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Review

CCL28 chemokine: An anchoring point bridging innate and adaptive immunity



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ARTICLE INFO

Keywords:

Chemokines
Mucosal responses
Adjuvants
Antimicrobial proteins
Innate immunity

ABSTRACT

Chemokines are an extensive family of small proteins which, in conjunction with their receptors, guide the chemotactic activity of various immune cells throughout the body. CCL28, β - or CC chemokine, is involved in the host immunity at various epithelial and mucosal linings. The unique roles of CCL28 in several facets of immune responses have attracted considerable attention and may represent a promising approach to combat various infections. CCL28 displays a broad spectrum of antimicrobial activity against gram-negative and gram-positive bacteria, as well as fungi. Here, we will summarize various research findings regarding the antimicrobial activity of CCL28 and the relevant mechanisms behind it. We will explore how the structure of CCL28 is involved with this activity and how this function may have evolved. CCL28 displays strong homing capabilities for B and T cells at several mucosal and epithelial sites, and orchestrates the trafficking and functioning of lymphocytes. The chemotactic and immunomodulatory features of CCL28 through the interactions with its chemokine receptors, CCR10 and CCR3, will also be discussed in detail. Thus, in this review, we emphasize the dual properties of CCL28 and suggest its role as an anchoring point bridging the innate and adaptive immunity.

1. Chemokines - An introduction

Chemokines are a family of chemoattractant cytokines with a molecular weight typically around 8 to 10 KD. Chemokines play a vital role in cell migration in response to a chemical gradient by a process known as chemotaxis. Chemokines are an integral part of host immunity and play important roles in many critical functions involved in regulating innate and adaptive immune responses. Chemokines modulate lymphocyte development, activation and effector functions, and play a vital role in immune surveillance. Historically, chemokines have been known by various names including the SIS family, SIG family, or SCY family of cytokines, the intercrines, and the platelet factor-4 superfamily. Chemokines are present in all vertebrate animals but none has yet been identified in non-vertebrates [1]. The action of a chemokine is mediated through chemokine receptors which are members of the G protein-coupled receptor (GPCR) family. These chemokine receptors are transmembrane receptors that are coupled to intracellular G-protein, which can stimulate signal transduction pathways inside the cell when they are activated [1,2]. Chemokines share structural similarity and usually contain four cysteine residues, which gives them their three-dimensional shape. Chemokines can be subdivided into four families as per the relative positions of their cysteine residues: CXC (α -chemokine), CC (β -chemokine), C (γ -chemokine), and CX3C (δ -chemokine).

Different chemokine families share 20–70% homology in their amino acid sequences. The CC and CXC chemokine families form the two largest groups. There are forty-seven known chemokines that bind to nineteen chemokine receptors with high degrees of affinity. Numerous chemokine receptors are highly promiscuous in their chemokine selectivity, and *vice versa*, numerous chemokines bind to more than one receptor [3,4].

The innate immune system includes cellular components as well as a host of soluble products such as antimicrobial peptides, complement fragments, cytokines, and chemokines. The adaptive immune response, which provides long-lasting protection, takes days to develop antigen-specific T cell receptors (cell-mediated immunity) and immunoglobulins (humoral immunity) [5,6]. Chemokines play an important role in both innate and adaptive immunity by inducing various immunological cascades. Recently, chemokines have been recognized to be involved in many other patho-physiological processes, e.g. cell proliferation, tumor immune escape, maintenance of homeostasis in the immune system, lymphocyte differentiation, chronic and acute inflammation, proliferation of hematopoietic progenitor cells, allergic responses, tumor growth, and modulation of angiogenesis [7]. These functions reflect chemokines' involvement in many immune processes through their antimicrobial, chemotactic, and immunomodulatory properties.

