Title:

Pleiotropic Immune Functions of Chemokine Receptor 6 in Health and Disease.

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Abstract

Chemokine C-C receptor 6 (CCR6) and its exclusive ligand CCL20 is an extremely important chemokine receptor-ligand pair which controls cell migration and immune induction during inflammatory disease. Not many scientific studies have been undertaken to study its immune mechanisms in detail, but its unique contribution to steady state cell chemotaxis in upholding immune tolerance and regulating immune homeostasis during inflammation is evident in multiple organ systems, including lung, liver, kidney, skin, brain, eye, joints, gonads and the gut in the human body. The role of CCR6 is constitutively expressed as a series of much debilitating severe inflammatory and autoimmune diseases, HIV and cancer metastasis. $CD4^+$ T cells, the central organizers of adaptive immunity, are stringently mobilized by the CCR6/CCL20 axis also induced by cytokines and a host of other factors in a carefully executed immune modulation scenario, to bring about a delicate balance between pro-inflammatory T_H17 cells and regulatory T_{reg} cells. Although the exact immune regulatory role is not elucidated yet, CCR6/CCL20 axis is implicated as a front runner which determines the polarization of T_H17 and T_{reg} cells and consequently the resolution or progression of many debilitating disorders. This review therefore aims at emphasizing the pleiotropic significance of the chemokines CCR6 and CCL20 in immunologic function in multiple organ systems thereby hoping to accentuate its value in future therapeutic modalities.

Keywords: CCR6; CCL20; T_H17; T_{reg}, Inflammation; Multiple Organs; Health and Disease

1.0 Introduction

Chemokines

Chemokines represent an exclusive cell directing system in the body consisting of signalling proteins of the immune system. The primary role of chemokines in infections is to attract immune cells bearing their cognate receptors to sites of inflammation. The best example is corroborated by the role of chemokines in mucosal immunity where epithelial cells of the mucosa activated by an inflammatory stimulus releases the chemokine ligands constitutively inducing chemotaxis of the leukocytes bearing their corresponding receptors, towards them [1]. This is necessarily how chemokines mediate immune modulation and maintain cell migration during immune homeostasis or inflammation. Chemokines have also been described by other names, such as specific 'cell positioning' system [2] or a potential 'cell navigating' system [1] which brings about immune modulation in multiple organ systems of the human body.

About 50 different chemokines and around 20 receptors have been identified so far and are located on the surface of a range of immune and non-immune cells [2]. Chemokines and receptors are present on T and B lymphocytes, dendritic cells, macrophages, monocytes, neutrophils, eosinophils, basophils, innate lymphoid cells, neurons, epithelial and endothelial cells [3].

Chemokines, abbreviated for chemoattractant cytokines, mediate their biological effects through 7- transmembrane domain - G protein-coupled receptors [1]. These specific cell surface receptors are differentially expressed on diverse cell types. At the biochemical level, chemokine receptors act as guanine nucleotide exchange factors, restricted mainly to the pertussis –toxin sensitive G1 class of G proteins. At the immunological level, they coordinate development, differentiation, anatomical distribution, chemotactic migration and effector capabilities of leukocytes [4]. The biological impact of chemokines produces multiple outcomes; (I) embryonic development (ii) angiogenesis (iii) T-helper subset development (iv) leukocyte homeostasis (v) wound healing (vi) lymphatic organ development (vii) inflammatory diseases (viii) tumour growth and metastasis (ix) B lineage maturation and antigen-driven B cell differentiation [1, 4, 5, 6, 7, 8].

Chemokines are small proteins having a molecular weight between 8-14 kDa [9]. The structure reveals three beta pleated sheets and a carbon terminal alpha helix with disulphide bonds connecting cysteine residues and consists of structurally related secreted proteins of 67- 127 amino acids. They can exist as a monomer, dimer or tetramer, but the functional form is a monomer [1]. Chemokines are named based on their receptors and each sub class, CC, CXC, CX3C or XC is based on the number and spacing of conserved cysteines, have R to denote receptor followed by a number indicating the chronological order of discovery [9].

Thus CC chemokines bind with CCR chemokine receptors. Some chemokines bind specifically to one receptor such as the cognate receptor, CCR6 and its exclusive ligand, CCL20. Chemokines can bind more than one receptor or many chemokine

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receptors can bind more than one chemokine, among which the CCR6 and its ligand CCL20 forms an exclusive monogamous pair [1]. Chemokines are known to actively recruit immature or effector cells to affected sites during maintaining immune tolerance or promoting inflammation but interestingly, inhibition of chemokines have shown to develop anti-inflammatory properties encouraging the exploration of novel therapeutic breakthroughs in medical treatment [10].

Chemokine mediated chemotaxis of T cells has been functionally linked to distinct immune-mediated diseases. Leukocyte migration from blood to tissue is a multi-step process. It involves selectin-mediated rolling on endothelium and chemoattractant-mediated integrin activation followed by leukocyte extravasation and chemotaxis up a chemoattractant gradient [4]. Chemokines are secreted from the cell after synthesis and become tethered to glycosaminoglycans, a group of sulphated polysaccharides present in the extracellular matrix or surface to form a stable chemokine gradient which enables binding with its receptor [1].

