitors like Infliximab (Remicade), adalimumab (Humira) and anti α4-integrin like certolizumab pegol (Cimzia) are also playing important role in controlling the pathogenesis of IBD [56] **(Table 2).**

Table 2: Therapeutics for IBD, their mode of action and possible side effects

Sr. No.	Therapeutics	Examples	Mechanism of action	Side effects
1	Aminosalicylates	Mesalazine, Sulfasalazine, Olsalazine, Balsalazide	1. Activation of PPARγ 2. Inhibition of macrophage chemotaxis 3. Inhibition of NF-κB, STAT & AP-1	Diarrhea, nausea, vomiting, headache, abdominal pain, hepatic abnormalities, Arthralgia and myalgia
2	Corticosteroids	Budesonide, Dexamethasone, Hydrocortisone, Methylprednisolone, Prednisolone, Prednisone	1. Down-regulation of NF-κB 2. Inhibition of cytokine production 3. Blocking the recruitment of immune cells 4. Inhibition of expression of adhesion molecules	Osteoporosis, metabolic syndrome, cardiovascular disease, infections, osteonecrosis, and cataract
3	Antibiotics	Metronidazole, Ciprofloxacin,	1. Decrease bacterial load 2. Suppress the intestine's immune system	Numbness, tingling, muscle pain, weakness, tendon rupture
4	Thiopurines	Azathioprine, 6-mercaptopurine	1. Immune system suppressors 2. Inhibition of protein synthesis 3. Inhibition of Lymphocyte proliferation 4. Induce apoptosis of activated T cells 5. Blocking of TRAIL, TNFRS7 & α4-integrin	Suppression of desired immune responses means loss of resistance to infection, very less chance of developing lymphoma and skin cancers, adverse effects on liver and pancreas,
5	Folic acid antagonists	Methotrexate	Inhibition of folate pathway and purine and pyrimidine synthesis	Hepatotoxicity, nausea, fatigue, pneumonia, bone marrow suppression, cancer.

6	Anti-TNF	Infliximab, Adalimumab, Certolizumab pegol, Golimumab	Inhibition of cytokine TNF-α thus act as anti-inflammatory	Costly, treatment failure, infusion reactions, infections, autoimmunity, lymphomas
7	Anti-α4-integrin	Natalizumab, Vedolizumab	Inhibition of T-cell migration	Progressive multifocal leukoencephalopathy
8	Probiotics	Lactobacillus species, Bifidobacterium species, E. Coli Nissle, yeast Saccharomyces boulardii	1. Inhibition of disease causing bacteria from sticking to the lining of the intestines 2. Inhibit inflammation 3. Re-maintenance of natural micro flora	So far, no serious side effects have been reported

8. Probiotic as therapy

It is evident from various studies that microflora pays key role in initiation or modulation of inflammation influencing the onset or progression of IBD. And the fact that disruption in the microbiotic balance evokes IBD opens new avenues for use of bacterial inoculation (probiotic) to target the IBD by restoring the balance. Probiotics are claimed to be safer, easier to produce and administer to patients than the pharma drugs and immune-modulators. The side effect of biologics and their ability to modulate the immune system is well known therefore alternative better strategies should be preferred. Many experimental models and clinical studies suggest the suitability of probiotics for targeting IBD and also the infection rate due to probiotics is negligible i.e., between 0.05% and 0.4% cases (58). The perfect mechanism by which probiotic alleviates IBD is still not clear. *Madsen* et al found that probiotic VSL#3 (Non-pathogenic *Salmonella* strains) act on epithelial barriers and improve the resistance to IBD induction [59]. Kaila et al proposed antibody production by *Bifidobacterium breve* [60], and Majamaa et al proposed enhanced cell mediated phagocytosis by the use of *Bifidobacterium lactis* probiotic [61]. Modulation of NF-kB pathway, prevention of epithelial apoptosis, antimicrobial activity and immune tolerance were observed by use of VSL#3, *Lactobacillus rhamnosus* GG, *Enterococcus faecium* and *Bifidobacterium lactis* respectively [58, 59, 62]. Each strain acts differentially and it was found from a study conducted by R. N. Fedorak et al [58] that five mechanisms might be responsible for the action of probiotics in alleviating IBD: [1] probiotics compete with microbial pathogens for receptors present on the surface epithelium; [2] immunomodulation of gut-associated lymphoid and epithelial cells; [3] suppression of pathogen growth through release of antimicrobial factors by probiotics [4] enhancement of mucosal barrier function; and [5] induction of T-cell apoptosis [58].

Clinical studies using probiotics to target CD demonstrated highly encouraging results. Gupta et al. used *Lactobacillus* GG and observed significant improvement in the Paediatric CD activity index within one week. McCarthy et al. showed that 76% patients avoided other treatments after 3 months of *Lactobacillus salivarius* therapy [63]. Apart from investigating the control of active disease many clinical studies were performed to evaluate the maintenance

of medical- and surgical- induced remission [58]. The studies demonstrated that probiotics not only alleviate CD but also beneficial for UC and remission maintenance. The studies performed by *Rembacken* et al [64], *Fedorak* et al [65], *Guslandi* et al [66] and *Borody* et al [67] with *E. coli Nissle*, VSL#3, *Saccharomyces boulardii* and fecal enemas - show very promising results. Genetically modified microorganism for probiotic, *Lactococcus lactis*-modified to secret and thus accumulate IL-10 at the mucosal surface was found to prevent IBD [14]. The overall findings support that probiotic therapy helps maintain the natural gut microflora and thus a suitable candidate for the treatment of IBD.

9. Conclusion and future perspective

The emerging cases during last two decades and severity associated with IBD need focused efforts to develop suitable therapeutics. The gut microbial composition and its relationship with immune system are the key considerations for IBD. IBD is targeted by various means including antibiotics, drugs and immune modulators. These agents show side effects, highly expensive and production is not adequate to reach to the large population. Although the therapeutic agents act through various mechanisms to target IBD, it is critical to maintain the balance of microflora in the gut. As the modulation of T cells shape the microflora, a prerequisite to maintain a healthy gut, we should use probiotic to treat IBD.

10. References

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