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Title:

Modulation of the CCR6 - CCL20 Axis: A potential therapeutic target in inflammation and cancer.

Authors: Ranmali Ranasinghe[1] and Rajaraman Eri[1]

1 School of Health Sciences, College of Health and Medicine,

University of Tasmania, Launceston, Tasmania, Australia.

First Author: Ranmali Ranasinghe

Correspondence:

Rajaraman Eri

Email: rajaraman.eri@utas.edu.au

Dr Rajaraman Eri
Associate Professor & Head of Discipline of Biomedical Sciences
Cell Stress, Inflammation and Immunity research
Associate Head (Learning & Teachingl)
School of Health Sciences, College of Health and Medicine
University of Tasmania
Office: Health Sciences Building (Newnham-C), 2nd Level, Room M217
Mail: School of Health Sciences, University of Tasmania, Locked bag 1322, Launceston TAS 7250, Australia
Tel: +61 3 6324 5467/0430029656**Fax:** +61 3 6324 5555**Email:** rderi@utas.edu.au
Web: http://www.utas.edu.au/profiles/staff/health/Raj-Eri
http://www.utas.edu.au/health/research/groups/cell-stress,-inflammation-and-immunity

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Abstract

Prototypical functions of the chemokine receptor CCR6 include immune regulation by manoeuvring cell chemotaxis and selective delimiting of the pro-inflammatory T_H17 and regulatory T_{reg} subsets during chronic or acute systemic inflammation. Inhibition of CCR6 is proposed to attenuate disease symptoms and promote recuperation of multiple inflammatory and autoimmune disorders. Prescription medicines with pharmacodynamics involving the inhibition of the chemokine axis CCR6-CCL20 is very limited. Developing such therapeutics is still at an early experimental stage which has mostly utilized pre-clinical models and neutralizing mono or polyclonal antibodies against either partner, CCR6 or CCL20. Other methods have been constitutive use of small molecules as peptide inhibitors or small interfering ribonucleic acid (siRNA) to interfere with transcription at the nuclear level. We in our review aim at introducing the wide array of potential CCR6-CCL20 inhibitors that have been tried to date in the research field with accent on attendant immune-modulator capacity and which are immensely promising compounds as forerunners of future curatives. 16 different tractable inhibitors of the CCR6-CCL20 duo have been identified to possess high medicinal potential to the drug developers worldwide to treat autoimmune and inflammatory diseases. A multitude of antibody preparations are already available in the current pharmaceutical market as patented treatment for diseases in which the CCR6-CCL20 axis is operative, yet must be used only as supplements with existing routinely prescribed medication as they collectively produce adverse side effects. Novel inhibitors are needed to evaluate this invaluable therapeutic target which holds much promise in the research and development of complaisant remedies for inflammatory diseases.

Key words: CCR6, CCL20, Inhibitors, TH17, Treg, Inflammatory diseases, Cancer

1.0 Introduction

Chemokines are chemoattractant cytokines consisting of small molecular proteins integral to the immune system. Quintessential characteristics of chemokines include balancing immune system integrity during steady state homeostasis and maintaining host defense during an inflammatory onslaught. Chemokines bind with their cognate receptors to discharge their immune

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modulatory functions in a concerted fashion, aided by the local cytokine milieu, adhesive and co-stimulatory molecules within the tissue microenvironment. Chemokines may be non- promiscuous, binding with a sole ligand or redundant, where multiple receptors bind to one ligand or *vice versa*. Around fifty chemokine ligands and twenty receptors have been identified to date having a molecular weight between 8-14 kDa. Chemokines and their receptors are essential for the trafficking of leukocytes during immune responses, and orchestrating immune cell chemotaxis to inflammatory sites among a host of other functions such as embryonic development, angiogenesis, wound healing, T helper subset development, B cell maturation and differentiation. Chemokines and receptors are present extracellularly on T and B lymphocytes, dendritic cells, macrophages, monocytes, neutrophils, eosinophils, basophils, innate lymphoid cells, neurons, epithelial and endothelial cells. Chemokine-mediated immune cell migration also contributes to multiple autoimmune and chronic inflammatory diseases as well as cancer metastasis and infection by HIV [1, 2, 3].

Among the chemokine repertoire, the CC chemokine receptor–ligand pair, CCR6 and CCL20 is becoming recognized for its invaluable therapeutic potential in immunological research. CCR6 is a 7- transmembrane domain - G protein-coupled receptor and CCL20 is known by several names such as, macrophage inflammatory protein MIP – 3α, Exodus -1 and liver and activation regulated chemokine, LARC. CCL20 is expressed only by T_H17 cells and not by regulatory T cells or other T helper subsets. Importance of the CCR6-CCL20 axis was emphasized with the discovery of the CCR6-expressing $CD4^+$ T helper cell sub populations, T_H17 and regulatory T_{reg} cells forming a platform which stringently regulates immune tolerance in healthy individuals. Disruption of this delicate alliance would tip the balance in favour of either the pro-inflammatory signature cell population, T_H17, or its immune regulatory partner, the regulatory T_{reg} cells being avidly recruited to the sites of infection or injury [4, 5].

