



Chemokine neutralization as an innovative therapeutic strategy for atopic dermatitis

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Atopic dermatitis is a chronic inflammatory skin disease with no specific treatment, affecting 10–20% of children and 1–3% of adults in developed countries. Current therapies alleviate symptoms and include emollients, glucocorticoids and calcineurin inhibitors. The limited efficiency and side-effects of these medicines call for better treatment, and a cure for atopic dermatitis represents an unmet medical need. The chemokine/chemokine-receptor network constitutes an attractive target for drugs in atopic dermatitis. However, the highly intricate nature of the chemokine network makes it difficult to identify a clear receptor to target. After a short description of atopic dermatitis and its current therapies, this review presents the current knowledge of the chemokine/chemokine-receptor system role in the regulation of inflammatory cell recruitment into the skin. We discuss the limitations of classical chemokine receptor blockade and introduce the concept of neutralization of chemokine ligands. This strategy represents a potential breakthrough in developing therapeutic agents to treat atopic diseases.

Introduction

^{Q3} The skin provides a physical and immune defense against allergens, bacteria and viruses. Tissue injury results in mediator release and the recruitment of cells to the skin, leading to the development of four cardinal signs of inflammation: redness, heat, swelling and pain. In a balanced situation, redness and swelling will subside after the invaders are eliminated. When this balance is lost, the uncontrolled physiological process drives chronic inflammation. This occurs through failure to resolve acute inflammation or through persistence of inflammatory stimuli [1]. Here, we focus on the chemokine network that is known to recruit immune cells in skin inflammatory diseases such as atopic dermatitis (AD). We discuss current therapies compared with innovative approaches related to chemokine neutralization, a convincing strategy to treat inflammatory disease.

Atopic dermatitis

AD is a chronic inflammatory skin disease with an increasing prevalence (15–30% in children and 2–10% in adults) in industrialized countries. It is characterized by typical features including pruritus, dry skin as well as red, crusted and relapsing skin lesions [2]. Owing to the absence of specific laboratory markers, the diagnosis of this disease is made on clinical observations, and is based on Hanifin and Rajka criteria (Table 1). ^{Q4}

It is now recognized that AD pathophysiology results from the aberrant interaction between environmental factors, a skin barrier dysfunction, a genetic predisposition and an immune system deregulation [2]. For instance, pollens, house dust mite (HDM) and irritants are important triggers for this pathology. In addition, genetic studies have identified different chromosomal regions associated with AD. There has been a particular focus on mutations of the *FLG* gene encoding filaggrin protein (essential for skin-barrier formation).

AD is generally associated to other allergic conditions such as asthma and allergic rhinitis [3] that are characterized by a T helper

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TABLE 1

Diagnosis features of atopic dermatitis based on Hanifin and Rajka criteria

Major criteria	Minor criteria
• Pruritus • Typical distribution and morphology: flexural lichenification in older children, adolescents and adults, face, neck and extensor involvement in infants and children • Chronic or relapsing course • Personal or family history of atopic diseases (asthma, allergic rhinitis, AD)	• Xerosis • Ichthyosis, palmar hyperlinearity or keratosis pilaris • Immediate (type 1) skin-test reactivity • Raised serum IgE concentrations • Early age of onset • Tendency toward cutaneous infections (especially <i>Staphylococcus aureus</i> and herpes simplex) • Tendency toward nonspecific hand or foot dermatitis • Nipple eczema • Cheilitis • Recurrent conjunctivitis • Dennie–Morgan infraorbital fold • Keratoconus • Anterior subcapsular cataracts • Orbital darkening • Facial erythema • Pityriasis alba • Anterior neck folds • Itch when sweating • Intolerance to wool and lipid solvents • Perifollicular accentuation • Food intolerance • Course influenced by environmental or emotional factors • White dermographism or delayed blanch

2 (Th2) cell recruitment, hyper-eosinophilia and elevated levels of immunoglobulin E (IgE). The mechanisms by which these reactions occur are complex, but usually start with a barrier permeability dysfunction. Indeed, a disrupted skin membrane permits allergens and microbes to cross the epidermis and trigger a cascade of inflammatory reactions. Epidermal keratinocytes release tumor necrosis factor (TNF)- α and other proinflammatory cytokines like thymic stromal lymphopoietin (TSLP), which activates dendritic cells (DCs) such as Langerhans cells (LCs) present in the epidermis. After taking up allergens, these cells undergo a maturation process in which they migrate to lymph nodes (LN). There, they present allergens to naive T cells and thereby contribute to their differentiation into Th2 cells. Once generated, Th2 cells produce interleukin (IL)-4, IL-5 and IL-13. IL-4 and IL-13 mediate isotype switching to IgE synthesis, whereas IL-5 is involved in eosinophil survival [4]. These proinflammatory cytokines also induce adhesion molecule and chemokine expression, supporting cell infiltration into the skin (Fig. 1). Noteworthy, although the acute phases are characterized by a Th2 pattern, chronic AD lesions display a Th1-type cytokine production, such as interferon (IFN)- γ .

Current atopic dermatitis therapies

None of the current AD therapies has proven to be a magic bullet thus far. The major unmet medical need is a cure for the severe forms of this disease. Table 2 effectively covers several therapeutic approaches used to treat AD. Furthermore, in this section, we discuss the possible side-effects of these treatments.

