See related Commentary on page v

Role of the Chemokine Receptor CCR4 and its Ligand Thymus- and Activation-Regulated Chemokine/CCL17 for Lymphocyte Recruitment in Cutaneous Lupus Erythematosus

Joerg Wenzel, Stephanie Henze, Eva Wörenkämper, Etiena Basner-Tschakarjan, Malgorzata Sokolowska-Wojdylo, Julia Steitz, Thomas Bieber, and Thomas Tüting
Department of Dermatology, University of Bonn, Bonn, Germany

Skin-infiltrating T lymphocytes are thought to play a major role in the pathogenesis of cutaneous lupus erythematosus (CLE). In this study, we investigated the role of the chemokine receptor 4 (CCR4) and its ligand thymus- and activation-regulated chemokine (TARC/CCL17) for the recruitment of T cells in inflamed skin of patients with CLE. We found significant numbers of CCR4+ T lymphocytes in the skin of all patients with CLE. Interestingly, a subset of patients with disseminated scarring skin involvement were characterized by both lesional and circulating CD8+ T cells expressing CCR4. Destruction of epidermal and adnexal structures was histomorphologically associated with CCR4+ cytotoxic T cells invading basal layers of the epidermis where keratinocytes showed apoptotic death. The CCR4 ligand TARC/CCL17 was strongly expressed in skin lesions and elevated in the serum of CLE patients. The functional relevance of lymphocytic CCR4 expression could be confirmed by TARC/CCL17-specific *in vitro* migration assays. Our investigations suggest that CCR4 and TARC/CCL17 play a role in the pathophysiology of CLE. In particular, cytotoxic CD8+ T cells expressing CCR4 appear to be involved in scarring subtypes of CLE.

Key words: CC receptor 4/cutaneous lupus erythematosus/skin homing/TARC/T cell
J Invest Dermatol 124:1241–1248, 2005

Lupus erythematosus (LE) is an autoimmune disorder with a broad clinical spectrum reaching from primarily cutaneous manifestations (CLE) up to systemic disease (SLE). Frequent cutaneous subtypes of LE are the scarring chronic discoid LE (CDLE) and the non-scarring subacute CLE (SCLE). Patients suffering from CDLE are characterized by scarring discoid plaques, ranging from localized to disseminated variants. SCLE patients are characterized by nummular or gyrated erythematosquamous lesions, predominantly in sun-exposed areas without scarring. Histological analyses of CLE lesions typically reveal dense CD3+ junctional and periadnexal infiltrates (Tebbe *et al*, 1995). These supposedly autoaggressive T lymphocytes are thought to play a significant role in the pathogenesis of CLE lesions.

A little more than 10 y ago, studies addressing the immunobiology of T cell trafficking provided the first insights into the molecular regulation of T cell homing to the skin (Robert and Kupper, 1999). It could be shown that naïve T cells express adhesion molecules like L-selectin on their surface, which allow them to enter lymph nodes via high endothelial venules expressing appropriate ligands such as peripheral node adressin. Once T cells become activated by skin-derived antigen-presenting dendritic cells in draining lymph nodes, they proliferate, express activation and effector molecules, and undergo the transition to memory T cells (Sallusto *et al*, 2000). During this transition, some effector and memory T cells acquire new molecular keys such as the cutaneous lymphocyte antigen (CLA), which allow them to preferentially enter inflamed skin where the appropriate ligands are expressed on cutaneous microvascular endothelial cells (Tietz *et al*, 1998). It could be shown that the majority of T cells in inflamed skin lesions such as atopic dermatitis, psoriasis vulgaris, lichen planus, discoid lupus erythematosus, and cutaneous T cell lymphoma indeed express CLA on their cell surface (Heald *et al*, 1993; Nakamura *et al*, 1998). The majority of CLA+ memory T cells express the chemokine receptor (CCR)4 (Campbell *et al*, 1999). With regard to T cell trafficking into inflamed skin, recent studies have highlighted the role of CCR4 and its ligand thymus- and activation-regulated chemokine (TARC/CCL17) (Schon *et al*, 2003). Increased expression of CCR4 was demonstrated in atopic dermatitis (Wakugawa *et al*, 2001), psoriasis (Inaoki *et al*, 2003), and in cutaneous T cell lymphoma (Kallinich *et al*, 2003). Interaction between CCR4 and its ligand TARC/CCL17 on activated endothelial cells mediates T cell extravasation by stimulating integrin-dependent adhesion of CLA+ T cells to intercellular adhesion molecule-1 (Campbell *et al*, 1999).

In this study, we investigated the expression of CCR4 on T lymphocytes in both skin and peripheral blood of patients suffering from CLE. We show that significant numbers of

Abbreviations: CDLE, chronic discoid lupus erythematosus; CLA, cutaneous lymphocyte antigen; CLE, cutaneous lupus erythematosus; dCDLE, disseminated CDLE; lCDLE, localized CDLE; LE, lupus erythematosus; SCLE, subacute cutaneous LE; SLE, systemic LE; TARC/CCL17, thymus- and activation-regulated chemokine

lesional T lymphocytes express CLA and CCR4. Importantly, the coexpression CLA+ and CCR4+ on lesional and circulating CD8+ T lymphocytes defines a subset of CLE patients characterized by disseminated scarring lesions. Destruction of epidermal and adnexal structures was associated with infiltration of CD8+ T cells largely expressing the cytotoxic molecule granzyme B. Increased TARC/CCL17 levels were found in lesional skin and serum of CLE patients. Importantly, CCR4-expressing lymphocytes isolated from the blood of CDLE patients showed TARC/CCL17-specific *in vitro* migration, confirming the functional relevance of our findings.

