Title:
CCR6-CCL20 Signalling Networks in Inflammatory Bowel Disease

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ABSTRACT

Inflammatory bowel disease (IBD) has evoked a significant interest in human immunobiology given its tactical immune evasion methodologies resulting in acute immune destabilization. IBD comprising of Crohn's disease and Ulcerative colitis manifest as chronic inflammation in the gut mucosa, leading to complexities involving immune dysregulation in the T helper lymphocyte arm effecting disease pathogenicity. The mucosa of the alimentary canal is constantly exposed to a myriad of food antigens and luminal microorganisms for which a consistent host-protective mechanism is operative in healthy people. Lowered mucosal immune expression which allows penetration of the epithelial barrier by infective pathogenic microbes, elicits both innate and adaptive immune responses in the gut culminating in aberrant intestinal inflammation. Interestingly, IBD leukocyte repertoire is significantly entwined with chemokine assisted chemotactic navigation into the sites of inflammation which is also thought to generate favourable immune suppressive responses. The functions of the cognate chemokine receptor, CCR6, which binds with its unique ligand CCL20, are expected to tilt the balance between upregulation of homeostatic tolerance and inflammatory pathophysiology. This review aims at critically examining the CCR6 driven immune pathways; T_H1/T_H2, T_H1/T_H17, T_H17/ T_{reg}, IL-23/IL-17, Akt/ERK-1 /2, ILC3 for systematic investigation of its underlying mechanisms in the future and to underpin its importance in resolving IBD aetiopathology. Thus, CCR6 occupies an exclusive position in gut immunology which renders it an invaluable therapeutic tool for the production of novel medicaments to treat IBD.

1.0 Introduction

Immune-compromised diseases of the gastrointestinal tract has been a topic which has attracted active interest for decades and is one that continues to test the robustness of our immune system. Among the many disease-related models of human immune-inactivation, IBD has been a pertinent system within the gut mucosal biology. Importantly, a new immune concept involving the chemokine receptor 6 is gaining momentum with chemokine activated immune mechanisms coming to the fore. Chemokines are a superfamily of immune modulatory small protein molecules which regulate leukocyte migration to inflammatory sites through their chemoattractant properties. Among a plethora of functions, chemotactic navigation of effector T helper cell cohorts to the gut mucosa from lymphatics appears to be primarily a CCR6 driven mechanism which synchronises immune homeostasis during IBD pathophysiology. CCR6- CCL20 axis has been a prominent immune modulator in both innate and adaptive immune responses of a wide range of inflammatory diseases. It is associated with tissue damage and injury, human immunodeficiency virus (HIV), ophthalmic disorders, lung and kidney disorders, autoimmune diseases, brain disease, arthrosclerosis, obesity, diabetes and cancer [1].

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2.0 Chemokines

Chemokines are a specific group of cytokines which are small molecular proteins known to perform several different functions in the human immune system. Salient functions include T helper lymphocyte differentiation and cell chemotaxis aiding cell migration towards inflammatory locations. Remaining functions of chemokines could be listed as angiogenesis, development of embryos, B cell maturation and differentiation, lymphatic organogenesis, wound healing, inflammation and cancer metastasis. Chemokines preferentially aid leukocyte homeostasis by directing cell movement towards sites of injury and have been referred to as a specific cell navigational mechanism positioned within the immune system. The total chemokine repertoire consists of 50 chemokines and they belong to receptor and ligand cohorts. Chemokines consists of 4 subgroups named XC, CX3C, CXC and CC in which the naming is based on their receptors. A receptor is denoted by R and therefore, CC chemokines bind with CCR chemokine receptors. Chemokines display a common molecular structure of having three beta pleated sheets with an alpha helix at the carbon terminal in which the cysteine motifs are joined by disulphide bonds. The entire chemokine is composed of about 67-127 amino acid residues. Chemokine receptors are exhibited on the cell surface and they are described as seven transmembrane domains joined to guanine nucleotide coupled G1 class protein receptors. One chemokine receptor can bind with many ligands or several ligands can bind with one receptor or simply one receptor would bind with one sole ligand in which case chemokine receptor 6 has only one monogamous partner, the chemokine ligand CCL20. Chemokines play a prominent role in inflammation and spreading of cancer and interestingly, chemokine inhibition has yielded anti-inflammatory attributes in inflammatory disorders [1].