Emollients

Xerosis (dry skin) is one of the cardinal AD symptoms, resulting from decreased lipid levels (in particular ceramides) and enhanced

transepidermal water loss (TEWL) in the skin [5]. The application of topical emollients containing ceramides and/or flaggrin breakdown products retards the TEWL and increases skin hydration. However, their use reduces AD symptoms in mild but not in severe AD.

Corticosteroids

Corticosteroids have been the mainstay of anti-inflammatory treatment with efficiency in the control of AD [6]. They act by suppressing the release of proinflammatory cytokines. The absorption of potent topical corticosteroids (TCs) presents the risk for several systemic side-effects, such as hypothalamic–pituitary–adrenal axis suppression. The local adverse effects include skin atrophy, telangiectasia, hypopigmentation and allergic contact dermatitis [7]. Systemic corticosteroids are required for severe AD patients. However, acute flares can occur when these medications are stopped.

Topical calcineurin inhibitors

Topical calcineurin inhibitors (TCIs) tacrolimus and pimecrolimus are anti-inflammatory drugs used to treat AD. They inhibit the activation of immune cells involved in AD. TCIs are not atrophogenic and can be safely applied on thin skin. However, skin cancer and viral skin infections appear when such agents are used in long-term treatments [8].

Antimetabolites

Azathioprine, mycophenolate mofetil and methotrexate are immunosuppressive agents used for the treatment of moderate-to-severe AD. They interfere with purine and pyrimidine synthesis, and block cell proliferation. Nausea, bone marrow suppression,

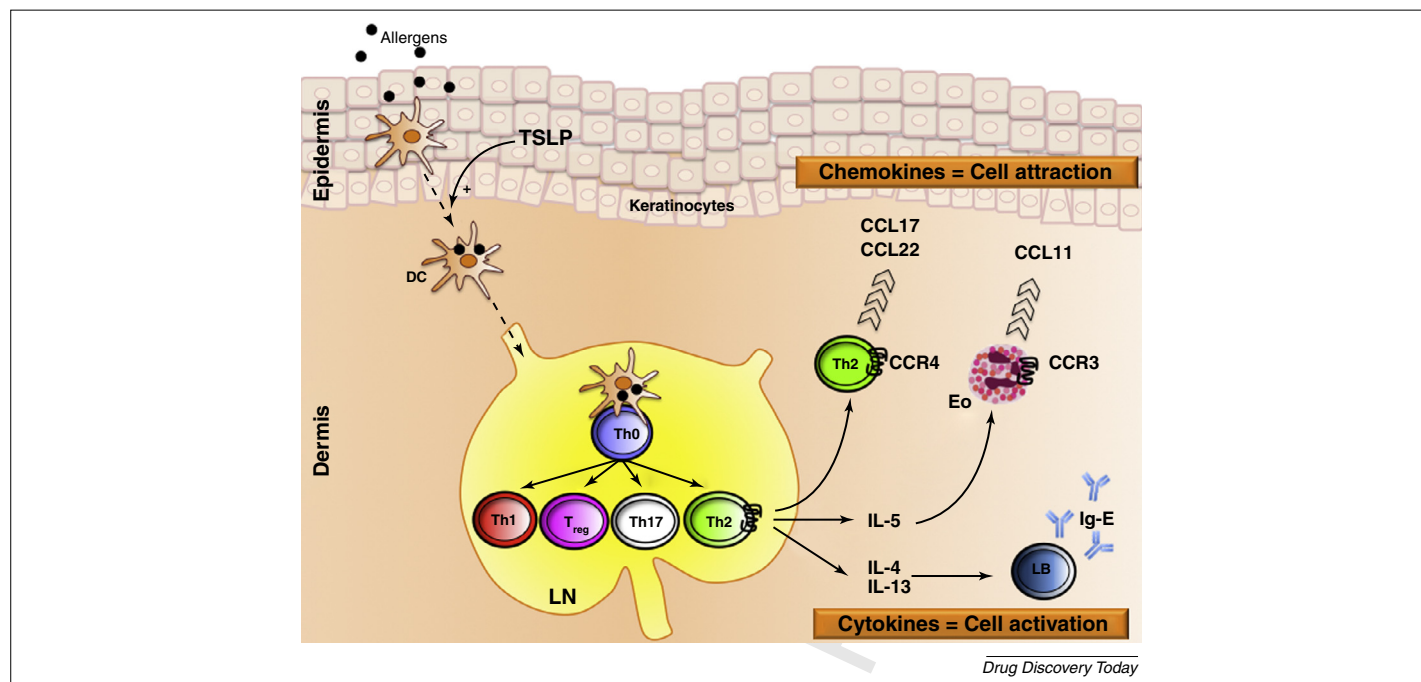


FIGURE 1

Pathogenesis of atopic dermatitis. Environmental allergens cross the skin barrier and activate epidermal keratinocytes, which subsequently secrete proinflammatory signals such as thymic stromal lymphopoietin (TSLP) cytokine. Overexpression of TSLP in the skin allows the maturation of Langerhans cells (LCs), which are antigen-presenting dendritic cells (DCs) present in the epidermis. Then, these cells migrate to lymph nodes (LN) and present allergen to naive T (Th0) cells. Generally, Th0 cells can differentiate into Th1, Th2, T_{reg} or Th17 cells. However, in the case of atopic dermatitis, Th0 cells differentiate into effector Th2 cells producing interleukin (IL)-4, IL-13 and IL-5. IL-4 and IL-13 activate B cells (LB), which differentiate into plasmocytes secreting immunoglobulin type E (IgE). CCR4-expressing Th2 cells migrate into the skin in response to CCL17 and CCL22 chemokines, which are secreted in the enflamed skin. By contrast, eosinophils activated by IL-5 express CCR3 and migrate to the skin in response to many chemokines, in particular CCL11. Once arrived to the skin, these immune cells secrete proinflammatory mediators able to enhance the immune response and damage the skin.