CCR6 and CCL20 have exhibited characteristics in tune with both immune homeostasis and immune activation. It is already established that the immunological impact of this chemokine receptor-ligand partnership have far reaching consequences in health and disease, that affect multiple organs of the human body. A plethora of research studies have demonstrated that CCR6 and CCL20 axis directly influences the nervous, respiratory, gastrointestinal, excretory, skeletal, and reproductive systems via pleiotropic immune mechanisms, manifesting as diseases causing high mortality. Given the exclusive role CCR6 and CCL20 play in clinical pathophysiology, this chemokine pair is

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considered as a potential therapeutic target, which by the blockade or inhibition of either partner is expected to produce successful pharmacotherapy as treatment for its diseases [6, 7, 8].

2.0 Inhibitors of CCR6-CCL20

A CCR6- CCL20 axis inhibitors consist of (i) antibody that binds CCL20, (ii) antibody that binds CCR6, (iii) small molecule inhibitor of CCL20, (iv) small molecule inhibitor of CCR6, (v) small interfering RNA (siRNA) that hybridizes to a nucleic acid encoding CCL20 and (vi) siRNA that hybridizes to a nucleic acid encoding CCR6 [9]. Most of the studies undertaken up to now had depended heavily on utilizing neutralizing monoclonal or polyclonal antibodies.

Current prescription medicines consist of disease-modifying drugs which ameliorate symptoms and pathology through non-specific suppression of inflammatory pathways. Blockade of inflammatory cytokines and products which up-regulate pro-inflammatory chemokines is known to further induce inflammation. Biological agents Infliximab, Tocilizumab and Etanercept, have been used to block CCL20 which is otherwise known to markedly increase inflammation in autoimmune and inflammatory disorders. Infliximab is an anti-TNF –α monoclonal antibody used to neutralize the inflammatory cytokine, tumour necrosis factor – alpha, etanercept has an anti-TNF- α modified as a fusion protein and Tocilizumab is an antibody produced against IL-6R, all three of which have demonstrated significantly reduced CCL20 concentration below serum baseline values and these are combined as supplements with routine pharmacotherapy. Infliximab depresses TNF-induced CCL20 upregulation via nuclear factor kappa B (NF-kB) pathway in synoviocytes causing rheumatoid arthritis. Blockade of IL-6 modulates the CCR6 – CCL20 axis by preventing differentiation of T_H17 cells from $CD4^+$ T cell populations [10].

Early studies on specific blocking of either CCR6 or CCL20 include the transfer of $CD4^+$T cells from Sakaguchi mice into severe combined immunodeficiency SCID mice and administering a monoclonal antibody (mAb) against CCR6 in a rheumatoid arthritis model. The mAb administered mice had exhibited lowered migration of T_H17 cells to the joints with a recognizably reduced disease severity. The other research has been mostly pivoted around $CCR6^{-/-}$ mice pointing in the direction of a disrupted CCR6-CCL20 axis which affects immune cell chemotaxis and a discernible change in immune homeostasis. In the gut of inflammatory

bowel disease (IBD) models, Ccr6 deficiency indicated pronounced changes in the overall histology at the Peyer's patches [10].

Chemokines bind with their G protein coupled receptors (GPCR) via an extensive protein-protein interface that includes domains at the extracellular surface and a deep pocket within the transmembrane domain (known as the orthosteric site) at the N terminus. Native chemokines which are constitutive GPCR agonists, can elicit intracellular responses including directional cell migration through the activation of heterotrimeric G proteins and β- arrestin - mediated signal transduction pathway. CCR6 is a seven transmembrane domain GPCR which binds with its sole ligand, CCL20 and innervates tactical lymphocyte migration to sites of inflammation the same way. The therapeutic strategies explored so far have employed inhibition of chemokine signalling by small molecule or peptide antagonists, which are engineered to typically block GPCR signalling by binding at the orthosteric site and preventing activation by the corresponding chemokine ligand [11].

Partial or biased agonists of chemokine receptors which exhibit a loss of efficacy in certain types of signalling pathways can also be engaged as potent inhibitors, in which AOP- RANTES, (amino oxy pentane - regulated on activation, normal T cell expressed and secreted) a modified form of RANTES/CCL5 (regulated on activation, normal T cell expressed and secreted/CC chemokine ligand 5) is being reported by Getschman and colleagues. AOP-RANTES induces CCL5 –mediated calcium signalling but lacks pro-migratory signalling and has altered receptor recycling properties. Increased efficacy of engineered chemokines with partial agonist activity is displayed by greater anti-HIV potency of AOP-RANTES compared to native RANTES and act as alternatives to small molecule GPCR antagonists [11]. Changing the chemokine's oligomeric state can significantly alter its signal transduction properties. *In vivo* chemokine function essentially depends on binding to glycosaminoglycans present in the extracellular matrix and the self –association of chemokines is known to relatively enhance this binding activity. Although full GPCR activity happens in the monomeric state, two conserved types of dimerization exists in CC and CXC chemokine subfamilies and as proof of dimeric functionality, two CCL20 variants designed to form dimers had acted as partial agonists for the chemokine receptor, CCR6. In a murine model of IL-23 dependent psoriasiform dermatitis, two novel dimeric CCL20 variants induced (i) intracellular calcium release (ii) minimized chemotactic activity (iii) inhibited CCR6 –mediated T cell migration (iv) up-

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regulation of IL-17 and IL-22 and (v) prevented psoriatic inflammation, thus highlighting CCR6 as a controllable therapeutic target [11].