2.1 Chemokine receptor 6 (CCR6) and chemokine ligand CCL20

CCR6 is denoted by a string of different molecular identities such as; CD196, CKRL3, GPR29, CKR-L3, CMKBR6, GPRCY4, STRL22, BN-1, DCR2, DRY6, CCR-6, CC-CKR-6 or C-C CKR-6 and is said to transduce signals that mobilize intracellular calcium ion flux upon binding to its ligand, CCL20. Apart from CCL20, human beta defensins which are a group of microbicidal peptides have been identified as additional epitopes although it needs more comprehensive validation. CCL20 was recognized as the principal ligand through calcium ion flux experiments in a K562 cell line devoid of other chemokine receptors but transfected with CCR6. CCL20 has other synonyms, such as LARC (liver and activation regulated chemokine), MIP-3α (macrophage inflammatory protein) and Exodus-1. CCR6 is predominantly expressed in appendix, pancreas, lymph nodes and spleen with reduced expression in foetal liver, testis, colon, small intestine and thymus. Expression of CCL20 is upregulated by intestinal enterocytes responsive to bacteria displaying flagellar movement as well as antigen presenting dendritic and macrophage subsets, Langerhans cells in the skin, endothelial cells, natural killer (NK cells), neutrophils, B cells and T_H17 cells. CCR6 is signatory on T lymphocyte subdivisions; T_H17 and T_{reg} cells, innate lymphoid cell (ILC) -3, neutrophils, NK

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T cells, B cells and immature dendritic cells. Distinctively CCR6 is thought to adopt the PI3-Kinase (Phosphoinositide 3 kinase) signal transduction cascade which leads to phosphorylation that provides energy for cell chemotaxis [1].

3.0 Importance of IBD

IBD consists of an immune-compromised disease complex that includes two sub phenotypes; Crohn's disease (CD) and Ulcerative colitis (UC) in the human gut specified by chronic inflammation within the intestine. These are multi-factorial, heritable diseases which have around 200 single nucleotide polymorphisms (SNP) including *NOD2, ATR16GL, XBPI, IL23R, STAT3, JAK2* and *CCR6* that predominantly manifest in genetically predisposed individuals [2]. Recently heritability was endowed with only 25% probability for being recognized as the cardinal factor causing disease because a multitude of environmental factors are also now held accountable [3]. 50% similarity of CD in monozygotic twins, rising incidence of disease in the past 60 years without changes in the genetic makeup, IBD being less common in underdeveloped countries and development of IBD in immigrants to countries of high prevalence have cast light over multi-parametric environmental influences on the inception of disease although it is still treated as a polygenic disorder [4]. Rather than blaming it all on genetic predisposition and the microbiome, the trend now is to focus on new signalling pathways and to name one is the ER stress apoptosis due to the unfolded protein response resulting in autophagy of Paneth, goblet and intraepithelial cells [5].

Population based studies reveal IBD as a global disease of the 21st century with highest prevalence in Europe and North America. A USA based study had revealed a recent 1.3% increase from usual American IBD statistics and correlated the disease more to persons who are unemployed, living in poverty and lacking a high school education. IBD has shown a rising trend in the newly industrialized countries in Asia, Africa, South America and the Middle East since the 1990s [6]. A recent population survey positioned Australia as a country reporting the highest prevalence alongside Canada, Denmark and New Zealand with CD being more common than UC amongst the Australians [7]. Data from confirmed reports state IBD is becoming more severe and complex in Australia with a projected estimate of 100,000 patients in 2022 [8]. Among the Australians surveyed, factors such as smoking, childhood immunological events like exposure to tonsillectomy or chicken pox and intake of frequent fast food are causatives which help develop IBD but frequent drinking of caffeine and owning pets provided protection against developing the disease. Mostly young adults of the age ranges of 15-29 are said to be vulnerable to contracting IBD with another peak evident in the 60 – 70 age group with the most susceptible age being over 45 years [9].

Although the aetiology of IBD is not completely understood yet, it is commonly attributed to gastrointestinal (GI) tract stimulation by excessive and abnormal adaptive immune responses via induction of pro-inflammatory cytokines. Such immune activation is produced against the 100 trillion or so luminal microbial flora inhabiting the intestine which spans about 200 – 400

m^2 in extent. Dysregulated innate and adaptive immunity followed by microbial dysbiosis due to disruption of the septic mucosal barrier which exposes persons to a store of luminal antigens, play a significant role in disease development [10]. Multiple causative factors newly defined as the 'exposome', consisting of all the environmental contributory agents are now implicated in IBD and include the gut microbiota; both commensal and pathogenic, excessive usage of antibiotics, nutrition, smoking, industrialisation with more exposure to pollutants, a westernized lifestyle, poor sanitation, sleep disorders, anxiety and depression, appendectomy, exposure to sunlight and vitamin D [3]. Consumption of milk protein, animal protein, polyunsaturated fats and high sugar foods is known to increase the hazard of IBD with inhalation of tobacco posing as another risk factor in CD [11]. Passive inhalation of smoke either during pregnancy or childhood is thought to increase incidence of CD [3].