liver abnormalities, bacterial infections and malignancy are the most common adverse effects [9].

Omalizumab

Omalizumab is a humanized monoclonal antibody against IgE. Thus, IgE does not bind to its high-affinity Fcε receptor (present on mast cells and basophils), preventing the release of proinflammatory mediators. The major caveat for omalizumab use is the cost-effectiveness considerations [10].

Cytokine inhibitors

Researchers have explored whether the inhibition of IL-4 and IL-13 could provide a new treatment [10]. Dupilumab (a monoclonal antibody binding the IL-4α receptor subunit) effectively blocks IL-4 and IL-13 signaling. In moderate-to-severe AD, it has demonstrated clinical improvement. The adverse effects are nasopharyngitis, headache and skin infections. It has also been shown that anti-IL-5 antibodies (reslizumab and mepolizumab) decreased serum and airway eosinophilia. Notably, only 5% of the patients had clinical improvement [11,12]. Other clinical trials have shown therapeutic benefit in patients treated with anti-TNF-α antibodies (e.g., infliximab). However, infections and malignancy cases were observed with such strategies targeting TNF-α [13,14]. As a consequence of the limitations of all these treatments, research focusing on AD pathophysiology has identified other promising strategies targeting the chemokine/chemokine-receptor system that will be explored in detail in the following paragraphs.

Cutaneous chemokine/chemokine-receptor network in atopic dermatitis

Chemokines are a type of cytokine that are involved in leukocyte trafficking during homeostatic and inflammatory processes [15]. They are divided into four classes (C, CC, CXC and CX3C) on the basis of the N-terminal conserved cysteine (C) residue arrangement. In addition, these proteins exert their biological effects by activating seven transmembrane (TM) receptors mostly belonging to the G-protein-coupled receptor (GPCR) family and inducing signal transduction *via* Gαi protein. Within this network, there is a considerable redundancy because multiple chemokines bind a single receptor and alternatively.

AD is characterized by leukocyte migration into the skin, driven in a large part by excessive chemokine production in the site of inflammation. Numerous chemokines, such as CCL1/I-309, CCL5/RANTES, CCL11/eotaxin, CCL17/TARC, CCL18/PARC, CCL22/MDC, CCL26/eotaxin-3, CCL27/CTACK, CXCL12/SDF-1 and CX3CL1/fractalkine, have been associated with an AD phenotype [16] (Table 3). Among these, well characterized chemokines such as CCL17 and CCL22 chemokines are produced by keratinocytes, DCs and dermal fibroblasts in response to proinflammatory signals [17]. Both proteins share a common receptor, CCR4, that is predominantly expressed by Th2 and regulatory T (T_{reg}) cells. Other cell types express CCR4, like eosinophils, macrophages, basophils, DCs, platelets and natural killer (NK) cells. Consequently, several studies highlighted the association of CCR4 and its ligands, with Th2 responses and atopic diseases [18]. Moreover, it