Chemokine receptors have shown to employ several different pathways in inflammatory and autoimmune disorders and hence, it is advisable to simultaneously target more than one receptor-ligand pair to evaluate their efficacy in disease suppression. Fully humanized, IgG-like bispecific antibodies (BsAb) have been developed against CXCR3 and CCR6 and in testing its validity as a promising therapeutic target which (i) regulates cell migration of the T_H17 subset of CD4+ T lymphocytes, (ii) had demonstrated that it effectively blocks cell chemotaxis and (iii) induces specific antibody-dependent cell-mediated cytotoxicity *in vitro*. Dual targeting of chemokine receptors with a fully humanized BsAb in this instance had thus provided a potent interventional approach for the treatment of inflammatory and autoimmune diseases. Depletion of various pro-inflammatory subsets of pathological lymphocytes is another therapeutic approach but broadly targeting immune cell migration could turn out to be immune compromising. Thus, a combination of blocking agents may be required to prevent the recruitment of mixed leukocyte subsets to inflamed tissue in order to reap the maximal therapeutic benefits [12].

The use of anti-inflammatory botanical inhibitors as antagonists of CCR6 and CCL20 has been studied. Epigallocatechin gallate (EGCG) a, polyphenol compound found in green tea has been reported to inhibit CCL20-mediated cell chemotaxis by a significant 20%, in a study involving recombinant and chemically synthesized rhesus macaque chemokines developed for studying interactions between CCR6 and CCL20. 100% inhibition of CCL20-driven chemotaxis was observed with gallotannin, a phenolic metabolite (tannin) that occurs naturally in foods such as chick and cowpeas, nuts, mangoes and rhubarb and is associated with scavenging of free radicals and interference of both herpes virus and HIV. The same study also reported that CCR6 receptor peptide mimetics ECL-2 inhibits CCL20 induced migration. Extracellular loops of chemokine receptors called receptor peptide mimetics or ECL-X (where X refers to the number of the sequential extracellular loop) can bind directly to chemokine and inhibit chemokine-induced migration [13].

3.0 CCR6-CCL20 inhibition in multiple organ systems

3.1 *Nervous system*

CCR6 plays a specific role in neuroinflammatory response in experimental autoimmune encephalitis (EAE), validated by the delayed onset and reduced disease observed in $CCR6^{-/-}$ mice compared to the wild type (WT). In an experimental model of EAE, CCR6 was implicated not so much in the effector function but in an enhanced priming role of T helper cells. The study demonstrated impaired development of EAE in Ccr6 deficient mice and mice treated with a neutralizing anti-CCR6 antibody (Ab) or a novel CCR6 antagonist bearing synthetic truncated CCL20 peptides. Three functional outcomes were determined by this research summarizing it into (i) CCR6 being critical for the priming phase of EAE (ii) recruitment of immature DCs to tissue is CCR6 dependent and acts as a limiting factor for T cell priming and (iii) CCR6 regulates lymphocyte egress from peripheral lymph nodes, during active immune stimulation [14].

No effective monoclonal antibody (mAb) inhibitors against CCR6 yet exists for use in mouse models of inflammation but has been circumvented by transgenic mice expressing human CCR6 (hCCR6) under the control of its native promoter (hCCR6-Tg/mCCR6-/-). Anti hCCR6 mAb was recognizably effective in reducing disease severity in EAE, by remarkably attenuating clinical symptoms of myelin oligodendrocyte glycoprotein (MOG) induced EAE, a model in which antigen-specific B cells contribute to disease pathogenesis, and with reduced infiltration of inflammatory cells in the central nervous system (CNS). CCR6 is upregulated in T_H17 cells and innate lymphoid cells that produce IL-17 and IL-22 which suggests that CCR6 inhibition could lead to depressing T_H17 type of inflammatory reactions. Further, antagonizing CCR6 with a mAb should be an effective strategy for the treatment of T_H17 or T_H22 mediated inflammatory autoimmune diseases giving us the opportunity to selectively inhibit inflammatory cytokines like IFN-γ and IL-21, produced by $CCR6^+$ T_H17 cells during inflammatory conditions [12].