These dual diseases vary depending on the affected location in the gastrointestinal tract, severity of inflammation appearing in the intestinal wall and peculiarities in pathophysiology. CD is a chronic, transmural, segmental inflammatory disease that involves any part of the gut from mouth to anus but mostly affects the terminal ileum. CD forms ulcers, fistulas, stenosis and granulomas with intermittent periods of relapse and remission. UC is also a chronic, inflammatory disease which affects only the mucosa of the colon and the rectum. Symptoms common to both CD and UC are chronic relapsing flares associated with rectal bleeding, abdominal pain, tenesmus, urgency to evacuate, prolonged intermittent diarrhoea, anorexia, fatigue and weight loss [12]. Extra-intestinal manifestations have been reported at joints, some types of rashes in skin, eyes, liver and kidneys. Severity of the symptoms varies from mild to severe in patients not responding well to treatment. The most common complication of CD is the blockage of intestine due to swelling which results in the thickening of the bowel wall. Afflicted persons often encounter problems related to malnutrition, triggered by poor nutrient absorption [11]. A dearth of serological markers exist in the diagnosis of IBD and there is an acute need for more effective serological diagnostic tools to be introduced. No single blood test is diagnostic of IBD but abnormalities such as anaemia, elevated inflammatory markers, electrolyte abnormalities due to diarrhoea, vitamin deficiencies as seen in CD and low albumin indicative of both inflammation and poor absorption of nutrients are also used as diagnostic tools [12]. Those relapsing during clinical management and develop complications usually require surgical intervention whilst UC requires the removal of the large intestine known as colectomy after which there is no relapse but in CD, the disease could still recur after surgery [13]. Long standing CD and UC is a high risk factor for intestinal cancer [14]. IBD patients also become highly susceptible to chronic immune disorders such as HIV, psoriasis, primary sclerosing cholangitis and ankylosing spondylitis. IBD associated mortality has been reported by some studies while person's suffering from CD displaying comorbidities with cardiovascular and respiratory disease are also documented [15].

4.0 Immune mechanisms of CCR6- CCL20

Principal mechanisms of adaptive immunity encompass $CD4^+$ T cells and its subsets in enforcing immune activation in the gut. Naïve T_H0 helper cells are transformed into effector T helpers upon antigen sampling by dendritic cells and or macrophages in the mesenteric lymph nodes. Effector T helpers consist of four congenial immune sub types, T_H1, T_H2, T_H17 and regulatory T_{reg} cells although recent interest has been rigorously focused on the two subgroups; T_H17 and T_{reg} cells in the immune induction of IBD. These two subgroups have demonstrated distinctively opposing functional roles primarily attributable to their alienated cytokine profiles represented by; inflammation –triggering IL-17 and inflammation-dampening IL-10 respectively. In a nutshell, T_H17 induces disease activation by releasing inflammatory cytokines leading to tissue damage while T_{reg} cells promote immune tolerance induced by inflammation suppressive cytokines aiding tissue restitution [16, 17]. The underlying mechanisms favouring these two opposing roles are not clear yet but the expression of the chemokine receptor, CCR6 is considered the cardinal determinant of their selective proliferation in maintaining immune homeostasis. The CC-motif chemokine receptor 6 initiates chemoattractant migration of leukocytes towards the intestinal epithelium which binds with its partnering chemokine, CCL20 produced by intestinal epithelial cells when provoked by microbial stimulation [18]. This underpins the concordant, yet enigmatic role the CCR6 – CCL20 axis performs in primarily resolving the IBD immune mechanism [19]. The discovery of factors which skew the selection of T_H17 and T_{reg} polarization in the gut mucosa would be a novel breakthrough in IBD therapy [20].

Multiple cytokines and transcription factors serve to upregulate CCR6 expression on T_H17 cells. TGF-β, IL-6, IL-17, IL-21 and IL-23 as well as the lineage-selective master transcription factors RORγt and RORα are important regulators in upholding the immune induction of CCR6 which is the hallmark of the T_H17 cell cohort and they are strongly associated with autoimmune disease [21]. IBD invariably falls under the direct influence of the pro-inflammatory apparatus driven by CCR6 including T_H17 cell differentiation and proliferation. Well documented research reports that neutralising IL-17 as well as T_H17 lacking CCR6 receptors had served to markedly inhibit several autoimmune disorders [22]. Intriguingly, CCR6 represents a double edged sword by its tenacity to mobilize the immune suppressive T cell population, the natural regulatory Treg cells induced by the transcription factor FoxP3. Treg cells are highly effective in modulating autoimmune disease progression by actively suppressing pro-inflammatory T cell proliferation.

Concomitantly CCR6 drives chemotactic recruitment of T_H17 and Treg cell subsets to sites of infection in a self-sustained feedback loop because T_H17 cells also have the ability to express CCL20, induced by the cytokines TGF- β and IL-6 and STAT3. Given the dubious role played by CCR6 in disease amelioration, there obviously remains hitherto unidentified factors such as cytokines, adhesion and Co-stimulatory molecules and a whole repertoire of innate immune induction factors associated with CCR6 which contribute towards the selective

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delimitation of T_H17 cells Vs Treg distribution at the sites of inflammation. The most recently introduced immunological concept on IBD links deregulated T_H17 and Treg axis as the pivotal point which decides the fate of IBD resolution [19, 23].