TABLE 2

Summary of atopic dermatitis therapeutic approaches and their side-effects

System	Target	Pharmacological intervention	Potential outcome	Disadvantage and/or side-effects
Skin barrier function	Lipids (ceramides) Filaggrin	Topical moisturizers Emollients Occlusive agents	Moisturizing Reduced TEWL Reduced pruritus and inflammation	Expensive treatment Not efficient (especially for severe AD forms) Contain potentially sensitizing agents (fragrances, perfumes, irritants)
Innate immune system	Proinflammatory cytokines	Corticosteroids	Anti-inflammatory effects Decrease in proinflammatory cytokines Reduction of skin colonization with <i>Staphylococcus aureus</i>	Usage of systemic corticosteroids in patients with severe AD Flares of AD High absorption Systemic effects (hypothalamic–pituitary–adrenal axis suppression, cataracts, glaucoma and growth retardation) Hyperglycemia and hypertension Local adverse effects (development of striae, skin atrophy, telangiectasias, hypopigmentation, rosacea, perioral dermatitis, acne and allergic contact dermatitis) Not applied to thin skin sites (face, neck, and other skin folds) Wound healing and re-epithelialization impairment Topical corticosteroid gel formulations contain propylene glycol that can be irritating
Innate immune system	Calcineurin	Calcineurin inhibitors (tacrolimus and pimecrolimus)	Decrease in proinflammatory cytokine expression Increase of antimicrobial peptide expression Not atrophogenic (they are safely used for facial and eyelid eczema) Treatment for patients who are poorly responsive to topical steroids or have steroid phobia	Stinging and local irritation Pimecrolimus can increase the risk of viral infections (eczema herpeticum and molluscum contagiosum) Risk of skin cancers and lymphoma observed in patients who received systemically (rather than topically) administered calcineurin inhibitors
Innate immune system	<i>Staphylococcus aureus</i> Herpes simplex virus	Antibiotics and antivirals	Usage for the treatment of secondary infections	Topical antibiotics are not efficient Risk of antimicrobial drug resistance Severe skin rash owing to smallpox vaccination (eczema vaccinatum)
Innate immune system	Histamine receptors	Antihistamines	Pruritus decrease	Sedation effects Topical antihistamines are useless and can induce cutaneous sensitization and drowsiness
Innate immune system	Phosphodiesterases	Topical phosphodiesterase inhibitors	Anti-inflammatory effect Reduction of pruritus	Undergoing clinical trials Nausea and emesis
Innate immune system	Cyclophilin	Cyclosporin A	Immunosuppression effect Anti-inflammatory effect Inhibition of cytokine (IL-2 and IFN- γ) transcription	Relapse flares Raised serum creatinine Substantial renal impairment Hypertension Usage limited to patients with severe refractory AD Nausea Abdominal discomfort Hypertrichosis Paresthesia Hyperbilirubinemia
Innate immune system	Purine synthesis	Azathioprine	Anti-inflammatory and antiproliferative effects	Usage in severe AD Myelosuppression Hepatotoxicity Increased susceptibility to infections Hypersensitivity reaction (malaise, myalgia, fever, gastrointestinal symptoms, maculopapular rash, urticarial, vasculitis, erythema multiform or erythema nodosum) Risk of malignancy (Kaposi's sarcoma)

TABLE 2 (Continued)

System	Target	Pharmacological intervention	Potential outcome	Disadvantage and/or side-effects
Innate immune system	Purine biosynthesis	Mycophenolate mofetil	Immunosuppression in organ transplantation Treatment of refractory inflammatory skin disorders	Nausea, vomiting, diarrhea and abdominal discomfort Mild increase in serum levels of liver enzymes Viral and bacterial infections Risk of malignancy Bone marrow suppression
Innate immune system	Purine and pyrimidine synthesis	Methotrexate	Effective in patients with moderate-to-severe AD Inhibition of cytokine synthesis and cell chemotaxis	Nausea Bone marrow suppression Gastrointestinal toxicity Liver function abnormalities
Adaptive immune system	T cells	Recombinant IFN- γ	Th2 downregulation Clinical improvement Decreased blood eosinophilia	Influenza-like symptoms
Adaptive immune system	T cells	Intravenous immunoglobulin	Immunomodulatory activity	No clear benefit
Innate immune system	Total IgE	Anti-IgE antibody (omalizumab)	Reduction of plasma IgE	Conflicting results Limitation of paediatric data Usage in adults and children older than 12 years Not directed toward AD patients with low levels of IgE
Adaptive immune system	B cells	Anti-CD20 antibody (rituximab)	Treatment of severe AD Clinical improvement	No significant efficacy of etanercept in children
Adaptive immune system	T cells	Alefacept and efalizumab (humanized monoclonal antibodies against CD11a)	Clinical improvement Inhibition of T cell activation Reduced cellular infiltrate in AD lesions	Phase II has been completed, and drugs have been withdrawn from market Lack of clinical effect with efalizumab Increased risk of infections Increased risk of multifocal leukoencephalopathy
Innate immune system	TNF- α	Anti-TNF- α antibody (infliximab)	Anti-inflammatory effects	Risk of infections (tuberculosis) and malignancies
Adaptive immune system	TSLP, IL-4, IL-5, IL-13, IL-31	Other biologicals (or cytokine inhibitors)	Reduction of pruritus Inhibition of Th2 cells Improvement of skin barrier function Expression of antimicrobial peptides	Approaches that only target IL-4 were not effective, and developments have been halted (pascolizumab) IL-5 inhibitors are efficient for a small number of patients

Abbreviations: AD, atopic dermatitis; IFN- γ , interferon-gamma; IgE, immunoglobulin type E; IL, interleukin; TEWL, transepidermal water loss; Th, T helper; TNF- α , tumor necrosis factor- α ; TSLP, thymic stromal lymphopoietin.

has also been shown that the skin-specific CCL27 chemokine is expressed under homeostatic conditions by keratinocytes and is further induced by proinflammatory cytokines in AD [19]. CCR10, its only receptor described to date, is mostly expressed by cutaneous lymphocyte antigen (CLA)⁺ memory T cells. *In vivo*, the CCL27–CCR10 interaction regulates memory T cell recruitment into the skin. More recently, CCL1 and its specific CCR8 receptor have been elegantly demonstrated to direct LC migration to the draining LN in a murine model of AD [20]. Otherwise, CCL11, CCL24 and CCL26 bind to the CCR3 receptor, which is expressed by eosinophils, and is also present on Th2 lymphocytes, mast cells and basophils [21]. Their expression is obviously increased in AD lesions and probably contributes to CCR3⁺ cell recruitment into skin. Beside the CC subgroup, the CXC chemokine CXCL12 is also involved in skin-selective homing of immune cells. It has been shown that this chemokine is produced by dermal fibroblasts and attracts mature LCs (expressing its CXCR4 receptor) from the epidermis to the dermis of the enflamed skin [22].