Posterior uveitis is an intraocular inflammatory disease affecting the uvea and the retina which can impair vision. Bromodomain extraterminal (BET) proteins have been recognized as potential inhibitors of EAE and now, of uveitis. In EAE, BET proteins act via the suppression of $CD4^+$ T_H1 cells in reducing disease

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severity. BET proteins are gene regulators which block the activity of transcription factor T-bet which in turn suppress proliferation of T_H1 sub population. A recent study on uveitis has revealed pharmacological blocking of T_H17 cell differentiation by using BET proteins as inhibitors, which had been successful in attenuating inflammation in uveitis. Using both human and mouse *in vitro* cell cultures, they provided evidence that BET inhibitors suppress the expression of RORγt and a significant downregulation of T_H17 –associated genes IL-17A and IL-22. The key finding is that BET inhibition markedly upregulated $FoxP3^+$ expression accompanied by lowered pathogenicity *in vivo*, suggesting that BET inhibition may switch retinal $CD4^+$ T cell polarity from a T_H17 to T_{reg} phenotype. Thus it may represent a viable therapeutic entry point for inflammatory and autoimmune disorders which primarily depend upon the T_H17/Treg axis for disease resolution [15].

Allergic conjunctivitis is an inflammatory disorder of the ocular surface characterized by Ig E and T_H2 driven allergic responses marked by eosinophilic infiltration of the conjunctiva and involve the chemokine receptor CCR6. In mice induced with experimental allergic conjunctivitis (EAC), disease severity was compared between WT and CCR6 knockout (KO) models which showed absence of CCR6 suppressed allergen-specific Ig E secretion, decreased mast cell and eosinophil accumulation and therefore, minimized allergic conjunctival inflammation. Reduced inflammation was ascribed to reduced cytokine secretion from T_H2 cell type in draining lymph nodes. Neutralization of CCR6 ligand, CCL20 with CCL20 neutralizing antibodies, clearly repressed disease evaluating parameters indicating that CCR6 might be important for the optimal development of T_H2 responses as well as inflammation in EAC [16].

3.2 *Integumentary system*

Psoriasis is a chronic autoimmune skin disease which affects up to 3% of the world's population, characterized by infiltration of inflammatory T cells to the skin in response to injury or autoantigens. Skin inflammation is caused by the cytokines of accumulated $CCR6^+$ T cells and dendritic cells which migrate towards the chemokine ligand, CCL20 and other chemokines, produced by keratinocytes and endothelial cells [11].

Animal models of psoriasis have already established the importance of the IL-23/T_H17 axis related to the CCR6-CCL20 receptor-ligand pair extending it also on

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to human disease. Human psoriatic skin invariably displays higher upregulation of CCR6/CCL20, especially with higher CCR6 expression on circulating PBMCs than in normal skin. Psoriasiform skin inflammation induced by the drug, Imiquimod (IMQ) when treated with an anti- hCCR6 mAb, had a striking effect in preventing epidermal hyperplasia and dermal inflammation. This result is consistent with increased infiltration of IL-17 producing γδ T cells to psoriatic skin which was abrogated by anti-CCL20 mAb treatment. Inhibition of $CCR6^+$ cell migration is considered more advantageous than anti-IL17 /IL-17R therapies because then, other cytokine pathways such as IL-22, GM-CSF and β-defensin binding could be targeted with the specific inhibition of $CCR6^+$ cells [11].

CCR6 inhibition by a hitherto unidentified small molecule inhibitor named CCX9664 is published by a team of researchers experimenting with psoriasis, reporting that the dominant T_H17 receptor, CCR6 when inhibited by CCX9664, an orally available inhibitor, has shown to be very effective in a preclinical *in vivo* model with (i) reduced skin inflammation correlated with (ii) reduced numbers of IL-17 secreting T cells. Apparently, CCX9664 has proven efficacy in models equivalent to results achieved with an antibody to the IL-17 receptor, which is described as a known human therapeutic target [17]. Another article by the same author has documented protection against psoriasiform –like disease in mice by a CCR6 antagonist which exhibited limited infiltration of $CCR6^+$ leukocytes into the epidermis.

CCL20 neutralizing antibody was developed by Bouma and colleagues to test its efficacy in recruiting $CCR6^+$ cells to sites of inflammation using an experimental suction-blister model. In a randomized, placebo-controlled first-in-human study, Bouma *et al* had employed GSK3050002, a humanized IgG1k antibody, a reported to be potent CCR6 agonist, to assess target engagement and the ability to inhibit pro-inflammatory CCR6 – expressing immune cell trafficking to the site of injury. Results demonstrated selective dose-dependent inhibition of $CCR6^+$ T_H17 cells by this novel antibody to support further development of such CCL20 antagonists to treat autoimmune and inflammatory disorders [18].

3.3 *Skeletal system*

Data available from preclinical studies of research again highlights CCX9664 as a novel CCR6 antagonist capable of producing low disease severity in rheumatoid arthritis, a common autoimmune disease which affects the joints in the skeletal

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system. This chemokine receptor antagonist selectively blocks CCR6 mediated recruitment of T_H17 cells to the sites of inflammation providing an orally administrable inhibitor with minimal side effects [19]. Jaen et al, the same group of researchers published another promising CCR6 antagonist, identified as CO339589 which had inhibited CCL20 mediated chemotaxis and binding in a human natural killer (NK) cell line and $CCR6^+$ enriched human peripheral blood mononuclear cells (PBMC). It was identified as a highly potent molecule on both human and mouse CCR6 suitable to use for treatment of diseases, rheumatoid arthritis, psoriasis and multiple sclerosis (MS) [20].