4.1 Immune mechanisms of CCR6-CCL20 specific to IBD

The small intestine is the primary site of inflammation as it is exposed to a load of bacterial antigens and nutrients and is covered by a single layer of epithelium containing, epithelial cells, Paneth cells, Entero-endocrine cells, Microfold (M) cells and mucous secreting Goblet cells. Most of the immune action takes plays in Peyer's patches where the follicle associated epithelium (FAE) is crossed by antigen samplers which stimulate naive T and B cells to develop into effectors. Effector T and B lymphocytes are always found patrolling the mucosal surfaces in the gut. Inflammation is part and parcel of the intestinal mucosa to help maintain a healthy protective immune response to the numerous colonies of symbiotic microbes which crowd within the gut. Gut tolerance mainly occurs in Peyer's patches and isolated lymphoid follicles. Antigen uptake in the GALT – gut associated lymphoid tissue mostly occurs via M cells in the FAE in Peyer's patches by dendritic cells. CCR6 bearing dendritic cells are attracted towards CCL20 producing epithelial cells and thus migrate towards the mucosa associated connective tissue named the Lamina Propria (LP), to activate naive T and B lymphocytes. Primed effector cells drain out of afferent lymphatics to the mesenteric lymph nodes from which they enter the thoracic duct and then joins the bloodstream and return through the network of vessels back to the intestinal mucosa [24]. Activated T helper cells stimulate naive B cells into antibody producing plasma cells. Antigen-primed B lymphocytes are transformed into class switching B plasma cells that secrete IgA in the gut mucosa upon antigen presentation and activation by dendritic cells [25].

CCR6 deficient models displayed an overall change in the architecture of the intestinal epithelium with smaller Peyer's patches; lesser number of sub epithelial domes; absence of isolated lymphoid follicles; few intestinal M cells; high resistance to bacteria which enters through M cell conduits. The other significant IBD-relevant responses included marked elevation in the number of T_H17 cells in the spleen and lymph nodes; less preference for migration to inflamed sites and less suppressive capabilities of Treg cells; DSS and TNBS induced colitis producing moderate and severe disease respectively; and reconstitution of $Rag2^{-/-}$ SCID mice with naïve T cells from healthy mice resulting in acute inflammation [19, 26, 27, 28, 29, 30].

5.0 Immunologic pathways in IBD involving CCR6

An interesting feature of immune –regulated diseases highlighted by GWAS is gene loci similarity across a wide range of such disorders that pinpoint shared aetiology. Meta-analysis of shared single nucleotide polymorphisms (SNP) referred to as the "Immunochip", consisting of a microarray of SNPs have been designed to reveal deep replication and fine mapping among immune-controlled pathogeneses. Among the large number of disorders

surveyed, many displayed co-occurrence and familial incidence suggesting that a person afflicted with one immune –mediated affliction runs a high risk of contracting another of the same type. This overlapping aetiology is attributed to common clinical and immunological parameters concomitantly shared by those disorders which led us to a systematized examination of the various immune-activation pathways involving the chemokine receptor 6 as shown in figure 1 that culminate in immune- mediated disease pathogenicity in IBD [31].

