Paradoxical Increase in Skin Inflammation in the Absence of CCR4

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The chemokine receptors are seven transmembrane, G-protein-coupled surface receptors that play key roles in the migration and localization of leukocytes to the skin during physiologic and inflammatory states. Their ligands, chemokines, are small secreted proteins that initiate leukocyte chemoattraction. Recent data indicate that known subsets of T helper (Th) cells express signature chemokine receptors (e.g., CXCR3, CCR3/4, and CCR6) that help to define individual subsets such as Th1, Th2, and Th17 cells, respectively, although there is some degree of overlap among these T-cell subsets. In this issue, Lehtimäki *et al.* use an oxazolone-induced contact hypersensitivity (CHS) model to show that T cells (as well as neutrophils and eosinophils) from CCR4$^{-/-}$ mice accumulate just as (if not more) efficiently in inflamed skin as compared with the same population of leukocytes from wild-type (WT) mice. Although somewhat unexpected, their results can be explained if CCR4 attracts both proinflammatory and suppressive T cells into skin in addition to serving functions that are partially redundant with those of CCR10. Finally, we discuss other possible roles for CCR4 in the homing of T cells to skin.

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The chemokine receptors are seven transmembrane, G-protein-coupled surface receptors that play key roles in the migration and localization of leukocytes to the skin during physiologic and inflammatory states. Their ligands, chemokines, are small (8–11 kDa) secreted proteins that are predominantly involved in regulating leukocyte chemoattraction (Lonsdorf *et al.*, 2009). Within this chemokine network, there is considerable redundancy; multiple chemokines may bind a single receptor and vice versa. Functionally, chemokine receptors facilitate directional migration toward an increasing gradient of specific ligand(s). Moreover, chemokines activate leukocyte surface integrins, facilitating firm adhesion of white blood cells to vascular endothelium through immunoglobulin superfamily members at sites of inflammation and initiating a crucial step in the multistep paradigm of leukocyte recruitment. The release of specific chemokines at inflammatory sites in a time-dependent manner determines the order in which leukocyte (or T-cell) subsets enter inflamed sites such as skin. Recent data indicate that known subsets of Th cells express signature chemokine receptors (e.g., CXCR3, CCR3/4, and CCR6) that help to define individual subsets such as Th1, Th2, and Th17 cells, respectively, although there is some degree of overlap among these T-cell subsets. Thus, chemokine receptor repertoires define T-cell subsets phenotypically and functionally, determining their tissue tropism. This concept has importance physiologically and pathologically, given that distinct effector T-cell subsets are preferentially recruited in different inflammatory skin diseases.

Skin-homing T cells express glycosylated antigenic epitopes known as "cutaneous lymphocyte-associated antigens" (CLAs), enabling their specific binding to E-selectin, an adhesion molecule highly expressed on inflamed endothelium in skin. Binding to E-selectin initiates T-cell rolling interactions on vascular endothelium, the first step in the multistep recruitment paradigm (Fuhlbrigge *et al.*, 1997). A large majority of peripheral blood CLA$^+$ lymphocytes co-express CCR4 (Campbell *et al.*, 1999), with approximately 40% showing additional expression of CCR10 (Hudak *et al.*, 2002). The ligands for CCR4 are CCL17 and CCL22. Multiple skin-derived cell types have been shown to synthesize CCL17, including keratinocytes, activated macrophages, dendritic cells (DCs), and endothelial cells. The significant role of this molecule in skin homing is supported by immunohistochemical evidence showing constitutive and inducible expression of CCL17 by cutaneous venules as well as by keratinocytes in the epidermis. Additionally, integrin-dependent arrest of CLA$^+$ T cells on cutaneous venules is triggered by CCR4 and its ligands (Campbell *et al.*, 1999).

It should be noted that CCL17 is only one of many chemokines expressed in skin (Lonsdorf *et al.*, 2009). CCL27, for example, is also expressed in the basal layer of the epidermis, and its receptor, CCR10, is produced by a subset of CLA$^+$ T cells. Homey *et al.* (2002) have shown that targeting CCL27 can ameliorate allergen-induced inflammation. Some investigators have proposed that CCR4 function in cutaneous recruitment may overlap with CCR10 because inhibition of both CCR4 and CCR10 function was required to block contact dermatitis fully in mice (Reiss *et al.*, 2001). Recent data indicate that under some conditions, however, CCR4 appears to have unique functions in the recruitment of skin-homing T cells that are not shared with CCR10 (Campbell *et al.*, 2007). Based on these models, CCR4 exhibits varying degrees of contribution to skin trafficking, depending on the inflammatory model used.