3.4 *Reproductive system*

There is evidence that CCR6-CCl20 axis may operate via the MAPK (mitogen-activated protein kinases, originally called ERK – extracellular signal-regulated kinases) and stress-activated Protein kinase C- JNK (jun kinase) pathway, which contributes to inflammatory responses in mammals. The MAPK/ERK pathway is a chain of proteins in the cell that communicates a signal from an extracellular receptor to the nuclear DNA of the cell initiating transcription. In endometriotic tissues and peripheral blood collected from patients having ovarian endometriomas T_H 17 cells expressed CCR6 whereas CCL20 was secreted by the epithelial and stromal cells thus promoting immune cell chemotaxis of the receptor-ligand pair. In this study, CCL20 caused selective migration of T_H17 cells in the peripheral blood in a migration assay while increased secretion of CCL20 was detected upon *in vitro* stimulation with IL-1β, TNF-α and IL-17A in endometrial stromal cells. Interestingly, inhibition of the proteins, p38 and p42/44 MAPKs and stress-activated protein kinase c-Jun kinase had suppressed the secretion of CCL20 increased by the pro- inflammatory cytokines IL-1β, TNF-α and IL-17A. This is suggestive of the fact, CCR6/CCL20 system is involved in T_H17 migration to endometriotic tissues and set up inflammation upregulated by inflammation-inducing cytokines in the development of endometriosis. The protein inhibitors utilized in these experiments shed light on to a possible therapeutic application that could become useful for curbing inflammatory disorders produced through CCCR6-CCL20 activation [21].

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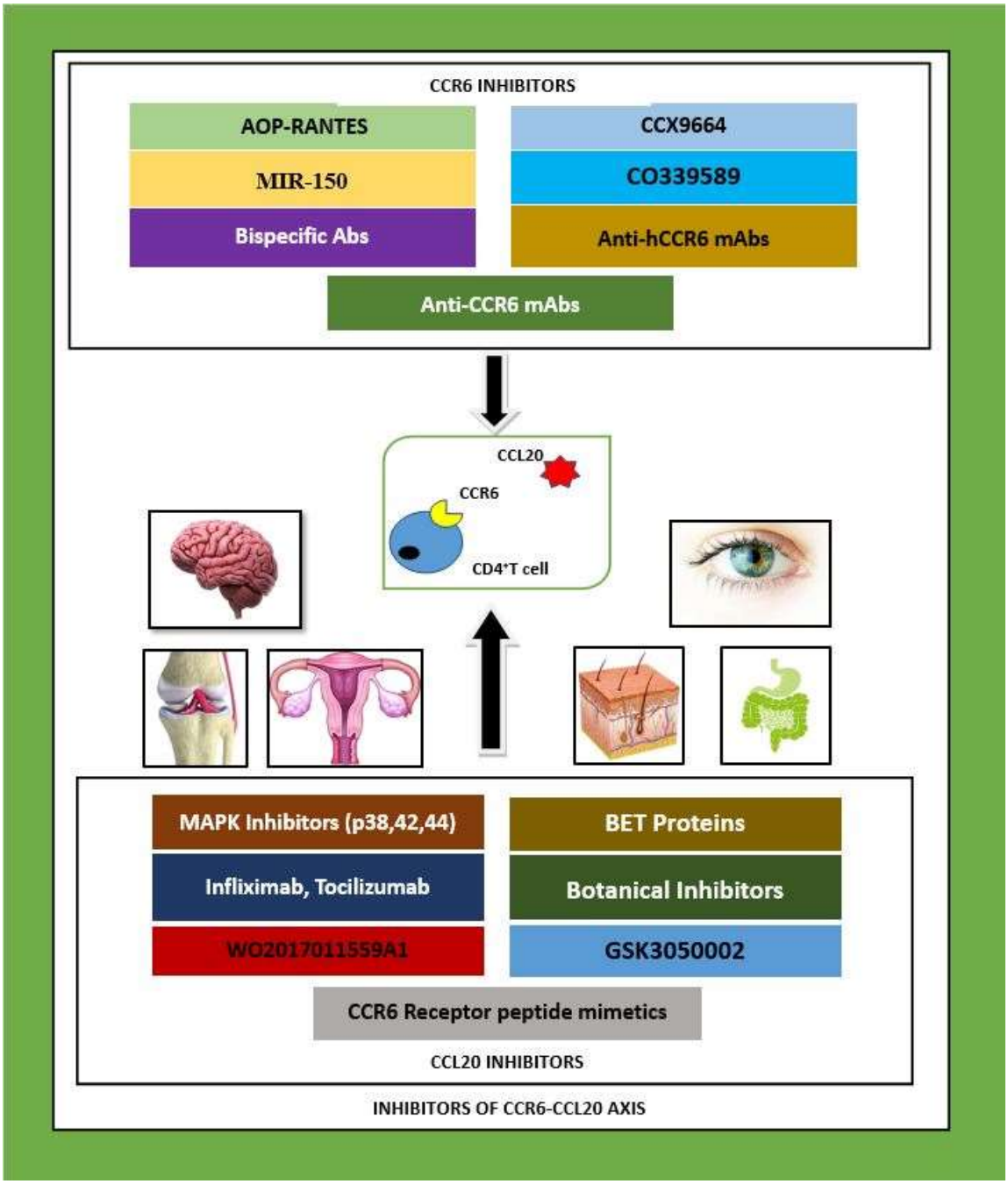