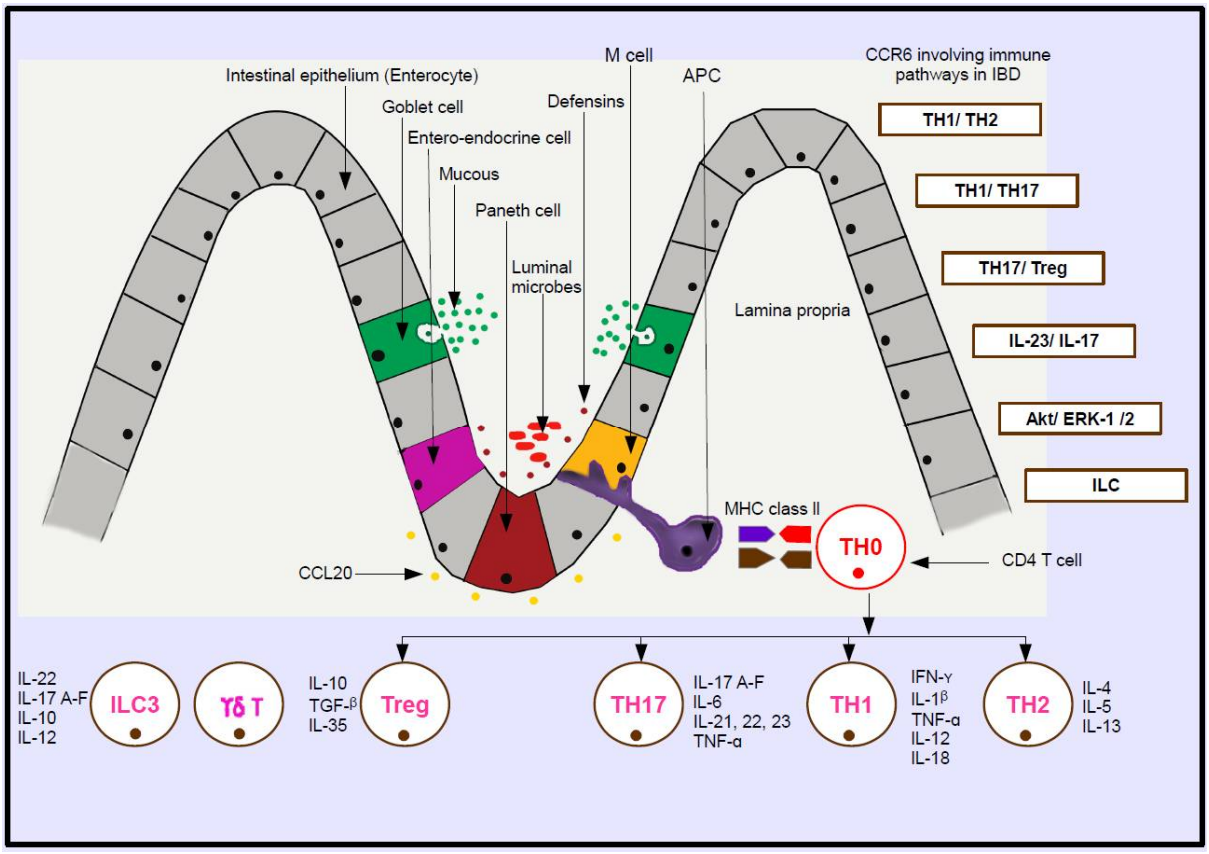

Figure 1: Schematic representation of the CCR6 involving immune pathways in IBD in the gut mucosa and priming of naïve CD4 T cells into effectors in mesenteric lymph nodes with their associated cytokines.

5.1 *TH1/TH2 pathway*

The immunological background of CD and UC in the past had been ascribed to the traditional dichotomy of TH1 (IFN-γ, IL-1β, IL-18, TNF-α, GM-CSF) and TH2 (IL-4, IL-5, IL-13, IL-22) cytokine pathways in which disease susceptibility to CD was promoted by TH1 associated cytokines where UC was modulated by TH2 cytokines. This old version has been challenged recently by a more modernized concept of TH17 vs Treg imbalance paradigm, playing a greater, more convincing role in determining IBD pathogenesis [32]. In certain autoimmune diseases including IBD, TH1 cells are known to upregulate the receptor CCR6 which could be fairly assumed to play a pathogenic role given the pro-inflammatory cytokines secreted by this cell subtype [1].

5.2 *TH1/TH17 pathway*

IL-17 is considered as a major role player in IBD given its release from the TH17 cells, a prominent cellular marker of IBD pathogenesis. Blockade of TH17 cells has strongly demonstrated decreased inflammation in the gut leading to lowered severity in acute colitis. IL-17 is well documented as a pro-inflammatory cytokine which has several isoforms, IL-17A to IL-17F whose mRNA levels had been persistently high in both CD and UC patients. IL-17 is proactive at the innate immunity level by stabilizing tight junctions in the intestinal epithelium and in demonstrating adaptive immunity are reports of IL-17 acting to recruit T cells into the LP during an inflammatory episode. The pro-inflammatory TH17 cells are characterized by transcription factor RORγt and surface markers IL23R and CCR6, and upregulate the cytokines IL-17A, IL-17F, IL-21, IL-22, IL-26 and chemokine ligand CCL20. TGF-β which is essential for the development of both murine and human TH17 cells, is also known to reciprocally regulate both TH17 and regulatory Treg development. GWAS have confirmed TH17 differentiation is under the influence of the genes coded by *IL-23R*, *IL-12B*, *JAK2*, *STAT3*, *CCR6* and *TNFSF15* which are directly linked to increase the risk of contracting CD and partly, UC. Collectively, inflammation in CD is selectively mediated through the polarization of TH1 and TH17 pathways as evidenced by immune therapy utilizing anti IL-12/IL-23p40 antibodies which produced reasonable efficacy in treating CD [31, 32, 33].