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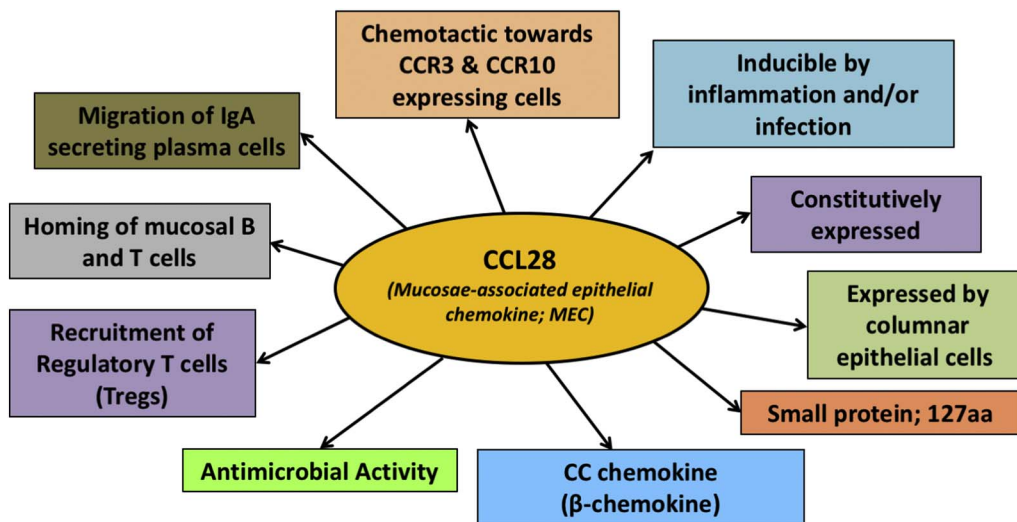


Fig. 1. Top 10 properties of CCL28.

2. CCL28

CCL28 (Mucosae-associated epithelial chemokine; MEC), is a recently described CC chemokine (β -chemokine) signaling via CCR10 and CCR3 [8,9]. CCR10 was originally identified as the receptor for CCL27 which is also called IL-11R α -locus chemokine or cutaneous T cell attracting chemokine. CCR3 is the receptor for eotaxin/CCL11 and many other chemokines known to act on eosinophils [10,11]. CCL28 is constitutively expressed in a wide variety of tissues and inducible through inflammation and infections [12,13]. CCL28 is expressed by columnar epithelial cells in the gut, lung, breast and the salivary glands. It drives the mucosal homing of T and B lymphocytes that express CCR10 and the migration of eosinophils expressing CCR3 [14–16]. This chemokine is constitutively expressed in the colon, but its levels can be increased by pro-inflammatory cytokines and certain bacterial products implying a role in effector cell recruitment to sites of epithelial injury [17]. CCL28 is constitutively expressed and has also been implicated in the migration of IgA expressing cells to the mammary gland, salivary gland, intestine and other mucosal tissues [12,18,19]. We have mentioned top 10 key features of CCL28 including their physical, expression and functional properties in Fig. 1. In this review, we will discuss in detail the antimicrobial and immunomodulatory features of CCL28.

3. Antimicrobial peptides/proteins (AMPs)

AMPs are important weapons utilized by the host defense against microbial infection and function as the key components of the innate immune system [20]. These peptides/proteins have been identified in organisms as diverse as non-vertebrates, humans, and plants [21,22]. Almost all living organisms, ranging from vertebrates to bacteria, utilize AMPs to defend against microbial invasion. A wide variety of antimicrobial proteins have been identified such as histatins, defensins, and more recently certain chemokines including CCL28 [23].

4. CCL28 and its antimicrobial activity

The first report describing chemokines with antimicrobial function appeared in 2000 [24]. Researchers identified two antibacterial proteins in platelet granules and showed them to be variants of CXCL7 and CTAP-3. Some other chemokines such as CXCL9, CXCL10, and CXCL11 were also found to be potent AMPs against *E. coli* and *L. monocytogenes* [25,26]. Various studies have shown that most chemokines exhibit direct antimicrobial activity against gram-positive and gram-negative bacteria, fungi, and enveloped viruses [24,27–29]. The antimicrobial activity of CCL28 was initially identified due to the amino acid

sequence similarity between the C-terminal region of CCL28 and histatin-5, a histidine-rich candidacidal peptide in human saliva [30]. CCL28 has been shown to exhibit broad spectrum antimicrobial activity against gram-positive bacteria (e.g. *Streptococcus mutans* and *Staphylococcus aureus*), gram-negative bacteria (e.g. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), and some fungi (e.g. *Candida albicans*) [17]. High expression levels of CCL28 in epithelium and mucosal secretions such as milk and saliva may provide a constitutive innate immune defense against a variety of bacterial pathogens and strongly suggest its involvement in antimicrobial activity [13].