Limitations of chemokine receptor targeting

Many reports have taken advantage of genetically engineered animal models and have demonstrated that chemokine receptors have important roles in atopic diseases, and could be new pharmacological targets of anti-inflammatory molecules. Indeed, CCR3^{−/−} mice displayed impairment in the eosinophil recruitment to ovalbumin (OVA)-sensitized skin in AD models [23]. By contrast, the role of the CCR4 receptor in atopic diseases was debated because many studies have shown conflicting results about targeting this receptor. For example, Schuh *et al.* used CCR4^{−/−} mice in an *Aspergillus*-induced asthma model and showed a reduced AHR and eosinophil recruitment [24]. However, other groups demonstrated that CCR4^{−/−} mice showed no protection against airway inflammation, and adoptive transfer of antigen-specific Th2 cells from CCR4^{−/−} mice failed to traffic to the allergic lung of wild-type (WT) mice [25]. Consequently, other models indicated that CCR4 deletion promotes rather than prevents AD. They showed that T cells, neutrophils and eosinophils

TABLE 3

Atopic dermatitis-associated chemokines and their receptors

Chemokines	Cellular origin	Increased plasma levels	Corresponding receptors	Target cells
CCL1/I-309	Keratinocytes Endothelial cells Mast cells	Yes	CCR8	T cells LC precursors
CCL2/MCP-1	Keratinocytes Leukocytes	Yes	CCR2	Monocytes DC precursors
CCL3/MIP-1α	Leukocytes	Yes	CCR1 CCR5	Monocytes T cells
CCL4/MIP-1β	Leukocytes	Yes	CCR5	Monocytes T cells
CCL5/RANTES	Keratinocytes Leukocytes	Yes	CCR1 CCR3 CCR5	Eosinophils T cells Fibroblasts
CCL11/eotaxin	Keratinocytes T cells	Yes	CCR3	Eosinophils T cells
CCL13/MCP-4	Keratinocytes Macrophages T cells	–	CCR2 CCR3	Monocytes DC precursors Eosinophils T cells
CCL17/TARC	Keratinocytes Endothelial cells DCs Fibroblasts	Yes	CCR4 CCR8	Th2 cells
CCL18/PARC	Keratinocytes DCs	Yes	Unknown	T cells
CCL22/MDC	Macrophages DCs	Yes	CCR4	Th2 cells T _{reg} cells
CCL26/eotaxin-3	Keratinocytes Endothelial cells	Yes	CCR3	Eosinophils T cells
CCL27/CTACK	Keratinocytes	Yes	CCR10	T cells
CX3CL1/fractalkine	Endothelial cells	Yes	CX3CR1	T cells NK cells Monocytes DCs
CXCL12/SDF-1α	Dermal fibroblasts	–	CXCR4 CXCR7	Mature LCs

Abbreviations: DCs, dendritic cells; LCs, Langerhans cells; NK, natural killer; Th, T helper; Reg, regulatory.

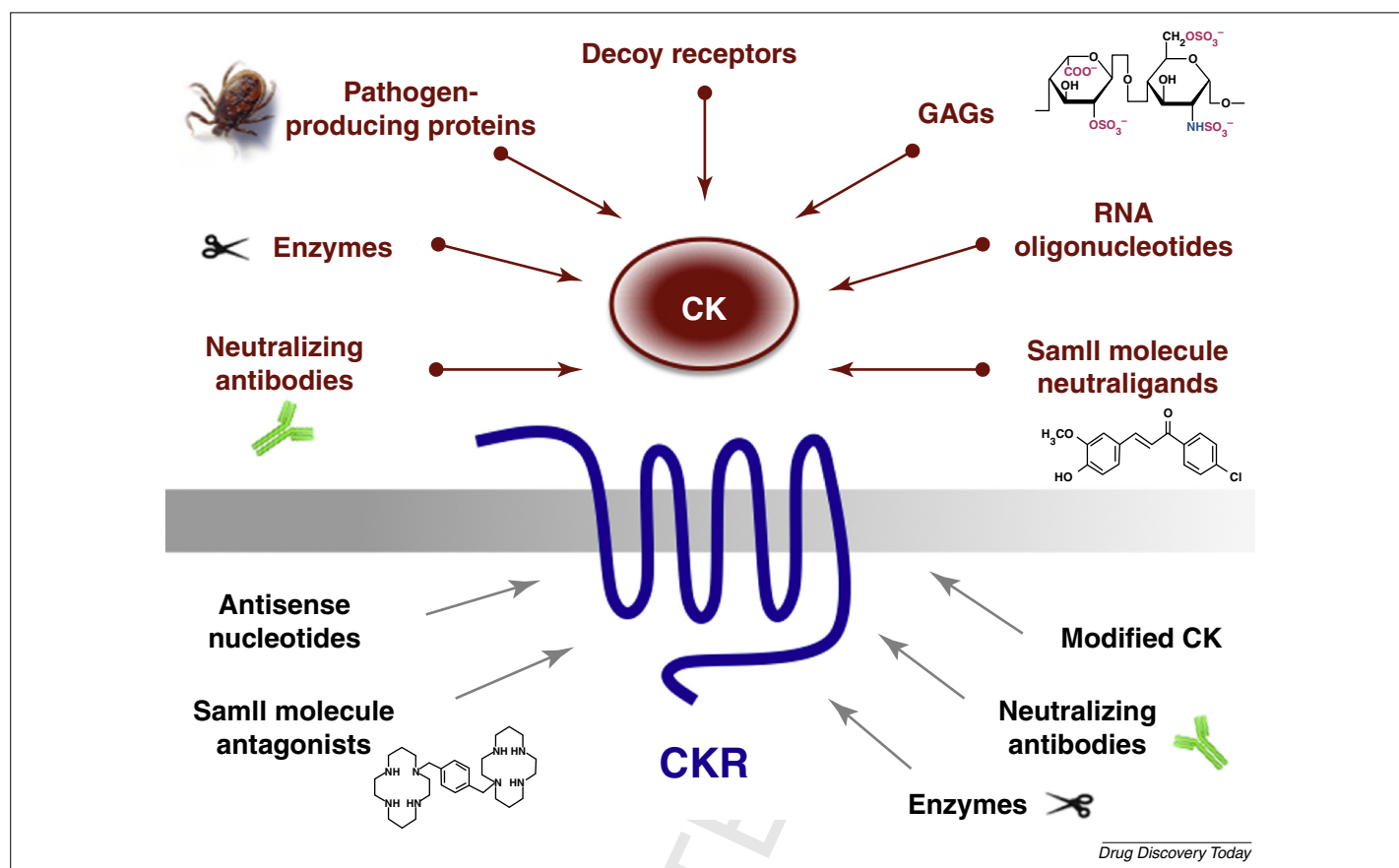
Q6 accumulate in the inflamed skin of CCR4^{−/−} mice [26,27]. These findings suggested that CCR4 was not sufficient alone for cell recruitment, and had redundant functions with CCR10, because the inhibition of both receptors was required to block AD inflammation in mice [28]. In addition, knockout mice have been crucial in studying the role that CCR8 has in leukocyte recruitment [29]. In fact, this receptor deletion impaired eosinophil recruitment and Th2 cytokine production in models of allergic airway inflammation. However, these *in vivo* observations were controversial and were contested by the work of other laboratories [30]. There are, however, other examples where chemokine-receptor-deficient mice (like CXCR4^{−/−} mice) have lethal phenotypes, precluding their study in AD disease [31].