Contradicting responses have been recorded for IL-17A where its inhibition produced attenuated inflammation as well as stimulation of experimental colitis. TH17 is an important immunological milepost in IBD pathogenesis as it drives the TH17/Treg imbalance paradigm which is the modernized version of the TH1/TH17 pathway central to delineating the IBD progression [34]. STAT3 innervated IL-17 pathway is notably relevant to colitis inflammation as it is a transcription factor specific to TH17 cells. Overexpression of STAT3 induces the differentiation and proliferation of TH17 cells whereas absence of STAT3 decisively depresses the differentiation of TH17 cells from naïve T cells [35].

IL-17 A-F are potent cytokines which preferably induce granulocyte recruitment, tissue damage and production of IL-21 and IL-22. Ex-vivo cell cultures taken from IBD mucosa produced higher levels of IL-17A than the controls. IL-21 is said to drive the TH1/TH17 mechanisms in the gut evidenced by two facts; blockade of IL-21 resulted in the inhibition of LP mononuclear cells from producing IFN-γ and IL-17A and secondly, IL-21 deficient mice became resistant to TH1/TH17 driven colitis [36, 37]. Interferon regulatory factor proteins, IRF5 and IRF8 which are also transcription factor proteins have been strongly linked with CD and UC in directing transcription of IL-23A, IL-12A and IL-12B as well as suppressing IL-10 which leads to a powerful TH1/TH17 response. IRF5 induces the M1 antimicrobial phenotype in macrophages while IRF8 is vital for the development of dendritic cells and monocytes and when its gene is mutated it develops primary immunodeficiency in man [31].

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5.3 *T_H17/Treg*

T_{reg} cells are a loosely defined large cohort of immune suppressive reactors comprising of several sub phenotypes such as T-helper 3 cells, $CD8^+$ suppressor cells, NK-like cells, Tr1 cells and some of γδ T cell populations. However the central players bearing the T_{reg} identity are classified into two common classical subtypes consisting of the $FoXP3^+$ $CD25^+$ $CD4^+$ T cells named the natural T_{reg} which develop within the thymus and the latter being the inducible or adaptive T_{reg} which can develop from naïve T cells in the peripheral lymphoid organs. These key T_{reg} cells function in two ways; In the first type, the cytokines, IL-10, TGF-β and IL-35 expressed by T_{reg} collectively suppress local effector T cells irrespective of antigen-specificity and in the second type, the original clone persists beyond its lifespan which can be adoptively transferred between animals and tend to generate potent interest in clinical transplantation and cell therapy against autoimmunity [37, 38].

There are four major suppressive mechanisms by which T_{reg} cells activate immunity; secretion of cytokines, surface molecule signalling, cytolysis and metabolic control. Loss of function mutations of FoxP3 has shown decreased numbers of functional T_{regs} and exacerbation of autoimmune disease [38]. Interestingly, CCR6-CCL20 axis plays a critical role in selecting the up-regulation of T_{regs} in the periphery following inflammation (20). In IBD, proliferation of T_{reg} cells as opposed to the pro-inflammatory T_H17 is a remarkable factor which determines induction of tolerance and therefore, anti-inflammatory behaviour (20). Number of $CD4^+$ $CD8^-$ $CD25^+$ T_{regs} was markedly increased in inflamed and non - inflamed tissue of IBD patients compared to healthy controls and were localized in the LP and the muscularis mucosa. T_{regs} isolated from the mucosa of IBD patients had shown potent suppressor activity *in vitro* [38].

5.4 *IL-23/IL-17 pathway*

Advanced clinical trials interfering with IL-23 has shown promise for successful treatment of colitis. IL-23 is the master cytokine which drives not only the differentiation of T_H17 cell repertoires but also is responsible for evoking remarkable antimicrobial responses in the gut. IL-23/IL-17 immune mechanistic pathway denotes a central tool which operates to heighten IBD related pathology and is also validated by the scans of GWAS. This pathway is known to involve many susceptibility loci of different genes of which the SNP of *IL23R* gene encoding for the large subunit of IL-23 receptor, has been a common defect in a large number of IBD afflicted individuals. *STAT3* and *JAK2* are two other perilous gene variants which produce chronic intestinal inflammation and are also linked to IL-23 signal transduction in the IL-23/IL-17 axis. The remaining high risk variants of this pathway are *IL12b* which initiates development of the common subunit of IL-12 receptor and *CCR6* which encodes the chemokine receptor that is preferentially expressed by T_H17 cells [26, 39]. Collectively, all of these genetic aberrations provide ample evidence that they act as sentinels which contribute towards IBD immunogenicity. It is of interest to note that in a murine arthritic model, IL-23 had induced IL-17 release in $CD4^+$ T cells via the activation of STAT3, JAK 2, PI_3K/Akt and -

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NFkB and which also disclosed a link between IL-1, IL-17 and IL-23 as the arthritic mice were an IL-1R antagonist deficient population [39].