5. Underlying mechanism of CCL28 antimicrobial activity

Due to CCL28's homology with histatin-5, it was originally expected that the mechanism of its antimicrobial activity would be similar to that of histatin-5, which selectively binds to the mitochondrial membrane after internalization into candida cells [30]. However, in a published study, researchers demonstrated this was unlikely because of the following observations: 1) histatin-5, is mainly effective against candida species while CCL28 shows a much broader spectrum of antimicrobial activity [31], 2) histatin-5 showed a relatively slow antimicrobial effect compared to CCL28 [32], and 3) FITC-labeled CCL28 mainly stained the cell surface of *C. albicans*, in contrast with the reported association of histatin-5 to mitochondria membrane within yeast cells [33]. Apparently, CCL28 exerts its antimicrobial activity by spontaneous membrane insertion and pore formation in target microbes [17], but more detailed study is needed to explain the same.

Recent reports demonstrate the contribution of the amino acid sequences in the antimicrobial mechanism of CCL28. The antimicrobial mechanism for other conventional cationic AMPs has been proposed to be charge-mediated, in which the positively charged AMPs interact with the negatively charged microbial membranes [34]. The chemokines CCL28 and CCL27 share 31% identity at the amino acid level and both mediate the migration of lymphocytes via interactions with the chemokine receptor CCR10 [10]. In contrast to CCL28, CCL27 exhibits no antimicrobial activity [17,26]. Interestingly, these two proteins share high homology at the N-terminus and low homology at the C-terminus. N-termini of chemokines have been implicated in mediating lymphocyte migration through chemokine receptor binding. It has been shown that the positively charged amino acids at the C-terminus of CCL28 significantly contribute to the antimicrobial activity of the protein [35]. Simultaneously, structure-activity analysis also supports that the CCL28 likely uses a charge based killing phenomenon for its antimicrobial activity. This conclusion was based on several independent experiments: 1) According to confocal fluorescent microscopic

observation, CCL28 binds to *S. typhimurium* phoP bacteria, but the highly similar, non-antimicrobial, chemokine CCL27 does not bind to it. This might be due to lack of the highly positively charged extended C-terminus, present in CCL28 sequence but absent from CCL27 [35]. 2) Mutant bacterial strains, such as phoP *S. typhimurium* and *Y. pestis* strains, with high negative charges on their membranes, are more sensitive than their wild type parent strains to CCL28-mediated cell death. 3) CCL28 binds and kills phoP *S. typhimurium* more efficiently in low salt concentrations than in high salt conditions. These findings support the conclusion that CCL28 shares many similarities with other, better studied and established AMPs.

Simultaneously, it has been suggested that other than its cationicity, the characteristic hydrophobicity and amphipathicity of CCL28 also contribute to its killing activity. However, the amino acid characteristics necessary for the antimicrobial activity have not yet been elucidated. Researchers also determined that the primary sequence features required for antimicrobial activity of CCL28. Using successive truncations, results illustrated that the holoprotein (108 amino acids) is necessary for activating the full antimicrobial activity of CCL28. Consecutive truncations resulted in an almost complete loss of activity following the removal of the 24C-terminal amino acids. Charge reversal as well as positive charge to neutral charge mutations, support the theory that the positively charged amino acids of the C-terminus contribute to the antimicrobial activity of CCL28 [35]. In determining which regions of the CCL28 protein are essential to antimicrobial activity, one of the findings strongly supports a vital role for amino acids 85–89 of CCL28. Results showed that the deletion of this region resulted in a dramatic reduction in the antimicrobial activity of CCL28. Positively charged amino acids in the first two positions of this sequence (K or R) is a trait strongly conserved in species ranging from rodents to primates and ruminants. In addition to this single indispensable region, charge neutralization and reversal experiments on C-terminal amino acid of murine CCL28 suggest that other positively charged amino acids at C-terminus contribute to the antimicrobial activity of the protein [36].