Figure **1**: CCR6-CCL20 axis inhibitors investigated in pre-clinical and clinical studies to date as possible therapeutics for autoimmune and inflammatory diseases in multiple organ systems; nervous, skeletal, integumentary and gastrointestinal sytems. CCR6 inhibitors are given above as a cluster and CCL20 inhibitors are given below as a cluster. Legend: MAPK – mitogen activated protein kinase, p- protein, CCL20 – CC chemokine ligand 20, mAbs – monoclonal antibodies, CCR6- CC chemokine receptor 6, hCCR6 – humanised CCR6, Abs- antibodies, IL- interleukin, BET – bromodomain extraterminal proteins, R- receptor, MIR-150 – micro ribonucleic acid - 150, AOP-RANTES – amino oxy pentane - regulated on activation, normal T cell expressed and secreted.

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Type of Inhibitor	**Name of Inhibitor**	**Nature of Inhibitor**	**Immune cells /cytokines Involved**	**Experimental outcome**	**Name of Disease/ Model**	**Ref**
Anti-hCCR6 mAbs		CCR6 Inhibitor	T_H17, B cells	Reduced inflammatory cell infiltration	Experimental Autoimmune Encephalitis (EAE)	12
Anti-CCR6 Abs	CO339589	CCR6 Inhibitor	NK cells PBMC	Lowered Disease	RA, Psoriasis, MS	20
Anti CCL20 Abs	WO2017011559A1	CCL20 Inhibitor	T_H17, Treg	Inhibits CSC activity, tumorigenesis	Carcinoma	9
	GSK3050002	CCL20 Inhibitor	T_H17	Reduced cell chemotaxis	Suction blister in skin	18
		CCL20 Inhibitor	γδ T cells	Reduced immune cell infiltration to skin	IMQ induced Psoriasiform Dermatitis	12
Anti-TNF-α Abs	Infliximab Tocilizumab	Indirect CCL20 Inhibitor	Synoviocytes	Inhibits TNF-α induced CCL20 upregulation	RA	10
Inhibitors of p38, 42, 44	MAPK Inhibitors	Indirect CCL20 Inhibitor	T_H17	Reduced pro-inflammatory cytokines, Suppressed CCL20	Ovarian Endometritis	21
Partial Agonists	AOP - RANTES	Partial CCR6 Inhibitor	T_H17	Reduced inflammatory cell chemotaxis	Psoriasiform Dermatitis	11
Bispecific Abs	Humanised IgG-like BsAb	CCR6 and CXCR3 Inhibitors	T_H17	Blocks cell chemotaxis	Murine model	12
BET Proteins		Gene regulator	T_H17, T_{reg}	Reduced inflammation	Posterior Uveitis	15

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Small Molecule Inhibitors	CCX9664	CCR6 Inhibitor	T_H17	Lowered Disease	Rheumatoid Arthritiis (RA)	19
Botanical Inhibitors	EGCG, Gallotannin	CCL20 inhibitor	T_H17	Inhibited cell chemotaxis	Rhesus Macaque model	13
Sphingosine 1 PO4 R agonist	FTY720		$CD4^+$T Cells	Inhibit egress of lymphocytes from LNs	Allergic Diarrhea	22
microRNA	MiR-150	CCR6 Inhibitor	CTCL cells	Reduced Metastasis	Cutaneous T cell lymphoma (CTCL)	25
Polyphenol compound	Resveratrol	Sirtuin - 1 activator	T_H17, Macrophage	Lower disease, inflammatory cytokines	Type-1 diabetes, RA, Lupus, EAE	23
CCR6R Peptide Mimetics	ECL-2	CCL20 Inhibitor	$CCR6^+$ cells	Reduced cell chemotaxis	Rhesus Macaque model	13

Table 1: Types of inhibitors used against the CCR6-CCL20 axis, their common names, immune cells involved, experimental outcome and diseases involved**.** Legend: MAPK – mitogen activated protein kinase, p- protein, CCL20 – CC chemokine ligand 20, mAbs – monoclonal antibodies, BsAb – bispecific antibodies CCR6- CC chemokine receptor 6, hCCR6 – humanised CCR6, Abs- antibodies, IL- interleukin, TNF-α – tumour necrosis factor – alpha, BET – bromodomain extraterminal proteins, PO_4 – phosphate, R- receptor, RNA – ribonucleic acid, AOP-RANTES – amino oxy pentane - regulated on activation, normal T cell expressed and secreted. NK – natural killer, PBMC- peripheral blood mononuclear cells, IMQ – Imiquimod, T_H17 – T helper subset 17, T_{reg} – regulatory T reg cell, γδ T cell – gamma delta T cell, EGCG-Epigallocatechin gallate, Ref – references to inhibitors