2.0 Chemokine Receptor CCR6 and its ligand CCL20

CCR6 in *Homo sapiens* is also known by BN-1, DCR2, DRY6, CCR-6, CD196, CKRL3, GPR29, CKR-L3, CMKBR6, GPRCY4, STRL22, CC-CKR-6 or C-C CKR-6 [11]. CCL20 was identified as the sole known ligand to bind with CCR6 using CCL20 induced calcium mobilization in K562 cells transfected with CCR6 and not with any other chemokine receptors, CCR1-5 [1]. Five other chemokines (CCL2 -5 and CCL17) failed to bind to CCR6 although there is documented research stating CCL18 as a possible binding partner [12]. When CCL20 binds to CCR6, the receptor is internalized with decreased cell surface expression [1]. The anti-bacterial peptides, β-defensins, have also been shown to elicit chemotaxis through CCR6 although its activity on CCR6 still remains experimental [13].

CCL20 is known by several names such as, macrophage inflammatory protein MIP – 3α, Exodus -1 and liver and activation regulated chemokine, LARC and was discovered using bioinformatics techniques. It is expressed only by Th17 cells and not by regulatory T cells or other T helper subsets [1]. CCL20 expression is up regulated in the intestinal epithelial cells only as a response to invasive or non-invasive flagellated bacteria and is much labelled as an inflammatory chemokine [14]. Inflammation-related cells have been shown to express CCL20 and they include endothelial cells, neutrophils, natural killer (NK) cells, Th17 cells, B cells, dendritic cells, macrophages and Langerhans cells [15].

CCR6 is constitutively expressed in both lymphatic as well as in non-lymphatic tissue: predominantly in spleen, lymph nodes, appendix and pancreas and to a lesser extent in thymus, colon, small intestine, foetal liver and testis [11]. CCR6 is further expressed on various leukocyte subsets, including immature dendritic cells, B-cells, T-cells (pro-inflammatory T_H17 cells, regulatory T_{reg} cells), NKT cells, innate lymphoid cell 3 and neutrophils [16].

The dominant role of CCR6 in inflammatory disease is underpinned by its influence on driving the T helper subset differentiation and maintaining leukocyte homeostasis.

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Naïve T helper cells resident in lymph nodes, upon antigen sampling will differentiate into its effector sub populations, T_H1. T_H2, T_H17 and regulatory T_{reg} cells mediated by the prevailing cytokine environment and a host of other factors. However a critical factor which determines the development of T_H17 and T_{reg} cohorts evidently becomes the upregulation of CCR6 as both these cell sub types are known to be CCR6 $^+$ CD4 $^+$T cells. Thus proliferation, migration and promoting pro-or anti - inflammatory effects of these helper sets might be primarily CCR6 dependant processes [17].

$CD4^+$ T helper cells are the most important key players in organising adaptive immunity. Naïve $CD4^+$ CD45 RB ^{high} T cell populations upon activation by antigen presenting cells differentiate into the mainstream T_H1 and T_H2 classical lineages. They regulate cellular immunity through their respective signature cytokine profiles, namely, IFN-γ, IL-1β and TNF-α in T_H1 and IL-4, IL-5 and Il-13 in T_H2. T_H1 is pro-inflammatory by nature while T_H2 mediates humoral and allergic responses [18]. T_H1 and T_H2 differ in immune function as well as migratory capability by expressing distinct chemokine e receptors which modulate selective recruitment into sites of inflammation. The other two T helper subsets, T_H17 and regulatory T cells (T_{regs}), specifically express CCR6 but not always by the T_H1 or T_H2 cells [19].

Research from different groups have conferred opposing roles to T_H17 and regulatory T_{reg} cells in inflammatory disease [1]. IL-17 induced T_H17 differentiation is known to promote inflammation and intriguingly T_H17 also release CCL20, the chemokine ligand of CCR6. With either an autocrine or paracrine secretion of CCL20, T_H 17 cells also trigger a self-perpetuating cycle at inflammatory locations in a positive feedback loop. IL-17 production also induces TNF-α and IL-1β, pointing towards an inflammatory pathway involving the nuclear transcription factor NF-kB [20]. FoxP3 bearing regulatory T_{reg} cells are primarily disease suppressive in function and promotes disease resolution by downregulating inflammatory T cell proliferation and is induced by the cytokines, TGF- β and IL-10 [21].

CCR6 expression on T_H17 cells is induced by the cytokines, TGF-β, IL-17, IL-6, IL-21 and IL-23 as well as the lineage-specific master transcription factors RORγt and RORα [4]. CCL20 can be induced in a variety of cell types by lipopolysaccharide (LPS) and also stimulated and up-regulated by the pro-inflammatory cytokines IL-1α and TNFα during acute inflammation. CCL20 expression is constitutively up-regulated by many other pro-inflammatory cytokines such as IL-1β, IL-17, IL-21, IFN-Ϋ [22]. In contrast, the anti-inflammatory cytokine IL-10 down-regulates CCL20 expression indicating that this chemokine does not very well favour the accumulation of regulatory T cells particularly at inflammatory locations. IL-4, IL-22 and surprisingly, IL-23 have reported negligible inductive effect on CCL20 expression [4].