6. CCL28 protein structure and its antimicrobial activity

The characteristic positively charged C-terminal amino acids in CCL28 are broadly conserved across species, suggesting that evolutionary pressures have continued to select for a positively charged antimicrobial C-terminal region since the divergence of humans and mice. Furthermore, studies have investigated the involvement of disulphide bridges within CCL28 in its antimicrobial activity. Antimicrobial chemokines are generally larger and more structurally complex than other AMPs. CC chemokines have a conserved structure throughout the N-terminus, with three anti-parallel β -sheets followed by a C-terminal α -helix. The α -helix of CC chemokines has been postulated to serve as a structural scaffold, and this region has been switched between chemokines without loss of chemotactic specificity [17]. The N-terminus of CC chemokines and the accompanying structural complexity has been demonstrated to be important for chemokine receptor binding and for the activation of leukocytes. Replacement of the canonical, consecutive, cysteines is predicted to destroy disulphide bonding within the chemokine, which in turn should significantly alter the tertiary structure of the protein. Mutating these cysteines in other chemokines has resulted in a loss of receptor binding and cellular activation. In these experiments, the antimicrobial activity of CCL28 was not influenced by any changes in the N-terminus sequences. These results suggest that if the tertiary structure of CCL28 is important in mediating its antimicrobial activity, this structure is formed independently of the disulphide bonding known to be vital for CCL28 receptor binding [37].

7. Evolutionary analysis of CCL28

In a recent study, a phylogenetic tree from the amino acid sequences

of chemokines and AMPs was generated using parallel prrp and phyplp programs [38]. CCL28 and CCL27 are closely related due to having sequence similarity. As such, the two chemokines share the receptor CCR10 and this suggests that the origin of these two genes was a gene duplication event followed by differentiation. Thus, these two chemokines may represent the most primordial CC chemokines in mammals. It should also be noted that the extended C-terminal domain of CCL28, which aligns side by side with histatin-5, is not encoded by a separate exon in the CCL28 gene [17]. Thus, CCL28 is not a chimeric protein generated through a new combination of exons during evolution. Therefore, one possibility is that the chemokine family and the AMPs have diverged from a common primordial molecule. CCL28 still retains its antimicrobial activities and functions as such. However, it is rather striking that CCL27, the chemokine most like CCL28, hardly displays any significant antimicrobial activity. Thus, another possibility is that the antimicrobial activity of CCL28, as well as its histatin-like C terminus, has been fortuitously generated through the convergent evolution of a chemokine to become an antimicrobial protein [39]. In either case, the functional and evolutionary relationships between chemokines and AMPs in innate and adaptive immunity will be an interesting subject in future studies.

8. Adjuvants/immunostimulators

Adjuvants are molecules or compounds that can enhance immune responses against co-administered antigens. One of the primary functions of an adjuvant is enhancing the immunogenicity of a vaccine by stimulating and eliciting a natural immune response and improving antigen processing, presentation, and recognition. Adjuvants can also improve immune responses in populations where responses to vaccines are typically reduced, such as infants, the elderly, and immunocompromised patients.