Several pharmacological strategies have been pursued to develop inhibitors for chemokine receptors and to understand their roles in pathological conditions. For instance, classic approaches such as those involving small-molecule antagonists, antibodies, modified chemokines, antisense nucleotides and enzymes were

envisaged to target the receptor itself, and to block its interaction with chemokines (Fig. 2). Despite these efforts, chemokine receptor blockade either shows no or modest anti-inflammatory activity, or induces off-target effects [32]. Thus, in this section, we highlight the different strategies blocking these receptors in the case of atopic diseases, and we discuss their successes and failures in drug development.

Neutralizing antibodies

A number of studies have proposed the targeting of chemokine receptors by designing neutralizing antibodies against them. For example, it has been shown that monoclonal neutralizing antibodies targeting either CCR3 or CXCR4 reduced lung eosinophilia and AHR in murine models of allergic airway inflammation [33]. By contrast, other antibodies neutralizing CCR8 receptor pose **Q7** some problems because they lack specificity and show cross-reactivity with several other chemokine receptors, which could cause some confusion about the role of this receptor in AD. Other studies

**FIGURE 2**

Neutralization strategies targeting chemokine ligands or their receptors. Chemokines are neutralized by antibodies (or even nanobodies), enzymes (such as dipeptidyl peptidase-4/CD26 neutralizing CCL22), RNA oligonucleotides (such as NOX-A12 neutralizing CXCL12), pathogen-producing proteins (such as tick-producing evasins), decoy receptors (such as D6 receptor scavenging chemokines), soluble glycosaminoglycans (such as heparin) and small molecules (such as chalcone 4 neutralizing CXCL12). However, chemokine receptors are blocked by antibodies, enzymes (such as metalloproteases inhibiting CXCR4 receptor), antisense nucleotides (such as ASM8 inhibiting the CCR3 receptor), modified chemokines (such as met-RANTES inhibiting the CCR5 receptor) and small-molecule antagonists (such as AMD3100 inhibiting CXCR4 receptor). Abbreviations: CK, chemokines; CKR, chemokine receptor; GAGs, glycosaminoglycans.

cast doubts on the CCR4 receptor validity as a target for allergic diseases. In fact, in a severe combined immunodeficiency (SCID) murine model of HDM-induced airway inflammation, a CCR4-neutralizing antibody abolished airway hyperresponsiveness (AHR), eosinophilia and IgE synthesis [34]. Likewise, CCR4 neutralization with a monoclonal antibody was ineffective in modulating the allergic response in guinea pigs [35]. Moreover, mogamulizumab, another CCR4-neutralizing antibody, was proven to be efficient against primary adult T cell lymphomas in clinical trials [36] but Phase II studies revealed side-effects (skin rash, Steven-Johnson syndrome and cytomegalovirus infection) with one fatal case. This could raise questions about the application of mogamulizumab to skin.