Until recently, CCR4 was thought to be expressed preferentially by Th2 cells (Sallusto *et al.*, 1998), but it appears that many T-cell subsets can express CCR4 to varying degrees. In some inflammatory models, CCR4$^+$ T cells express cytokine profiles consistent with Th1 and Th17 cells (Campbell *et al.*, 2007). CCR4 has also been found on Foxp3$^+$ regulatory T cells (Tregs) and is critical for skin localization and suppression of cutaneous

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inflammatory responses (Sather *et al.*, 2007). More recently, Duhen *et al.* (2009) have defined the Th22 subset, characterized by expression of CCR10, CCR4, CCR6, and CLA as well as by the production of IL-22, but not IL-17 or IFN-γ.

In this issue, Lehtimäki *et al.* describe their use of an oxazolone-induced contact hypersensitivity (CHS) model to show that T cells (as well as neutrophils and eosinophils) from CCR4$^{-/-}$ mice accumulate just as (if not more) efficiently in inflamed skin compared with the same population of leukocytes from wild-type (WT) mice. Furthermore, CHS in CCR4$^{-/-}$ mice (as measured by ear swelling response) was increased moderately compared with CHS in WT mice. These findings are similar to those published by Reiss *et al.* (2001), who demonstrated that CCR4-deficient mice had equivalent lymphocyte accumulation in ear skin and a modest increase in ear swelling compared with WT mice following sensitized challenge to the allergen DNFB. Surprisingly, in both of these models, similar modest increases in ear swelling and T-cell infiltration were found in the CCR4-deficient group relative to WT controls. Therefore, it appears that, under the conditions defined in these two models, loss of CCR4 may actually promote inflammation and T-cell accumulation within the skin. Reiss and colleagues (2001) have further shown that CCR4$^{-/-}$ mice treated with anti-CCL27-neutralizing antibodies (CCR10 ligand) have dramatically reduced cutaneous lymphocyte localization and ear swelling compared with CCR4 deficiency alone. Interestingly, the anti-CCL27 antibodies alone had a negligible impact on CHS by each of the quantifiable criteria used. Taken together, the results from these two publications suggest that CCR4 and CCR10 pathways may serve redundant functions; either alone is sufficient for skin homing and effector function in these CHS models. These results make sense in that CCR10 and CCR4 are (to varying degrees) expressed in the CLA^{+} population of skin-homing T cells. Intriguingly, these results suggest that the isolated loss of CCR4 promotes, rather than prevents, the development of skin inflammation and T-cell homing.

In other models, CCR4 may have an obligatory role in T-cell recruitment to skin. Specifically, Campbell *et al.* (2007) developed an ovalbumin peptide–specific TCR skin inflammation model that demonstrated that CCR4 is critical for T-cell homing to the skin and subsequent inflammation. Unfortunately, this model cannot be readily compared with the current study or other oxazolone/DNFB-induced CHS models.

> Chemokine receptors are both positive and negative mediators of skin inflammation.

Reconciling the role of CCR4 in skin physiology and pathology is not easy. The expression of CCR4 by Tregs could potentially help explain the surprising results yielded by Lehtimäki *et al.* (2010) and Reiss *et al.* (2001). Sather *et al.* (2007) showed that CCR4-deficient Tregs have impaired skin homing, leading to severe, tissue-selective inflammation and effector T-cell infiltration of the skin and lung. Lehtimäki *et al.* (2010), however, reported that, within the oxazolone CHS model, the number of Tregs was found to be significantly elevated within the skin of CCR4$^{-/-}$ mice relative to WT mice (i.e., Foxp3 mRNA was increased dramatically in inflamed skin, whereas CD4^{+}Foxp3^{+} cells were approximately doubled in skin samples by immunohistochemistry and moderately increased as demonstrated by flow cytometry). It appears that in the oxazolone model, skin trafficking of Tregs is not dependent on CCR4; however, redundant function of CCR10 in skin homing cannot be excluded because the authors report that 10% of Treg cells isolated from the lymph nodes of oxazolone-treated mice expressed CCR10. It has not been ruled out, however, that the increase in Tregs seen in CCR4$^{-/-}$ mice might simply reflect an enhanced recruitment of Tregs to skin through CCR10 (or other chemokine receptors) because of a secondary phenomenon in response to the vigorous skin inflammation present in CCR4-deficient mice.

Interestingly, the Treg effector cytokines, IL-10 and TGF-β, were elevated only minimally relative to the dramatic increase noted for Foxp3 mRNA in CCR4$^{-/-}$ mice, suggesting that, despite the increased numbers of Foxp3^{+} cells, regulatory function might be defective in the absence of CCR4. Rightly, the authors note that these cytokines are not restricted to Tregs and, therefore, Treg effector