CCR6 confers an antagonistic function in these T helper populations, the T_H17 and Treg cells, although it is unknown what other factors are responsible for tipping the balance in favour of disease progression. CCR6-CCL20 axis remains the pivotal point which determines reciprocal generation of these two cell types but simultaneously highlights the importance of CCR6 in T cell migration.

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A disrupted CCR6-CCL20 axis only leads to the development of inflammation because CCR6 is adequately expressed on regulatory T cell populations in the cure phenotypes. A study involving the transfer of wild type T_H17 cells and CCR6 deficient T cells in to a Rag1 $^{-/-}$severe combined immune deficient (SCID) mice resulted in severe intestinal inflammation and a subsequent reduction in T_H17 and T_{reg} populations occurring [1]. CCR6 expression is deemed to be more important to T_{reg} cells than to T_H17 cells because this subset suppresses inflammatory T cell proliferation and promote disease resolution. Research documented so far has assigned dual roles to CCR6 in inflammation and immune homeostasis [23].

3.0 CCR6 signalling pathway

Upon binding to CCL20, CCR6 on leukocytes initiates signal transduction *via* activation of the Gαi family of heterotrimeric G proteins. Like most classical chemokine receptors, the downstream signalling of CCR6 involves activation of calcium mobilization, PLC-β, phosphatidylinositol 3-kinase/Akt, ERK1/2 phosphorylation, and actin polymerization [24].

Cell responses to chemoattractants make them typically sensitive to pertussis toxin, which indicates heterotrimeric G proteins from the G1 class are coupled to the signalling pathway downstream of the receptor. As explained by Figure 1, activated G protein heterotrimers release guanosine di phosphate (GDP) and bind guanosine tri phosphate (GTP), which dissociates afterwards into Gβγ subunit complexes and Gα subunits. In leukocytes, Gβγ activates membrane-associated phospholipase C-β2 (PLC) and phosphoinositide 3-kinase (PI_3K). PI_3K catalyzes phosphatidylinositol 4, 5 biphosphate (PIP_2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) which is subsequently converted into inositol triphosphate (IP_3) and diacylglycerol (DAG) by activated PLC. IP_3 regulates the mobilization of calcium ions from intracellular stores and DAG acts in conjunction with calcium to activate various isoforms of protein kinase C (PKC). Activated PKC and various Ca –sensitive protein kinases catalyse phosphorylation, thereby activating a series of signalling events ending up in cell migration [4].

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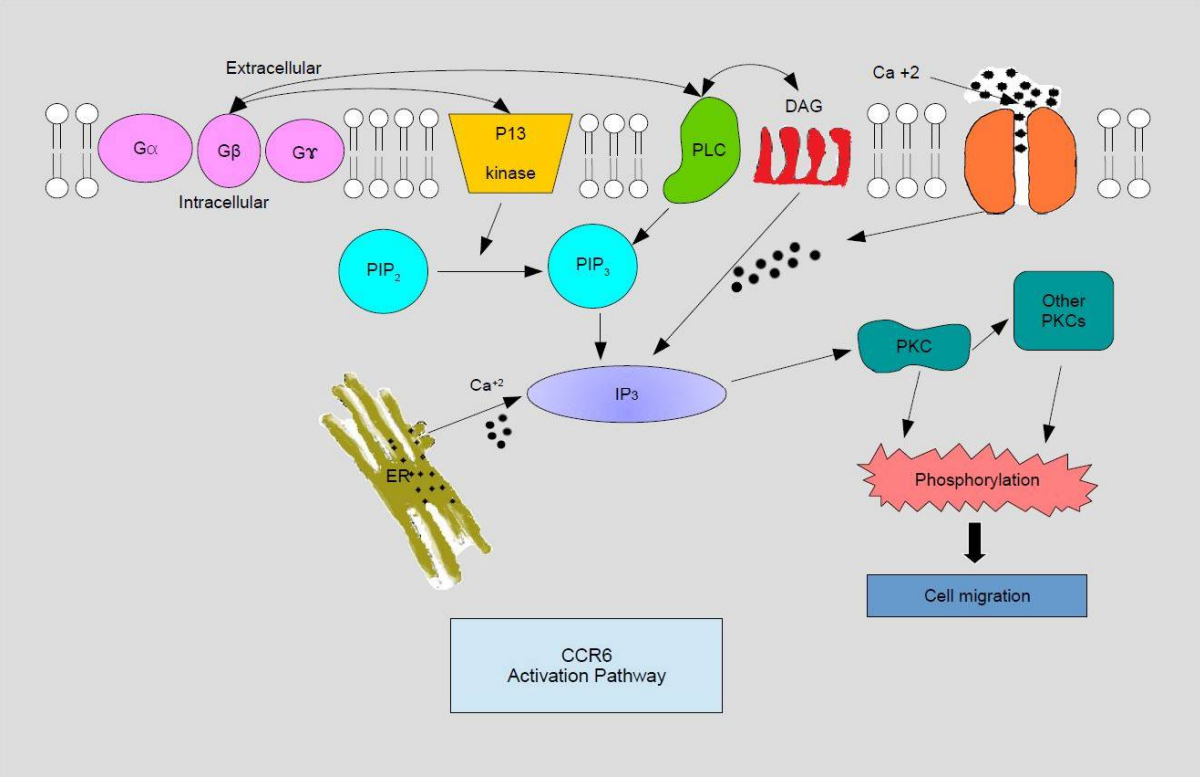