Results

Enhanced expression of CCR4 on lesional CD8+ T lymphocytes identifies a subset of patients with scarring CDLE CLE lesions are immunohistologically characterized by a dense junctional and perivascular T cellular infiltrate, primarily composed of CD4+ and CD8+ cells (Fig 1*a–c*). In initial studies, we further investigated infiltrating lymphocytes by immunohistochemistry for the homing molecules CLA and CCR4. As expected, large numbers of CLA-expressing lymphocytes were present in skin lesions of CDLE, SCLE, and SLE when compared with healthy controls. Figure 1 shows a semiquantitative analysis with numbers of cells per high-power field of view. Furthermore, as detailed in Fig 1*e*, significantly increased expression of CCR4 was also found in lesions of CDLE when compared with healthy controls ($p < 0.01$).

We consistently noted a prominent infiltration with CD8+ T cells in lesions from patients with scarring subtypes of CLE. To assess the cytotoxic function of these CD8+ T cells, we stained for the cytotoxic molecule granzyme B. As shown in Fig 1*f*, patients with CDLE displayed significantly increased expression of granzyme B ($p < 0.01$) when compared with other LE subtypes and healthy con-

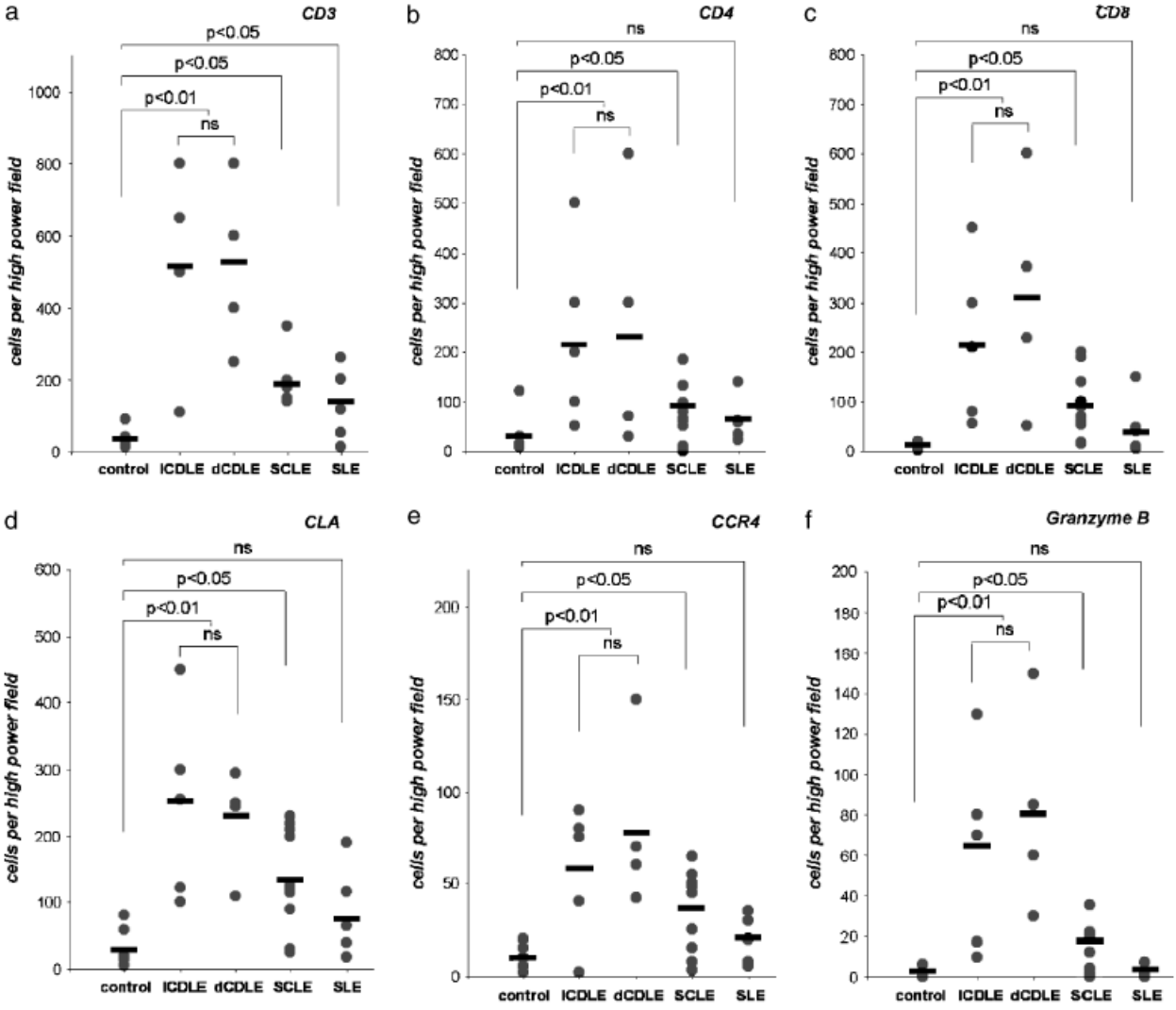

Figure 1
Immunohistological analyses of CD3, CD4, CD8, cutaneous lymphocyte antigen (CLA), CC receptor (CCR)4, and granzyme B expression. Lesional skin biopsies were taken from patients with localized chronic discoid lupus erythematosus (lCDLE) (n = 5), disseminated CDLE (dCDLE) (n = 4), subacute cutaneous LE (SCLE) (n = 11), systemic LE (SLE) (n = 5), and healthy controls (n = 5). Stainings were performed using the LSAB2 kit. Sections were analyzed by two experienced dermatohistopathologists independently. Expression was evaluated as cells per high-power field (× 200 magnification). One point in the figure represents the mean results of the two investigators.