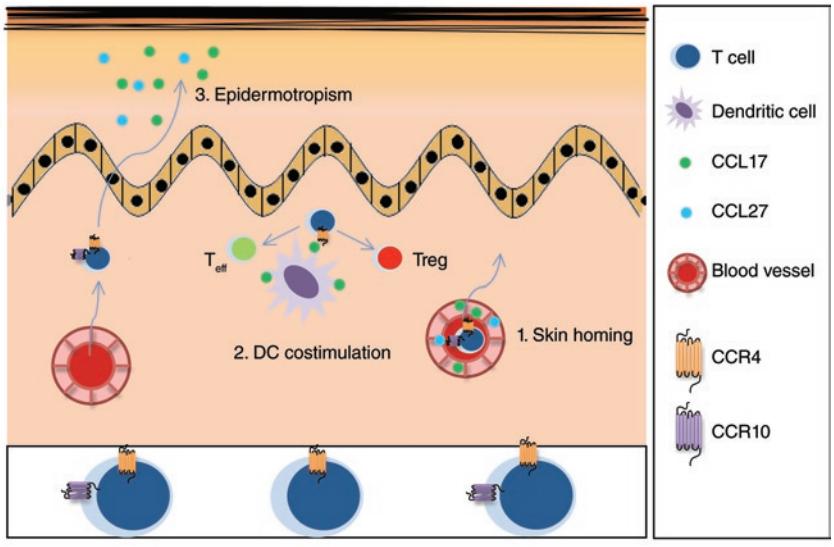

Figure 1. Possible mechanisms by which CCR4 might regulate skin inflammation. (1) CCR4 and/or CCR10 may directly influence migrations of effector (T_{eff}) or regulatory T (Treg) cells into skin through dermal blood vessels. In some model systems, CCR10 and CCR4 are redundant in terms of driving inflammation. (2) Attraction of relatively undifferentiated T cells with skin dendritic cells (DCs) may drive T cells toward suppressive or effector phenotypes. (3) Localization of T cells in epidermis may be influenced by CCL17 expressed in the epidermis. CCR10 may also have a role because its ligand, CCL27, is produced in basal keratinocytes.

function cannot be fully evaluated. Because Treg homing remains intact, we propose that a defect in Treg function (but not necessarily homing) might account for the results observed with the CCR4$^{-/-}$ mice. To test this hypothesis, *in vitro* T-cell suppressor assays may be performed using Tregs isolated from oxazolone-treated skin of WT and CCR4$^{-/-}$ mice.

Additional hypotheses may explain the unexpected results presented by Lehtimäki *et al*. (2010). As discussed previously, multiple cell types, including endothelial cells, DCs, and keratinocytes, produce CCL17. CCR4 may be dispensable with respect to recruitment of T cells into the skin (section 1 in Figure 1), with CCR10 playing a compensatory role in the absence of CCR4, but events downstream of initial recruitment may be important. Secretion of CCL17 by DCs is believed to mediate DC–T-cell interactions. Upon maturation, DCs have been shown to produce CCL17, which mediates attraction and adhesion of these DCs to antigen-primed T cells (Wu *et al.*, 2001). In particular, these interactions may be important for the *in situ* development of effector and regulatory T-cell activation (section 2 in Figure 1). The lack of interaction between DCs and Tregs due to lack of CCR4 on Tregs is another possible explanation for the enhanced inflammation seen in CCR4$^{-/-}$ mice. Finally, keratinocytes also synthesize CCL17, which may be important for epidermotropism of both memory effector T cells and Tregs (section 3 in Figure 1). Correct localization of T-cell populations within the epidermis might alter inflammatory responses. For these reasons, future studies should address events downstream of tissue homing: specifically Treg/DC and T-cell/keratinocyte interactions.

It is becoming clearer that chemokine receptors are not always positive mediators of skin inflammation, but that they can be critical for suppression of inflammation as well. What complicates the picture is that some receptors, as exemplified by CCR4, may act in both ways. Depending on the model of inflammation used, CCR4's effects can influence inflammation in different, almost contradictory, ways. Indeed, the nuances and complexities of chemokine receptor function in the skin are just beginning to be discerned at a fine level.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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Paying "Particle" Attention to Novel Melanoma Treatment Strategies

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Malignant melanoma remains the deadliest form of skin cancer because of its highly aggressive nature and the lack of effective treatments. Recent investigations into alternative treatment strategies have highlighted the exciting potential of nanoparticles to increase melanoma cell delivery and the efficacy of small interfering RNAs (siRNAs) and pharmacological inhibitors. In this issue, Chen *et al.* report a new liposomal nanoparticle for c-*Myc* siRNA delivery, noting it to be highly effective in reducing c-*Myc* expression and inhibiting melanoma tumor growth in mouse models. This preclinical study underscores the importance of investigating nanoparticle treatment options for chemoresistant melanomas.

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Introduction

The field of targeted therapeutic strategies for melanoma is entering exciting times. Malignant melanoma is the deadliest form of skin cancer and has for decades represented a paradigm for chemoresistance. Current therapeutic options are poor and no new US Food and Drug Administration (FDA)-approved drugs have emerged in recent years. Current melanoma treatment mainstays, such as the alkylating agent dacarbazine and the immune-stimulating agent IL-2, are plagued by a lack of clinical benefit in the majority of patients and numerous side effects. However, highly

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