9. Role of CCL28 in the regulation of immune responses

The highest expression levels of CCL28 can be found in the salivary glands, and deregulated levels of CCL28 have correlations with salivary gland tumors, Hodgkin's disease, and Sjögren's syndrome [40–42]. CCL28 can be found in nearly all mucosal tissues, including in the mucosal tissues of the mammary gland. Mammary glands only express CCL28 at the onset of lactation, and this expression of CCL28 in mammary glands parallels the migration of IgA antibody secreting cells (ASCs) into these glands [19]. Additionally, this migration of IgA ASCs can be prevented by the use of anti-CCL28 antibodies [19], demonstrating that CCL28 regulates the migration of IgA ASCs into mammary glands. CCR3 is also a potential receptor for CCL28, however it is not the primary receptor utilized in healthy conditions [9,43]. Studies have begun to suggest that CCR3 might interact with CCL28 more frequently during immunologically stressful conditions. One such study showed that CCL28 levels are increased in patients with atopic asthma and this results in the enhanced accumulation of IgE secreting plasma cells, which express CCR3 [9,12,44]. CCL28 interacts with the chemokine receptor CCR10, and this receptor is expressed in both mucosal and epithelial tissues with the goal of recruiting and localizing IgA ASCs and T lymphocytes to mucosal areas [12,18,45,46]. It comes as no surprise that CCL28 and CCR10 are important in the regulation of mucosal immune responses and immune cell recruitment to the mucosa. High levels of expression of CCR10 and CCL28 in gastrointestinal and bronchial sites have been shown to play important roles in controlling the recruitment and homing of immune cells to these locations upon pathogen exposure [9,47–50]. CCR10 responds to signals from CCL28 and aids in the localization of IgA secreting immune cells to mucosal sites [9,12]. CCR10 can also be found expressed on memory T cells expressing the CD4 + /CLA + marker. This marker is expressed in skin-homing lymphocytes in response to inflammation in cutaneous sites and the oral mucosa [49]. A study performed utilizing a mouse allergic

rinitis model showed increased expression levels of CCL28 along with enhanced homing of CD4 + T cells expressing CCR10 or CCR3 in response to antigen challenge [51]. This finding suggests that the interaction between CCR10/CCR3 and CCL28 are involved in the trafficking and recruitment of CD4 + T cells into nasal mucosal tissues [51]. It was discovered that CCL28, which is expressed on the surface of epithelial barriers, has its expression level upregulated in the nasal epithelia in response to antigen challenges [8,9]. Pro-inflammatory cytokines are responsible for inducing CCL28 expression in airway epithelial cells [52]. Additionally, CCL28 expression levels are significantly increased in the lung tissues of mice during airway inflammation in response to allergen challenges [14,53]. CCL28 expression levels have also been found to be increased in response to other inflammatory responses, including to rheumatoid arthritis and *Helicobacter pylori* induced gastritis [54,55]. The expression of CCR10 was enhanced during airway inflammation due to allergen induced asthma in mice [53]. Another study reported that the expression of CCL28 by epithelial cells in response to microbial products or IL-1 provides a signal to localize subsets of T lymphocytes expressing CCR10, which include CD4 + CD25 + Foxp3 + T cells or T regulatory cells (Tregs) at mucosal surfaces. CCL28 may provide a critical signal for positioning Tregs at inflamed epithelial surfaces and CCR10 expression defines a subset of Tregs that home to epithelial sites [56,57]. Thus, these studies support that the expression of CCL28 and its co-receptors is induced by pro-inflammatory cytokines and/or infections and play crucial roles in generating host immunity against pathogens and limiting auto-immunity and inflammation at mucosal sites.

10. CCL28 as a unifying immunostimulator at mucosal surfaces

CCL28 shows a high level of homology with another chemokine, CCL27 [8,9]. CCL27 is very selectively expressed in the skin, and has been shown to attract cutaneous lymphocyte antigen specific memory T cells via CCR10. On the other hand, CCL28 has been shown to selectively attract lymphocyte subsets, including IgA ASCs, and are involved in the mucosal homing of B and T cells. This chemo-attractive effect is achieved by the binding of CCL28 to the CCR3 or CCR10 chemokine receptors. Simultaneously, studies have revealed that CCL28 is involved in the robust recruitment of relevant immune cells involved in antigen recognition, immune priming, and pathogen clearance [49,58]. Seeking to increase the effectiveness of a vaccine with enhanced cross-protection, we recently investigated the effects of GPI-anchored CCL28 as an adjuvant with influenza virus-like particles (VLPs) in mice. GPI-anchored CCL28 were co-incorporated with influenza HA antigen on the same VLPs and compared with influenza HA VLPs (without GPI-CCL28) and a physical mixture of separate influenza HA and GPI-CCL28 VLPs. We observed that GPI-anchored CCL28 in influenza VLPs act as a strong immunostimulator at both systemic and mucosal sites, and boost a significant cross-protection in animals against heterologous viruses across a large distance [59]. Because of its specific role in orchestrating the localization of IgA ASCs at mucosal sites [19], we also analyzed the long-lasting mucosal adjuvanticity of GPI-anchored CCL28 co-incorporated into influenza VLPs. Data suggest that the GPI-anchored CCL28 induced significantly higher mucosal antibody responses involved in providing long-term cross-protection against H3N2 influenza virus when compared to other vaccination groups [60]. GPI-anchored CCL28 could be used to develop vaccines against other viruses such as: the human immunodeficiency virus-1 (HIV-1), simian immunodeficiency virus (SIV), Ebola virus, severe acute respiratory syndrome (SARS), coronavirus, and Rift Valley Fever virus (RVFV). Results support that GPI-anchored CCL28 could be used as an adjuvant with many forms of antigens; soluble, physically mixed, or co-incorporated. In a similar study, the intramuscular co-delivery of plasmids expressing CCL27 or CCL28 with a construct encoding influenza A/PR8/34 HA elicited antigen-specific humoral and T cell immune responses in the periphery as well as in the lung and exhibited improved protection from