Modified chemokines

Several studies reported N-terminal truncated or modified chemokines, because this region is crucial for chemokine receptor activation. It has been shown that Met-RANTES peptide (addition of a methionine residue to the CCL5 amino termini) inhibited the eosinophil influx in asthma and AD murine models [37]. However, these peptides have never been evaluated in clinical trials for AD.

Small molecules

Many academic laboratories and pharmaceutical companies have been devoted to find antagonists that block the recruitment of chemokine-receptor-expressing cells in allergic diseases. Several CCR3 small-molecule antagonists inhibited intracellular calcium mobilization and eosinophil chemotaxis induced by CCR3 agonists [38]. A number of these CCR3 antagonists have been patented, but few of them have entered clinical trials for allergic diseases. For example, DPC-168 was active in mouse and monkey models of allergic lung inflammation and entered Phase I testing for allergic diseases. However, this antagonist had off-target effects and showed poor selectivity over other receptors and transporters, such as cytochrome P450 (CYP)2D6 and human ether-a-go-go-related gene (hERG) channel, which is probably the reason why clinical trials were discontinued [38]. GW766994 inhibited CCR3 and was selective over the CCR1 receptor and the dopamine transporter [39]. Unfortunately, GW766994 was not efficient in a Phase III clinical trial for the treatment of allergic rhinitis. Small-molecule CCR3 antagonists also induce toxicology problems in rats. Regarding the CCR4 chemokine receptor, most small-molecule antagonists remained at a preclinical stage, despite all the efforts made and the promising results. So far, GSK2239633

entered Phase I clinical trials [40] but had a poor bioavailability, precluding its further development. Overall, these results about CCR4 neutralization should not prevent the exploration of this receptor as a therapeutic target in AD because there is clear evidence showing that CCL17 and CCL22 are key players in disease pathology.

Antisense nucleotides

ASM8 is an antisense oligonucleotide directed against human CCR3 mRNA and the common β chain of IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors, thereby downregulating their expression [41]. In animal models, ASM8 reduced AHR and inflammation after allergen challenge. In humans, ASM8 inhibited early asthmatic responses with a trend to diminish late responses [42]. Despite showing great therapeutic potential, antisense oligonucleotides present side-effects when using them in clinical trials such as hematologic toxicities and low intracellular uptake from the circulation.

Possible explanations for chemokine receptor neutralization failures

Chemokine receptors constitute one of the most relevant targets for atopic diseases. Surprisingly, inhibitors of chemokine receptors failed in preclinical and clinical trials [32]. We can envisage several reasons for this lack of success. For example, the failure could be due to the expression of some chemokine receptors (CCR4 and CCR8) on T_{reg} cells involved in immune response suppression. Targeting such receptors is efficient in cancer vaccine therapies but presents unwanted side-effects by worsening inflammation. As an example, mogamulizumab (anti-CCR4 neutralizing antibody) could be double-edged, enhancing antitumor immunity (by targeting T_{reg} cells) but also promoting severe immune-mediated side-effects in allergic diseases (by targeting Th2 cells) [10].

We now know that some chemokine receptors have crucial roles in inflammation and resolution of the immune response. Because CCR4 is involved in skin physiology and pathology, it could explain the conflicting results coming from the different *in vivo* models. Another possible reason for failure in targeting chemokine receptors is the translation from *in vitro* and *in vivo* studies into the clinic. Chemokine receptors impose challenges, such as species difference, animal model reliability and redundancy, making target validation relatively difficult. In the case of CCR4, the results

obtained from human studies have been corroborated with animal models (mice and guinea pigs). These observations suggest that the CCR4 biology could be different in rodents and humans.

Moreover, many molecules targeting chemokine receptors have been described with *in vitro* and/or *in vivo* efficiency, but not all have gone into clinical trials. Such hurdles are often attributed to the nature of the drugs themselves, such as lack of efficacy, poor pharmacokinetic profiles and safety issues. Some chemokine receptor antagonists have a quaternary nitrogen residue that interacts with the glutamate of the seventh TM helix of the receptor. Unfortunately, this leads to a pharmacophore formation similar to that of hERG inhibitors [43]. This could inhibit cardiac potassium channels and induce fatal cardiac arrhythmia.

Chemokine ligand neutralization: a promising therapeutic strategy for atopic dermatitis

Whatever the reason for therapeutic failures, the development of innovative strategies is actually needed for AD. We should learn simple lessons from nature. Bacteria, viruses and parasites have developed mechanisms to escape the immune system. They produce soluble proteins that bind to chemokines, thus circumventing the host response [44]. Of interest, such approaches targeting ligands could prevent cell chemotaxis by blocking their interaction with their receptors. Other chemokine ligand-neutralizing strategies exist: neutralizing antibodies and nanobodies [45], RNA oligonucleotides [46], glycosaminoglycans (GAGs), enzymes and decoy receptors scavenging the ligand [47,48], as well as small molecules [49,50] (Fig. 2).

These alternatives blocking the chemokine system were in accordance with results found using knockout mouse studies. For instance, mice lacking one of the CCL5, CCL11, CCL24 and CCL26 chemokines have impaired eosinophil trafficking to the skin and/or airways. Additionally, it has been shown that CCL17^{-/-} mice were protected from inflammation in murine models of OVA-induced AD and asthma. They significantly diminished eosinophils and CD4⁺ T cell infiltration, IgE and inflammatory cytokine production [51], which were not seen in CCR4-deficient mice. These results represent a prominent example of targeting the chemokine ligand (rather than targeting the receptor itself). In this section, we discuss in detail the different approaches targeting chemokine ligands that are used to treat AD (or other atopic diseases) (Table 4), and we shed light on their importance compared with conventional receptor inhibitors.