Figure.1 Schematic representation of CCR6 activation pathway

Gα, Gβ, Gγ – Subunits of G Protein coupled Receptors, PI3 kinase – Phosphoinositide 3 kinase, PLC- Phospholipase Cβ2, DAG- diacylglycerol, PIP2- Phosphatidylinositol 4,5- biphosphate, PIP3 – phosphatidylinositol (3,4,5)-trisphosphate, IP3- Inositol triphosphate, PKC – Protein kinase C, ER- endoplasmic reticulum, Ca+2 – Calcium ions

4.0 CCR6 and CCL20 in health and Disease

CCR6 and CCL20 chemokine receptor-ligand pair is implicated as a potential manipulator which creates a significant impact on human health and disease. In normal health, this pair performs an immune tolerogenic role by up-regulating immune suppression and hence the proliferation of FoxP3$^+$ regulatory T_{reg} cells aided by its cytokine milieu, when confronted with an inflammatory stimulus [25]. When this typical homeostatic function is disrupted, it is known to bring forth a marked increase of the T_H1/T_H17 axis thereby promoting adverse immunologic function of multiple systems culminating in a number of diseases such as sarcoidosis, idiopathic pulmonary fibrosis, chronic liver disease, experimental autoimmune encephalomyelitis, multiple sclerosis, rheumatoid arthritis, dry eye disease, psoriasis, glomerular nephritis, inflammatory bowel disease, HIV and an array of malignant cancers and their metastasis.

4.1 Lung

The adult human airways are not only a potential hub for harmful microbes, but also are exposed to allergens, pollutants, pulmonary emboli, complement deposition or autoantibodies because the lungs are said to inhale around 10,000 L of air every 24 hrs. A common occurrence in the progression of lung injury in response to these

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onslaughts is the local production and release of specific leukocyte chemoattractants which amplify the inflammatory response because inflammation is necessary in one way to defend, repair, protect against or subdue an infection. Thus activating leukocyte chemotaxis is vital to the development of antimicrobial host defense [4].

Research from Facco *et al* on pulmonary sarcoidosis had revealed CCR6 expression on T_H1 cells. CCR6 was co-expressed on alveolar macrophages in patients of sarcoidosis and alveolitis along with CXCR3 and CXCR6. CCR6 positive T cells infiltrated the lung insterstitium and were responsive to CCL20, CXCL10 and CXCL16. This observation amply demonstrates that T cells expressing CCR6 act co-ordinately with its ligand and T_H1 inflammatory cytokines during alveolitic disease [25]. Further, CCR6 possesses the capability to recruit immature and mature dendritic cells (DC) and other professional antigen presenting cells (APC) such as macrophages to sites of inflammation on the alveolar epithelium [26].

CCL18 released by alternatively activated macrophages, induced collagen synthesis by human lung fibroblasts, indicating CCR6 as the receptor for CCL18, in a screened phage-display library. Staining lung tissue indicated CCR6 expression by alveolar epithelial cells II (AEC-II) and fibroblasts in idiopathic pulmonary fibrotic (IPF) lungs that blocks airways, but not in tumour-free areas of squamous cell carcinoma patients inferring a role for CCR6-CCL18 involving fibroblasts in human lung disease [12].

In contrast, gene delivery of human CCL18 which has no known receptor, to the lungs of wild type (WT) mice induced pulmonary infiltration of T lymphocytes, but only less than 5% of the population had expressed CCR6. In the lungs of CCR6 deficient mice, CCL18-driven T lymphocyte trafficking was attenuated but not fully abrogated, concluding that CCR6 was not necessary for CCL18 –induced changes in mice *in vivo* and that CCR6 is not the main functional receptor for CCL18 in this model [27].

4.2 Kidney

T cells recruited to the kidney contribute to tissue damage in glomerulonephritis whilst chemokines and their receptors regulate T cell trafficking to sites of inflammation. CCR6 is expressed by renal $FoxP3^+$ $CD4^+$ regulatory T cells (Tregs) and IL-17 producing $CD4^+$ T (T_H17) cells while IFN-γ producing T_H1 cells were CCR6 negative. T_{regs} and T_H17 subsets had migrated attracted by CCL20 which was upregulated in renal biopsies of experimental murine nephritis and was followed by T cell recruitment, renal tissue injury, albuminuria and loss of renal function [28]. However, CCR6 deficiency too aggravated renal injury and increased mortality among nephritic mice because compared with the WT, CCR6 deficiency in mice had reduced infiltration of T_{reg} cells and T_H17 cells but not the T_H1 type. This is suggestive of the fact that an imbalance of T_{reg}/T_H17 paradigm associated with CCR6 mediation exists in the kidneys too, similar to the gut. Reconstitution with WT Tregs had protected $CCR6^{-/-}$ mice from aggravated disease confirming that CCR6 mediates renal recruitment of both T_{reg} and T_H17 cells whereas reduction of T_{regs} in the presence of T_H1 response, produced severe disease [29]. T_{regs} have been implicated in maintenance of tolerance to autoimmune renal disease, dampening

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renal inflammation and in preventing allogenic responses in renal transplantation [30].