3.5 *Gastrointestinal system*

T helper cell (T_H) 2 cytokine driven experimental food allergy was induced in a mouse model to evaluate both $CCR6^{+/+}$ and $CCR6^{-/-}$ cohorts taking allergic diarrhea as a pre-clinical parameter. $CCR6^{-/-}$ mice were protected from ovalbumin-induced diarrhea with significantly reduced T_H2 cytokines although allergen-specific immunoglobulin E (Ig E) production was not impaired,

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indicating that CCL20 regulates mucosal homeostasis and inflammatory trafficking of lymphocytes to the small intestine. Inhibition of $CD4^{+}$T lymphocyte homing by treatment with FTY720, a sphingosine 1-phosphate 1 receptor agonist shown to inhibit the egress of lymphocytes from mesenteric lymph nodes (MLN) and Peyer's patches, did not impair allergic diarrhea in $CCR6^{+/+}$ mice suggesting reactivation of CCR6 expressing T cells occurring locally within the small intestine. The study concluded a mast cell and Ig E independent role for $CCR6^{+}$ T cells in the pathogenesis of allergic disease in the gastrointestinal tract [22].

Resveratrol (*trans* -3, 4', 5-trihydroxystilbene) is a compound of the polyphenol group found abundantly in vegetables and various fruits such as grapes, which has shown a discernible therapeutic potential in treating type I diabetes in a NOD mouse model. It is an activator of the sirtuin 1, an NAD –dependent sirtuin family deacetylase which is known to suppress T cell immune responses by deacetylating c-Jun, an activating protein-1 family transcription factor. Gene array analysis had indicated a marked decrease in the expression of *Ccr6* which encodes for CCR6 in mouse splenocytes treated with resveratrol that also displayed reduced CCR6 abundance. To validate the therapeutic efficacy of resveratrol, in diabetes, IL-17 –producing cells and $CD11b^{+}F4/80^{high}$ macrophages had accumulated in the spleen and pancreatic lymph nodes but not in the pancreas itself, suggesting that resveratrol had blocked CCR6 signalling, and prevented migration of CCR6-bearing immune cells from peripheral lymphoid organs to the pancreas. Resveratrol is also known to prevent inflammation in mice via suppression of nuclear factor kappa B (NF-kB) pathway to lower inflammatory cytokine release and induce apoptosis. Further, resveratrol produces inhibitory effects on mitogen activated protein kinase (MAPK), plasma creatine kinase, Src family tyrosine kinase and phosphoinositide-3-kinase (PI3K) and has been used as medication in other autoimmune diseases - lupus, rheumatoid arthritis and EAE [23].

4.0 Carcinoma related studies

A recently submitted application to patent an anti- cancer therapeutic intervention was based on cancers comprising cancer stem cells (CSCs) that express CCL20 and/or CCR6 and includes a number of cancers, such as pancreatic cancer, colorectal cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancer, and breast cancer. Inventors of this novel compound had discovered overexpression of CCL20 in CSCs and targeted their

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invention to inhibit CCL20 activity in cancers exhibiting cells having a CSC phenotype. Furthermore, the same group of researchers introduced a dual anti-cancer mechanism by modulating CCR6/CCL20 axis in a tumour environment, stating therapeutic neutralization of CCL20, (i) directly inhibits CSC activity (ii) their tumorigenic ability and (iii) relieve immunosuppression. They found CCL20 secreted by tumour cells to strongly influence the frequencies of immune-suppressive regulatory T_{reg} cells and T_H17 populations, thereby supporting the fact that inhibition of CCR6/CCL20 duo presents a novel therapeutic strategy in cancer treatment [9].

As already evident, CCR6 and CCL20 have a role in the metastasis of advanced cutaneous T cell Lymphoma (CTCL), demonstrating that CCR6 activation innervates the STAT3 pathway mediating the transcription of CCL20, which leads to CTCL lymphomagenesis. Nutrient-dependent *In vitro* migration of CTCL cells were downregulated with transient knockdown of STAT3, CCL20, CCR6 and administering anti CCL20 antibody. In examining the *in vivo* effect of neutralizing CCL20 antibody in Xenografted SCID mice inoculated with CTCL cells, the group noted significantly prolonged survival where normally, they were expected to die with CTCL metastasis into multiple organs [24].

A second study on cutaneous T cell lymphoma documents inhibition of CCR6 by micro RNA -150 (MiR-150) leading to strong downregulation of tumour metastasis. MicroRNAs (miRNA) are a class of small regulatory RNA molecules which pair with target messenger RNAs to repress their productive translation and whose presence is significantly low in advanced CTCL cells. Altered expression of micro RNA results in haematologic malignancies (lymphomas/leukemias) and tumorigenesis. MiR 150 was found to downregulate CCR6 which in turn reduced metastasis in an immune deficient mouse model while CTCL cells displayed upregulation of IL-22 linked to increased stimulation of CCL20 and its binding to CCR6, thereby enhancing their multi-directional migration potential [25].