morbidity and mortality compared with non-adjuvant vaccinated animals [61].

CCL28's unique role of organ specific homing of immune cells in mucosal immunity has been well established [12,15,62]. This could potentially be explained by exclusive sets of chemokine receptors [63]. IgA plasma cells tend to localize in an organ specific manner because cells generated in the small intestine and colon home to those locations respectively, while those cells generated in response to nasal immunizations home into nasopharyngeal and other respiratory mucosal tissues [64–66]. It has been demonstrated that CCL28 is widely expressed by both intestinal and non-intestinal mucosal tissues including salivary glands, and its receptor CCR10 can be found expressed on nearly all IgA ASCs [8,9,17,46]. It was established that CCL28 can recruit IgA ASCs in both intestinal and non-intestinal mucosal tissue [12,15] and support organ specific ASC stimulation [67]. In some of the findings, enhanced expression of CCL28 in uterus and salivary glands was involved in attracting CCR10 + IgG plasma cells following mucosal vaccination and controlling cariogenic microbe, respectively [68,69]. Expression levels of CCR10 in intestinal T cells is almost non-existent, as only CLA + memory and/or effector T cells express CCR10, and these cells traffic preferentially to the epidermal keratinocytes in the skin [15,49,70]. It was recently reported that there is a positive correlation between titers of mucosal anti-HIV-1 IgA and the CCL28–CCR3/CCR10 system both in HIV-1 infected and HIV-1-exposed but sero-negative (HESN) individuals [71]. CCL28 is involved in attracting HIV-1-specific IgA secreting plasma cells in mice immunized with HIV-1 Env VLPs at various mucosal compartments [72] and it also augments antigen-specific immunity by mobilizing responsive immunocytes [73]. In a separate report, rhesus macaques vaccinated with SIV DNA and CCR9L (CCL25) or CCR10L (CCL28/CCL27) adjuvants showed significant protection from multiple low-dose intravaginal challenges with SIVsmE660 [74]. As CCL28 attracts IgA producing cells and promotes their migration to different mucosal sites, it was demonstrated that the use of CCL28 as an adjuvant has unique immunity modulating properties at various mucosal compartments [12,19]. Taken together, these data suggest that CCL28 is a vital component in the mucosal homing of IgA ASCs, which play a critical role in establishing mucosal immunity against pathogens. The significance of CCL28 in the directing of immune responses during infection/diseases is mentioned in the Table 1.

11. CCL28 bridging innate and adaptive immunity

In this review, we emphasized the dual roles of CCL28, i.e. antimicrobial and immunomodulatory properties. CCL28 was originally discovered through its ability to recruit various immune cells into different mucosal and inflammation sites [8,9]. High expression levels of CCL28 in epithelium and mucosal secretions such as milk and saliva may provide a constitutive innate immune defense against a variety of bacterial pathogens and strengthen its involvement in antimicrobial activity [13]. It is now clear that these molecules play a much wider role in antimicrobial activity, immune homeostasis, playing key roles in driving the maturation, homing, and activation of leukocytes [68,70]. First, the antimicrobial activity of CCL28 demonstrates its first line of defense [17] and second, the recruitment and homing of the B and T cells promote adaptive immune responses [67,71,72]. CCL28 and CCR3/CCR10 interaction orchestrates signaling to drive these processes or attract various immune cells present in the local neighborhood [67,69]. Recently, the recruitment of Tregs by CCL28 also demonstrates its role in the modulation of the immune system, maintaining tolerance to self-antigens, and prevention of the autoimmune diseases [56,57]. Taken together, we highlight the various points at which CCL28 contribute to both innate and adaptive immune responses and provide a connecting link between them.