CCR6 and its corresponding ligand CCL20 is also said to be involved in the recruitment of T and B cells to organized nodular infiltrates in human chronic renal inflammation. The mRNA expression of CCR6 and CCL20 quantified by real-time PCR in renal biopsies of various renal disease conditions had revealed this cognate chemokine receptor was expressed in $CD20^+$ B cells, $CD3^+$ T cells, tubular epithelial cells in inflamed kidneys and the endothelial cells of peritubular and glomerular capillaries although the functional role of endothelial CCR6 had not been evaluated. Similarly to CCR6-CCL20 being involved in the formation of gut-associated lymphatic tissue, it is postulated that the formation of nodular infiltrates in the kidney too happens in a CCR6-dependent manner [31].

4.3 Liver

Chronic liver injury results in hepatic inflammation leading to organ fibrosis. Patients with chronic liver disease had displayed intrahepatic up-regulation of CCR6 and CCL20 expression compared to healthy controls inferring that this chemokine receptor ligand pair may contribute to the migration of T_H17, regulatory (T_{reg}) and gamma-delta (γδ) T cells to sites of inflammation because CCR6 was explicitly required by IL-17 expressing γδ T cells for accumulation in the injured liver and produce disease resolution. Immunohistochemistry revealed accumulation of $CCR6^+$ mononuclear cells induced by CCL20 secretion of hepatic parenchymal cells in clinical liver disease whereas $CCR6^{-/-}$ mice developed more severe fibrosis with strongly enhanced hepatic immune cell infiltration compared to WT mice [32, 33].

4.4 Brain

T_H17 is strongly associated with autoimmune diseases as demonstrated by animal models of multiple sclerosis and rheumatoid arthritis and neutralising IL-17 as well as transfer of T_H17 lacking CCR6 receptors had markedly inhibited experimental autoimmune encephalomyelitis (EAE). Apart from autoimmune promoting, pro-inflammatory function of T_H17 it is also known to bring about disease resolution. Chemokines and adhesion molecules activate T cells into migrating to the central nervous system (CNS). The choroid plexus constitutively express CCL20 and is proposed to act as a gateway for CCR6 expressing $CD4^+$ T cells. EAE in animal models is used to study multiple sclerosis, which is an inflammatory, demyelinating disease of the CNS whose pathogenesis involves infiltrating T cells. Effector T_H1 and T_H17 subsets are found in MS lesions along with the expression of cytokines IFN-γ and IL-17. CCR6 plays a critical role in the entry of T_H17 which is said to induce EAE in the CNS. Direct analysis of CNS-infiltrating cells for CCR6 expression has revealed that in EAE, T_H1 cells outnumber T_H17 $CD4^+$ and CCR6 is expressed by both subtypes [34,35].

Cerebral ischaemia or stroke is ranked the second most common cause of death worldwide and is a much debilitating neurological disease condition. Immune-mediated tissue damage occurs in the first few days of suffering a stroke, mainly attributed to brain-infiltrating IL-17 producing γδ T cells which are largely positive for

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the chemokine receptor CCR6, triggering a highly conserved immune reaction. In a model of experimental stroke, genetic deficiency in *Ccr6* was associated with diminished infiltration of natural IL-17 producing γδ T cells and a significantly improved neurological outcome, outlining the role CCR6 plays in pro-inflammatory immune cell chemotaxis to inflamed sites in the brain [36].