Chemokine/chemokine receptor mediated activation of ERK (extracellular signal-regulated kinase) signalling cascade (Akt, ERK1/2, stress-activated protein kinase /c Jun N terminal kinase MAPKs) has been identified to play a crucial role in cancer cell proliferation and invasiveness. In a clinical study of lung adenocarcinoma, colony formation capacity, ERK signalling and chemokine production were assessed and the colony forming capacity of cells taken from patients was shown to increase by CCL20 stimulation and depended on part on

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the ERK phosphorylation pathway. Carcinoma-associated lung fibroblasts communicate locally derived signals by interacting with chemokines and their receptors resulting in carcinogenesis. Hence, the CCR6/CCL20 axis was found to be engaged in proliferation and migration of cancer cells via autocrine or paracrine mechanisms and they had suggested disruption of the functions of this chemokine duo may offer a promising strategy to treat cancers in the lung [26].

5.0 Conclusion

This review has summarized the role of different CCR6-CCL20 inhibitors investigated to date for their potency to modulate immune activation and therapeutic mechanisms in a number of inflammatory diseases and cancers. There is a dearth of deeper knowledge of the exact mechanisms of the CCR6-CCL20 axis. Extensive studies into immune and therapeutic functions of the CCR6-CCL20 axis with the use of novel inhibitors need to take momentum as there is a profound gap in immunological research into treatment options of autoimmune and inflammatory disorders. New interventions with synthetic cellular, molecular or nanoparticle inhibitors and derivatives of herbals and anti-toxic metabolites could be utilized to mimic or block the numerous biochemical pathways in which CCR6-CCL20 axis is involved in disease promotion. Given the austere immune regulation inducted by CCR6 and CCL20 partners in skewing the T_H17 and T_{reg} populations tilting immune homeostasis into inflammation, even invention of a vaccine may become possible to boost T and B memory lymphocyte subsets of choice coupled with selective inhibition of these two chemokines. Comprehensive randomized, placebo-controlled, clinical trials on the therapeutic use of the CCR6-CCL20 could become useful to thoroughly evaluate the benefits and disadvantages of the inhibitors of this axis in humans.

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7.0 Conflict of Interests

Authors declare no conflict of interests, commercial or financial in writing this article.

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9.00 Abbreviations

Abs	-	antibodies
Akt	-	protein kinase B
AOP	-	amino oxy pentane
B	-	bursa-derived lymphocyte
BsAb	-	bispecific antibodies
β-arrestin	-	beta arrestin
β defensin	-	beta defensin
BET	-	bromodomain extraterminal proteins
$CD4^+$ T	-	cluster of differentiation 4 positive thymocyte
CCR6	-	CC chemokine receptor 6
CCL20	-	CC chemokine ligand 20
CCL5	-	chemokine ligand 5
Ccr6	-	gene for CCR6
$CCR6^{-/-}$	-	CCR6 deficient
CNS	-	central nervous system
CSC	-	cancer stem cell
CTCL	-	cutaneous T cell lymphoma
DC	-	dendritic cell

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DNA	-	deoxyribonucleic acid
EAC	-	experimental allergic conjunctivitis
EAE	-	experimental autoimmune encephalitis
ECL	-	extracellular loop
EGCG	-	epigallocatechin gallate
ERK	-	extracellular signal-regulated kinases
FoxP3	-	forkhead box P3
γδ T cell	-	gamma delta T cell
GPCR	-	guanine/guanosine protein coupled receptor
GM-CSF	-	granulocyte macrophage colony stimulating factor
hCCR6	-	humanized chemokine receptor 6
IBD	-	inflammatory bowel disease
IFN-γ	-	gamma interferon
IgG	-	immunoglobulin G
IL	-	interleukin
IL-1β	-	interleukin one beta
IL-6R	-	interleukin –six receptor
IMQ	-	imiquimod
JNK	-	jun kinase
kDa	-	kilo dalton
KO	-	knockout
LARC	-	liver and activation regulated chemokine
m	-	mouse
mAbs	-	monoclonal antibodies
MAPK	-	mitogen activated protein kinase
MIP-3α	-	macrophage inflammatory protein – 3 alpha

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MiR	-	micro RNA
MOG	-	myelin oligodendrocyte glycoprotein
NAD	-	nicotinamide adenine dinucleotide
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
RA	-	rheumatoid arthritis
RANTES	-	regulated on activation, normal T cell expressed and secreted
RNA	-	ribonucleic acid
RORϒt	-	retinoic-acid-receptor-related orphan nuclear receptor gamma
SCID	-	severe combined immune deficiency
siRNA	-	small interfering ribonucleic acid
STAT3	-	signal transducer and activator of transcription 3
T	-	thymus-derived lymphocyte/ thymocyte
Tg	-	transgenic
T_H1	-	T helper 1
T_H2	-	T helper 2
T_H17	-	thymocyte helper 17
T_H22	-	T helper 22
TNF-α	-	tumour necrosis factor- alpha
T_{reg}	-	regulatory thymocyte cell

WT	-	wild type