TABLE 4

Summary of chemokine-ligand-neutralization strategies in atopic dermatitis

Neutralization strategy	Chemokines	Examples	Refs
Peptides or proteins	CCL11/eotaxin	Tick-producing Evasin 4	[52]
Glycosaminoglycans	CCL11/eotaxin	Soluble glycosaminoglycans (heparin)	[53]
Neutralizing antibodies	CXCL12/SDF-1 CCL17/TARC CCL22/MDC CCL27/CTACK		[22] [54–57] [54–57] [19,27,58]
Small molecules	CXCL12/SDF-1 CCL17/TARC CCL22/MDC	Chalcone-4 GPN279 GPN136	[49] [50] [50]

CCL11 neutralization

It has been shown that ticks produce evasin proteins that bind to and neutralize chemokines. Evasin-4 represents an example of CCL11 neutralization [52], and has anti-inflammatory properties in a murine model of contact hypersensitivity (CHS). In addition, soluble GAGs can form complexes with chemokines (such as CCL11) that are unable to bind and activate their cognate receptors [53] in asthma models. This is probably the result of the chemokine positively charged residues (lysine and arginine) that exhibit electrostatic interactions with negatively charged GAGs, which is of fundamental importance for leukocyte extravasation from the blood vessels into tissues.

CXCL12 neutralization

Anti-CXCL12-neutralizing antibodies reduced AD inflammation, by inhibiting DC migration from the epidermis to the dermis [22]. In addition, it has been shown that small molecules could neutralize CXCL12. Chalcone 4 was the first molecule discovered to bind to and neutralize CXCL12 biological effects. It is now called a 'neutraligand' by analogy to neutralizing antibodies. Chalcone 4 was proved useful in asthma models [49].

CCL17 and CCL22 neutralization

Recently, small molecule neutraligands showed a clear benefit in neutralizing CCL17 or CCL22. These chemical probes proved to be efficient *in vivo* by inhibiting inflammatory cell recruitment (eosinophils, CD4⁺ T cells and DCs) in a mouse model of allergic asthma [50]. Similarly, treatment with specific antibodies neutralizing CCL17 or CCL22 reduced the leukocyte recruitment to the lung following allergen challenge [54–57].

CCL27 chemokine neutralization

It has been shown that CCR4^{−/−} mice treated with anti-CCL27-neutralizing antibodies have significantly reduced cutaneous lymphocyte accumulation and ear swelling [27]. In addition, CCL27 neutralization by specific antibodies significantly inhibited inflammatory skin responses in mouse models mimicking AD [19,58].

Concluding remarks

Our review shows that a chemokine-neutralizing strategy could present benefits for the treatment of atopic diseases. To understand the chemokine function in diseases, it is a validated scientific strategy to block these chemotactic cytokines (rather than their receptors). This approach presents several advantages in comparison with traditional receptor inhibitors. It could be useful in the case of orphan chemokines (CCL18, CXCL14 or CXCL15), which have no receptor identified yet, to find neutraligands blocking

their activity. This anti-inflammatory approach could be extended to other biological systems such as cytokines.

Chemokine receptors can display constitutive activity. In contrast to receptor antagonists that have partial agonistic or inverse agonistic activities resulting in undesirable effects; chemokine-neutralizing agents do not affect the basal activity level of receptors [49,50,59]. In addition, a recent report suggested that CCL17 and CCL22 bind to distinct CCR4 receptor conformations on leukocytes, resulting in blockade of signaling *via* one conformation but not the other [59,60]. These findings represented a potential caveat against the development of CCR4 antagonists. Chemokine neutralization strategy could therefore be a rescue to target the CCR4 pathway. Moreover, some reports indicated that CCL22 but not CCL17 induced the recruitment of T_{reg} cells. So, the identification of molecules specifically neutralizing CCL17 (not CCL22) could provide a beneficial therapeutic option for the treatment of Th2 allergic diseases. This could be important in dialing out the off-target effects of inhibiting T_{reg} recruitment. The use of broad-spectrum agents hitting several chemokines might be appealing. The activity of tick-produced evasins is not limited to one chemokine but blocked several chemokines. They provide novel therapeutic opportunities for the treatment of allergy, enabling corticosteroid sparing.

Noteworthy, the discovery of chemicals neutralizing chemokines is of great importance. Small molecules do not bear the risk of immunogenicity like neutralizing antibodies. They are more stable than peptides, proteins and GAG-binding chemokines that are generally rapidly degradable. With such molecules, we could go on up to the *in vivo* level and evaluate their activity in clinical trials. Finally, the fact that chemokine neutraligands have been identified as a potential treatment for allergies has stimulated the field. There is a possibility that chemokine neutralization showing clinical efficiency will soon be forthcoming. These promising therapeutic candidates from preclinical studies will need to be validated and tested in humans to see whether they fulfill their initial promise.

Conflicts of interest

The authors declare no conflicts of interests.

Competing financial interest

The authors declare no competing financial interests.

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