5.5 *Akt/ ERK-1/2 pathway*

ERK-1/2 are abbreviations used to define Extracellular signal-regulated protein kinases 1 and 2 which belong to the mitogen-activated protein kinase superfamily prominently known to mediate cell proliferation. The Ras- Raf- MEK- ERK signal transduction leads to translocation of ERK 1 /2 to the nucleus to produce mitogenic responses and is a pathway which has much therapeutic potential. In a study which quantified CCL20 mRNA expression in IEC, correlated their results with inflamed lesions in the colon in CD and colorectal cancer (CRC), also measuring increased IL-8 proteins, CCL20-activated Akt /ERK- 1 /2 and SAPK/JNK MAP kinases emphasizing evidence of CCL20 mediation in these pathways. CCL20 activation recognizably had produced a 2.6 fold increase in cell migration of both IEC and CRC cells along with significantly enhanced cell proliferation of the same. They concluded that CCR6 mediates a critical aspect of intestinal homeostasis and intestinal inflammation by inducing chemotaxis of IEC and CRC cells in the gut [39, 40].

5.6 *Innate Lymphoid Cells (ILC)*

A recent cutting edge breakthrough in the study of innate immunity has been the discovery of innate lymphoid cells (ILC) which could provide an important mechanism to understand the causes of IBD. These are a specific set of immune cells exhibiting some interesting characteristics such as; (i) related to lymphoid tissue inducer cells in development and function (ii) morphologically similar to lymphoid cells but lack antigen-specific receptors (iii) absence of phenotypic markers usually present on immune cells and importantly (iv) produce cytokines relevant to IBD which offers protective immunity. Earlier these were thought to be related to natural killer (NK) cells but are now recognized as a group of novel cells which are useful in delineating IBD immune mediated pathways. Three subdivisions of ILC have been recognized, named ILC1, ILC2 and ILC3 and are based on their similarity of origin due to the sharing of transcription factors T-bet, GATA-3 and RORγt respectively. The prominent factor which highlights the closeness of ILC with T effector lymphocytes is that both these groups produce the same cytokines in IBD. ILC3 group displays the CCR6 receptor and fall into two cell lines named natural cytotoxicity receptor positive (NCR^+) and negative (NCR^-) and each of these categories produce different cytokines, the former secreting IL-22 while the latter being similar to T_H17 cells, secreting both IL-17 and IL-23. ILC3 cells which share similarities with T_H17, stimulate neutrophils via IL-17 to produce the enzyme elastase and oxygen free radicals which cause apoptosis in the intestinal epithelium. Mononuclear phagocytes are said to activate ILC in the gut. There is intercommunication between the ILC subgroups; NCR^+ILC3 cells tend to release IL-12 which in turn activates IFN-γ producing ILC1 subtype. ILC1s by producing IL-23 activate NCR^+ILC3 to express major histocompatibility complex class II epitopes which could initiate transformation of $CD4^+$ T cells into effectors. IL-22

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dependent CCR6 –mediated ILC3 trafficking to the Peyer's patches in the intestinal epithelium is known to exist and intriguingly, ILC3 are also able to produce IL-10, retinoic acid and TGF-β to aid the differentiation of T_{reg} populations. A decrease in IL-22 manifests as loss of immune tolerance and develops into IBD clinical pathologies [24, 41, 42, 43].

Table 1: CCR6 –driven different immune pathways, their mechanisms and disease outcome.

Pathway	**Link to CCR6**	**Mechanism**	**Outcome**	**Reference**
T_H1/T_H2	$CCR6^+$ T_H1 cells	Migration of $CCR6^+$ T_H1 cells to the intestine attracted by CCL20 produced by the IEC given their release of pro-inflammatory cytokines induce inflammation in the intestine.	Inflammation	32
T_H1/T_H17	$CCR6^+$ T_H17 cells	Induced by IL-23, RORγt and TGF-β, T_H17 cells upon differentiation release IL-17A-F which drives $CCR6^+T_H17$ cell recruitment towards intestinal epithelium attracted by CCL20 produced by IEC.	Inflammation	31-35
T_H17/T_{reg}	$CCR6^+$Treg cells	Induced by TGF-β and FoxP3, regulatory T_{reg} cells after differentiation release IL-10 which drives $CCR6^+T_{reg}$ cells towards the intestinal epithelium attracted by CCL20 produced by IEC.	Resolution	37-38
IL-23/IL-17	$CCR6^+$ T_H17	IL-12, STAT3 and IL-23 induced $CCR6^+$ T_H17 cells migrate towards the intestinal epithelium stimulated by IL-17A-F and attracted by CCL20 produced by IEC	Inflammation	26, 39