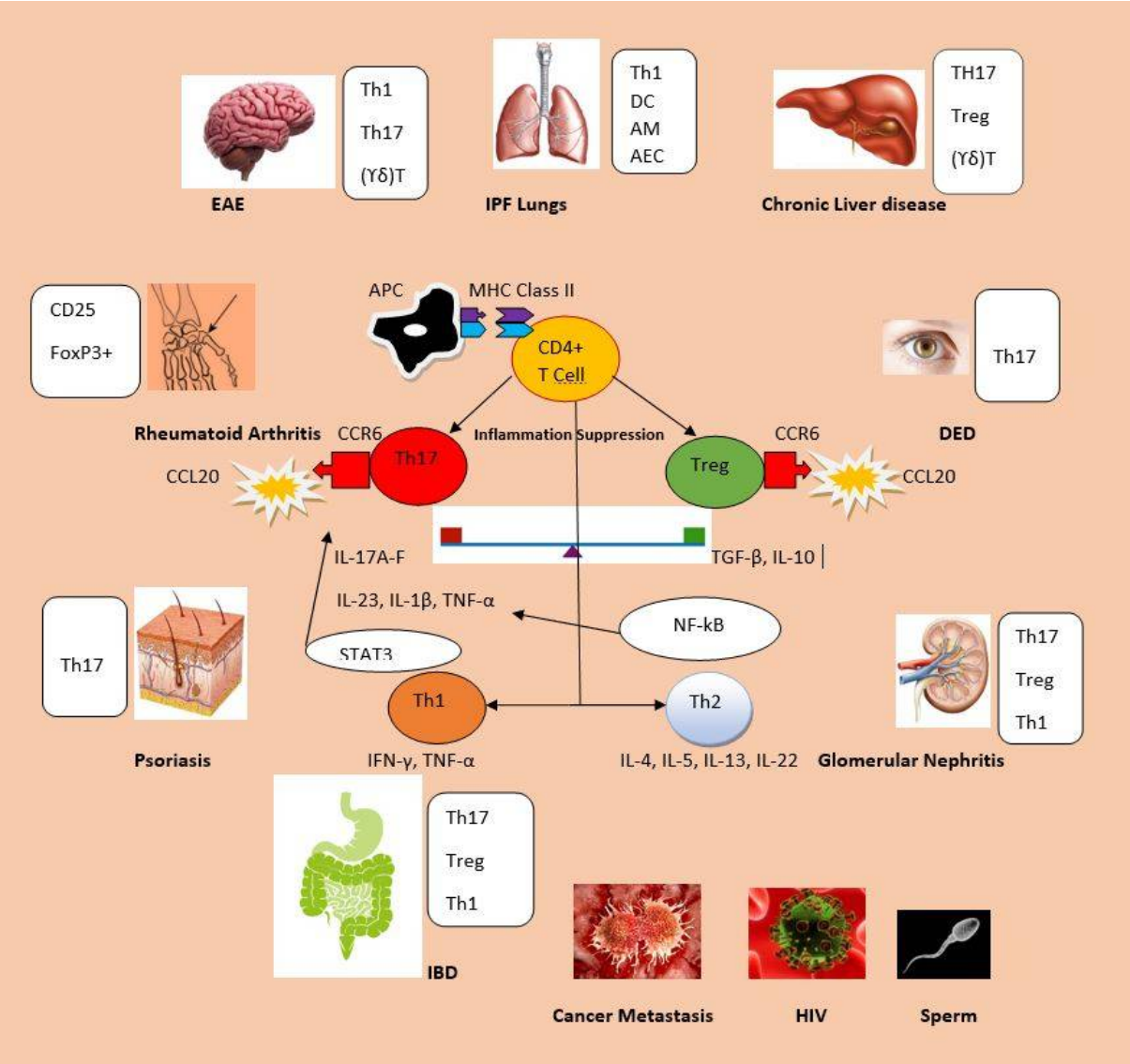

Figure.2: Schematic representation of the immunological impact of CCR6/CCL20 axis on multiple organs in the human body and the cells involved in promoting disease given next to each organ. EAE – Experimental Autoimmune Encephalomyelitis, IPF lungs – Idiopathic Pulmonary Fibrosis lungs, DED- Dry Eye Disease, IBD- Inflammatory Bowel Disease, HIV- Human Immunodeficiency Virus, APC- Antigen Presenting Cell, MHC Class II – Major Histocompatibility Complex Class II, AM – Alveolar Macrophages, DC – Dendritic Cells, FB- Fibroblasts, AEC- Alveolar Epithelial Cells, CCR6 – Chemokine receptor 6, CCL20 Chemokine ligand 20, IL – Interleukin Th1 – T_H1, Th2 – T_H2, Th17- T_H17, T_{reg}- Regulatory T_{reg} cells

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4.5 Eye

T_H17 cells are the primary effector cells causing pathogenesis of dry eye disease (DED), an immunoinflammatory disorder of the ocular surface that can even lead to corneal perforation. Local neutralization of CCL20 with antibodies administered subconjunctivally to DED mice had decreased T_H17 cell infiltration of the ocular surface producing improvement in clinical signs, suggesting that CCR6 interaction with CCL20 could direct T_H17 cell migration in the eye. Disruption of CCR6/CCL20 axis is therefore projected as a novel therapeutic approach to this condition [37].

4.6 Skin

The skin disorder, atopic dermatitis (AD) is characterized by a deficiency of keratinocytes in the skin which produces less CCL20 and similarly such patients also display a reduction in the expression of CCR6 which leave them exposed to viral infections leading to eczema herpeticum (ADEH) or eczema vaccinatum (EV). A population based study of European and African descent had recorded single nucleotide polymorphism (SNP) in CCL20 in native Europeans significantly associated with AD, suggesting that variants in CCL20 and CCR6 are highly relevant to AD and increase the risk of severe viral complications in this skin disease [38].