Table 1
Significance of CCL28 in the regulation of immune responses.

Diseases/infections	Description of the study	References
Influenza	<ul style="list-style-type: none"> Co-delivery of plasmids expressing CCL27 or CCL28 with influenza PR8 HA elicited antigen-specific humoral and T cell immune responses. GPI-anchored CCL28 worked as a strong immunostimulator with influenza VLPs to exhibit improved protection. GPI-anchored CCL28 in influenza VLPs promoted long-term mucosal protective immunity. 	[59–61]
HIV/AIDS	<ul style="list-style-type: none"> CCL28 with HIV-1IIIIB VLPs enhanced the migration of IgA ASCs to mucosal sites in a mouse immunization model. CCL28-including constructs prevented HIV infection of the gastro-intestinal mucosal lamina propria (MLP) via modulation of IgA ASC. CCL28 augmented HIV gp140-specific immunity by mobilizing responsive immunocytes. Rhesus macaques vaccinated with SIV DNA and CCR9L (CCL25 chemokine) or CCR10L (CCL28/CCL27 chemokines) adjuvants showed significant protection from multiple low-dose intravaginal challenges with SIVsmE660. 	[71–74]
Cancers	<ul style="list-style-type: none"> Enhanced expression of CCL28 in breast tissues correlated with breast cancer carcinogenesis and involvement. CCL28 encouraged the recruitment of Tregs which promoted tumor tolerance and angiogenesis. CCL28 enhanced breast cancer growth and metastasis through MAPK-mediated cellular anti-apoptosis and pro-metastasis. Reduced expression and secretion of CCL28 associated with the pathogenesis of pleomorphic adenoma and adenolymphoma. 	[42,75–77]
Atopic asthma	<ul style="list-style-type: none"> CCL28 enhanced the accumulation of IgE secreting plasma cells in patients with atopic asthma. 	[53]
Allergic rhinitis	<ul style="list-style-type: none"> A mouse allergic rhinitis model showed increased expression of CCL28 along with enhanced homing of CD4 + T cells expressing CCR10 or CCR3 in response to antigen challenge. 	[51]
<i>Helicobacter pylori</i>	<ul style="list-style-type: none"> CCL28 recruited gastric IgA secreting cells in human <i>Helicobacter pylori</i>-induced gastritis. 	[55]
Rheumatoid arthritis (RA)	<ul style="list-style-type: none"> Overexpression of CCL28 and CCR10 in RA synovial tissues played a pivotal role in the migration of endothelial progenitor cells into RA joints. 	[54]
Hodgkin's disease (HD)	<ul style="list-style-type: none"> Production of CCL28 by Hodgkin and Reed-Sternberg (H-RS) cells helped in tissue accumulation of eosinophils and/or plasma cells in classical HD. 	[40]

12. Future prospective

While CCL28 continues to demonstrate an ever-increasing role in mucosal immunity, several questions remain surrounding this fascinating chemokine. One such question is the mechanistic interactions between CCR3/CCR10 and CCL28. CCL28 prefers CCR10 in healthy, standard environments, but its interactions with CCR3 increase significantly during pathogenic assault. These controlled and nuanced interplays still are not fully understood. Additionally, a significant amount of current research is devoting time to investigating the therapeutic aspects of CCL28. One potential avenue is looking at the controlled-up regulation of CCL28/CCR10 and its effects. Alternative approaches are examining how CCL28 can be used to bolster mucosal immune responses when administered directly or as part of a vaccine regimen. In closing, further study into the CCL28 will yield significant breakthroughs in our understanding of host immunity.

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