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Akt/ERK 1 /2	CCR6$^+$ T_H17 / T_{reg}	CCL20 activates Akt/ERK 1 /2 and SAPK/JNK MAP kinases to increase cell proliferation and cell mobilization in the intestinal epithelium	Homeostasis	39, 40
ILC	CCR6$^+$NCR$^+$ ILC3	Induced by IL-22, CCR6$^+$NCR$^+$ILC3 release IL-12 and activate IFN-γ producing ILC1 cells	Inflammation	41, 42, 43
		IL-23-producing ILC1 activate NCR$^+$ILC3 to express MHC class II epitopes to initiate transformation of naïve T helper cells into effectors	Inflammation/ Resolution	
		Induced by IL-22 CCR6$^+$ ILC3 migrate to Peyer's patches attracted by CCL20 produced by IEC. Decrease in IL-22 results in loss of immune tolerance	Inflammation/ Resolution	
		Induced by retinoic acid, TGF-β and IL-10 released by ILC3 effect differentiation of Treg cells in the intestine	Resolution	

6.0 Future directions:

CCR6-CCL20 axis has not been investigated fully with respect to IBD pathogenesis. Although documented research has accomplished many IBD milestones, only little effort has been made to discover the absolute immune potency possessed by the chemokine receptor 6 in orchestrating adaptive immune responses during inflammation in the gut. There remains a dire need for spontaneous colitis models to investigate the immune mechanisms in spite of the extensive use of contrived mouse models treated with DSS and TNBS. Pre-clinical models such as CCR6/CCL20 double knockouts as well as humanized murine clones and randomized clinical trials utilizing antibodies and novel CCR6 inhibitors are suggested as future initiatives which might bring us closer to tracing the immunogenic contributions of the chemokine receptor 6. More investigations into the proteomics and metabolomics studies, combined with chemokine receptor 6 biology would perhaps be useful in identifying the multiple immune

pathways which deregulate IBD immunity. A comparison of immune mechanisms with those traced so far in other autoimmune disorders would also shed some light given their inclination to share genetic and molecular similarities in aetiology.

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8.0 Conflict of Interest

Authors declare no conflict of interests

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10.0 Abbreviations

Akt	-	protein kinase B
B	-	bursa-derived lymphocyte
β defensin	-	beta defensin
CD	-	Crohn's disease
$CD4^+$ T	-	cluster of differentiation 4 positive thymocyte
CCR6	-	CC chemokine receptor 6
CCL20	-	CC chemokine ligand 20
Ccr6	-	gene for CCR6
$CCR6^{-/-}$	-	CCR6 deficient
CRC	-	colorectal carcinoma
DC	-	dendritic cell
DNA	-	deoxyribonucleic acid
DSS	-	dextran sodium sulphate
ER	-	endoplasmic reticulum
ERK	-	extracellular signal-regulated kinases
FAE	-	follicle associated epithelium
FoxP3	-	forkhead box P3
GALT	-	gut associated lymphoid tissue
GATA	-	transcription factor
GI tract	-	gastrointestinal tract
GM-CSF	-	granulocyte macrophage colony stimulating factor
GWAS	-	genome wide association studies

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γδ T cell	-	gamma delta T cell
HIV	-	human immunodeficiency virus
IBD	-	inflammatory bowel disease
IEC	-	intestinal epithelial cell
IFN-γ	-	gamma interferon
IL	-	interleukin
IL-1β	-	interleukin one beta
ILC3	-	innate lymphoid cell 3
IRF	-	interferon regulatory factor protein
JNK	-	jun kinase
KO	-	knockout
LP	-	lamina propria
LARC	-	liver and activation regulated chemokine
M cell	-	microfold cell
MAPK	-	mitogen activated protein kinase
MEK	-	dual threonine and tyrosine recognition kinase
MIP-3α	-	macrophage inflammatory protein – 3 alpha
NCR	-	natural cytotoxicity receptor
NF-kB	-	nuclear factor kappa B
NK	-	natural killer
NOD	-	nucleotide-binding and oligomerization domain
p	-	protein
PBMC	-	peripheral blood mononuclear cells
PI3K	-	phosphoinositide-3-kinase
R	-	receptor
Ras / Raf	-	guanine nucleotide exchange factor

RNA	-	ribonucleic acid
RORϒt	-	retinoic-acid-receptor-related orphan nuclear receptor gamma
SAPK	-	stress-activated protein kinase
SCID	-	severe combined immune deficiency
SNP	-	single nucleotide polymorphism
STAT3	-	signal transducer and activator of transcription 3
T	-	thymus-derived lymphocyte/ thymocyte
T-bet	-	T box transcription factor
Tg	-	transgenic
T_H1	-	T helper 1
T_H2	-	T helper 2
T_H17	-	thymocyte helper 17
TGF-β	-	transforming growth factor – beta sulphate
TNBS	-	tri nitro benzene sulfonic acid
TNF-α	-	tumour necrosis factor- alpha
T_{reg}	-	regulatory thymocyte cell
UC	-	ulcerative colitis
WT	-	wild type