Psoriasis is a very common autoimmune disease condition of the skin that involves T_H17 associated signalling pathways. CCR6 deficient mice had failed to develop psoriasiform dermatitis in skin following IL-23 injections, because IL-23 is a growth and differentiation factor of T_H17 cells and hence a typical driver of T_H17 mediated inflammation, validated by previous research showing that recombinant IL-23 injections given to mice skin results in psoriasiform dermatitis that mimics human psoriasis in a short period as 5 days. A more recent experimental model has documented that IL-23 produced by dendritic cells act to sustain dermal CCR6 expressing T_H17 cells which release IL-22 that stimulates epidermal hyperplasia through STAT3 mediated mechanisms in human skin. Additionally, positive feedback was provided by epidermal and dermal production of CCL20, potentially recruiting more CCR6 expressing T cells or antigen presenting cells into inflamed psoriatic skin. Inhibition of CCR6 has been suggested to provide a possible therapeutic pathway to cure this disease [39, 40].

4.7 Joints

Rheumatoid arthritis is a chronic inflammatory autoimmune disease of the joints where chemokines regulate infiltration of synovial fluid by inflammatory cells. This disease is characterized by the increased release of CCL20 and accumulation of CCR6 expressing mononuclear T cells in the joints. An arthritis induced study model of $CCR6^{-/-}$ mice had not exhibited any clinical signs consistent with disease compared to WT controls but revealed that $CD4^+$ T cells, T_H17 cells and CD25 $FoxP3^+$ regulatory T cells showed up-regulation of CCR6 with RANKL which contributed towards disease, particularly osteoclastogenesis. A possible role in pathogenesis is thus highlighted in CCR6 in promoting inflammation at the joints [41, 42,43]. *Ccr6* single nucleotide polymorphisms (SNPs) have exhibited decreased

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basal and/or ligand induced Gαi protein signalling which predisposes individuals to disease such as rheumatoid arthritis (44).

4.8 Gonad

Capacitated human sperm is said to exhibit a directional movement towards CCL20 having the CCR6 receptor localized in the tail and a recent study revealed modifications in motility parameters of spermatozoa in the presence of chemokines. Chemokine receptor/ligand interactions in the male and female genital tracts promote sperm motility and chemotaxis under non-inflammatory conditions. Physiological reactions are thus mediated by CCR6 ligands in the male reproduction system which extends beyond a pro-inflammatory response and this observation underpins its significance in clinical reproduction and also possibly in contraception [45].

Organ	CCR6/CCL20	Cell Types	Disease	References
Lung	√	Fibroblasts, AEC, DC, T_H1 AM	IPF Lungs, Sarcoidosis	12, 25, 26
Liver	√	T_H17, T_{reg}, (γδ) T cells	Chronic liver disease	32, 33
Kidney	√	T_H1, T_H17, T_{reg}	Glomerular nephritis	28, 29, 30, 31
Brain	√	T_H1, T_H17, (γδ) T cells	EAE, Stroke	34, 35, 36
Eye	√	T_H17	Dry Eye Disease	37
Skin	√	T_H17	Psoriasis	39, 40
Joints	√	CD25- $FoxP3^+$	Rheumatoid Arthritis	41, 42, 43
Gut	√	T_H17, T_{reg}, T_H1	IBD	1, 50, 51, 52

Table 1: Organ systems and the diseases in which CCR6/CCL20 axis is functional

4.9 Gut

Animal models of the past and present to date have identified (i) genetic predisposition (ii) the composition of associated microbiome (ii) breakdown of innate immune barriers – disruption of the mucosal barrier due to decreased mucin synthesis, dysfunctional Toll-like and Nod-like receptor mediated pathways leading to increased pathogenicity, endoplasmic reticulum (ER) stress mediated apoptosis (iii) deregulated adaptive immunity and (iv) a plethora of environmental factors as multiple causes responsible for inflammatory disorders in the gastrointestinal tract (GI) and now, we can invariably include, disruption of the CCR6/CCL20 axis also as a significant contributory factor [46, 47, 48, 49].

GWAS have confirmed *Ccr6* as a risk allele of GI tract infections, among the 200 or so susceptibility loci already identified confirming that infectious diseases of the gut

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exhibit gene dependency and it also gives prominence to the CCR6 /CCL20 axis as a potential risk factor which determines disease outcome. CCR6 helps direct T_H17 cells to the small intestine upon immune induction and not only T_H17, but also $FoxP3^+$ regulatory Tregs are upregulated given the fact that CCR6 performs dual functions with regards to these two helper T subsets in gut associated lymphoid tissue (GALT). The observation that CCR6 deficient mice had T_H17 accumulated in spleen and bone marrow, unable to migrate due to the absence of this receptor and hence producing less intestinal inflammation, further supports its role in directing immune cell movement in the gut and also that T_H17 plays a pro-inflammatory role in intestinal disorders [50, 51, 52].

Intestinal microbiome which comprises of around 100 trillion cells is important for (i) colonization and maintenance of immune cells (ii) T_H17- Treg balance in the gut and (iii) protection against intestinal pathogens, evidenced by a decrease in T_H17 and an increase in Treg populations in mice given (i) antibiotics and (ii) bred in germ-free conditions. The disease outcome therefore, primarily depends upon the CCR6-CCL20 axis with microbiota featuring as another additional contributor [53, 54].

In inflammatory bowel disease (IBD) an autoimmune GI tract disorder which consists of Crohn's disease and Ulcerative colitis, Ccr6 knockout murine models had displayed (i) smaller Peyer's patches (ii) reduced number of sub epithelial domes (iii) absence of isolated lymphoid follicles (iv) reduced intestinal M cell numbers (v) increased resistance to bacteria which enters through M cell conduits (vi) increased Th17 cells in spleen and lymph nodes (vii) Reduced migration to inflamed sites and less suppressive capabilities of Treg cells (viii) moderate and severe disease in DSS and TNBS induced colitis respectively and (ix) transfer of naïve T cells to $Rag2^{-/-}$ mice resulting in aggravated disease [55, 56, 57]. SNPs in *Ccr6* have been reported to predispose individuals to Crohn's disease (44).

4.10 Cancer

It is now established that the chemokine receptor-ligand system is also used by cancer cells to direct lymphatic and haematogenous spreading and additionally has an impact on the site of metastatic growth of different tumours. Expression of CCL20 has been confirmed in various human cancer entities, such as leukaemia, lymphoma, melanoma, hepatocellular carcinoma, prostate cancer, colorectal adenocarcinoma, lung and oral squamous cell carcinoma as well as pancreatic carcinoma (PCA) [58, 59]. The expression of the CCL20/CCR6 system has been reported in PCA tissues and pancreatic cancer cell lines. Stimulation of the CCR6 bearing PCA cells with CCL20 led to an increased proliferation, migration and invasion and it was postulated that CCL20 may act via autocrine and paracrine mechanisms. Recent studies demonstrated that CCL20 may promote pancreatic tumour cell migration and invasion through the up-regulation of matrix metalloproteinase production [58].

A new interesting aspect of chemokine receptor biology is that chemokine receptors are implicated in cancer metastasis. Similar to CCR7 being critically important for the migration of T cells into lymph nodes, if chemokine receptors were to be expressed in tumour cells, they may be involved in organ-specific tumour metastasis. Specific

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signals must exist on the surface of tumour cells along with the establishment of chemotactic gradients that will allow them to migrate to common primary metastatic destinations of many cancers and in the metastatic spread to lymph nodes, various chemokine ligands are strongly expressed in these sites [59, 60, 61].

G protein coupled receptors (GPCR) represent ideal targets for the development of small molecular inhibitory epitopes because many best-selling anti-cancer therapeutics in the market today have targeted GPCR and it is probable that the chemokine superfamily will provide a useful target to find new important drugs [62, 63]. Inhibition of CCR6 signalling during or after medical or surgical treatment has been proposed would be useful in preventing liver metastasis of cancer by a study of BALB/c mice which showed overexpression of functional CCR6 and CCR7 on metastatic cells and tumour cell lines derived from the liver. They studied cell migration by tracking metastatic cells labelled with green fluorescent protein and measured CCR6 mRNA by RT-PCR which displayed preferential colonization of liver-sorted tumour cells when pre-incubated with the CCR6 ligand which is constitutively expressed by hepatocytes. This demonstrates chemotaxis via CCR6 might be a common mechanism adopted by malignant cancers when metastasizing to the liver [33]. Mutations in *Ccr6* also have been associated with a case of mucosa-associated lymphoid tissue (MALT) lymphoma [64].

4.11 HIV

Preferential infection by HIV of $CCR6^+$ T_H17 cells *in vitro* has been described in a study which had cultured both T_H1 and T_H17 cells sorted from healthy human peripheral blood in the presence of activated IL-1β and IL-23 to drive the expansion of T_H17 cells. Infection by HIV had produced minimal effects on T_H1 whilst causing marked depletion of T_H17 cells, increased infection of T_H 17 cells and cell death indicating a possible role for CCR6 in the internalizing of the virus within T helper populations [65]. In a more recent observation in relation to HIV infection of T_H17 cells, CCR6 was presented as a weak co-receptor in addition to the receptors CXCR4 and CCR5, which may act to provide an entry route to HIV invading the T helper cell subsets since preferential infection of T cells appeared to be independent of CCR6 expression. Nevertheless the importance of the CCR6/CCL20 axis is by no way diminished in HIV pathogenesis because this receptor- ligand pair is deemed to be actively recruiting T_H17 cells and DCs to the sites of infection thus propagating the virus to other sites of the body [66]. Envelope surface glycoprotein gp120 is known to up-regulate the expression of CCR6 in human B cells [11].

5.0 Conclusion

CCR6/CCL20 axis thus demonstrates a vital role in determining the disease outcome of inflammatory disorders and cancer metastasis in multiple organ systems in the human body. Inhibition of the CCR6/CCL20 axis is expected to open up new vistas for discovering novel therapeutics in the treatment of human disorders and a variety of therapeutic antibodies have already been made against the chemokine receptor 6. Effective blocking of the CCR6 signal transduction pathway using novel biochemical interventions might prove to be a useful therapeutic strategy in regulating pro-disease inducing cell chemotaxis within inflammatory microenvironments. Most of

the research has been centred around animal models which do not depict the true clinical picture, therefore there remains an urgent need for the evaluation of this chemokine receptor 6 and its ligand pair in expanded *in vivo* and *ex vivo* clinical studies.

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7.0 Conflict of Interest

Authors declare no conflict of interests

8.0 References

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