

The CD40/CD40 Ligand System in the Skin of Patients with Subacute Cutaneous Lupus Erythematosus

MARZIA CAPRONI, DANIELE TORCHIA, EMILIANO ANTIGA, BARBARA GIOMI, MICHELE MERCURI, WALTER VOLPI, and PAOLO FABBRI

ABSTRACT. *Objective.* To investigate whether CD40 and CD40 ligand (CD40L) is expressed in the skin of patients with subacute cutaneous lupus erythematosus (SCLE).

Methods. Six female patients with SCLE were studied. Skin biopsies were obtained from lesional and healthy sunprotected skin. Frozen sections were stained immunohistochemically using monoclonal antibodies to CD4, CD40, and CD40L. As controls we used 5 patients with discoid LE (DLE), 5 with dermatomyositis (DM), 3 with lichen planus (LP), and 2 with erythema multiforme (EM), as well as the normal-appearing skin of 5 healthy volunteers.

Results. The CD40 was intensely expressed in all SCLE, DLE, and DM lesions, and only focally in healthy sunprotected skin specimens. The number of CD40+ cells in SCLE dermis was lower than in DLE, similar to that in DM, LP and EM, and higher than in SCLE sunprotected skin. CD40L+ cells infiltrated the SCLE, DLE, DM, LP, and EM lesional dermis, and were more numerous in SCLE lesional skin than in SCLE healthy sunprotected skin.

Conclusion. We showed that the CD40/CD40L system may represent an important pathway of induction of SCLE lesions. The expression of such costimulatory system in healthy sunprotected skin also may signify that its abnormal activation is constitutive in SCLE, as previously observed in systemic LE. (First Release Nov 15 2007; J Rheumatol 2007;34:2412–6)

Key Indexing Terms:

SUBACUTE CUTANEOUS LUPUS ERYTHEMATOSUS
CD40 LIGAND

CD40
HEALTHY SUNPROTECTED SKIN

Subacute cutaneous lupus erythematosus (SCLE) is a widespread, photosensitive, nonscarring, nonindurated form of LE-specific skin disease¹. In individuals with a distinctive immunogenetic background [including the 8.1 ancestral HLA haplotype, C2/C4 deficiency, and tumor necrosis factor (TNF)- α -308A single nucleotide polymorphism], environmental factors, such as ultraviolet (UV) light and drugs, would trigger an autoimmune response. This includes the production of autoantibodies directed towards the nuclear antigen SSA/Ro that is exposed on the surface of UVB-induced apoptotic keratinocytes². Moreover, T lymphocytes, the main population of inflammatory cells in SCLE lesions^{3,4}, are likely to participate significantly in immunoinflammation. Thus, the tissue injury of SCLE lesions may result from autoantibody- and complement-mediated cell damage, direct T-cell-mediated cytotoxicity, TNF- α -induced apoptosis, and antibody-dependent cell-mediated cytotoxicity¹.

The CD40/CD40 ligand (CD40L) costimulatory system belongs to the TNF-TNF receptor superfamily. CD40 is ubiquitously expressed on the surface of immune and nonimmune cells, whereas CD40L is expressed preferentially by activated CD4+ T cells and platelets. The interaction between CD40 and CD40L represents a major costimulatory system that amplifies the immune response and can promote inflammation⁵. The main biological effects include the switch in recombination and synthesis of immunoglobulins by B cells, stimulation of dendritic cells to increase the cell-surface expression of other costimulatory molecules, upregulation of cell adhesion molecules, and production of various cytokines, such as TNF- α ⁵.

The CD40/CD40L system has been found to be involved in many immune-mediated diseases, including systemic lupus erythematosus (SLE)⁶. We recently showed that activated CD40L+ T cells infiltrate the cutaneous lesions of dermatomyositis (DM) and discoid LE (DLE)⁷, which, like SCLE, are skin manifestations of autoimmune connective tissue diseases.

Since no data concerning the expression of CD40 and CD40L in SCLE are available in the literature to date, our aim was to investigate whether such costimulatory system is expressed in SCLE lesions, as well as in healthy sunprotected skin taken from the same patients.

MATERIALS AND METHODS

Patients. The project was approved by the Hospital Ethics Committee. Having given informed consent, 6 patients with SCLE were studied. The

From the Departments of Dermatological Sciences and Experimental Pathology and Oncology, University of Florence, Florence, Italy.

M. Caproni, MD; E. Antiga, MD; B. Giomi, MD; M. Mercuri, MD; W. Volpi, MD; P. Fabbri, MD, Department of Dermatological Sciences; D. Torchia, MD, Departments of Dermatological Sciences and Experimental Pathology and Oncology, University of Florence.

Address reprint requests to Prof. P. Fabbri, Department of Dermatological Sciences, University of Florence, Via della Pergola 58/60, 50121 Florence, Italy. E-mail: fabbri@unifi.it

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patients were all women (mean age 51.8 yrs, range 28–63). At the time of the study none of the patients were undergoing topical or systemic treatment; only 1 patient, also with SLE, was taking oral corticosteroids plus cyclosporin A.

Skin biopsies were obtained from lesional skin and from gluteal sunprotected skin. As controls, with informed consent, we used biopsies taken from lesional and gluteal sunprotected skin of 5 patients with DLE (2 men, 3 women, mean age 30 yrs, range 23–38) and 5 with DM (4 men, 2 women, mean age 59.3 yrs, range 40–79), and from lesional skin of 3 patients with lichen planus (LP) and 2 with erythema multiforme (EM). The normal-appearing skin, taken from the abdominal skin of 5 healthy volunteers (2 men, 3 women, mean age 46 yrs, range 29–66) who underwent minor surgery, in whom no cutaneous or systemic inflammatory diseases were diagnosed, also served as a control. Biopsies were performed under local ring anesthesia and covered in cryoprotectant (Tissue-Tek, Miles Inc., USA) before storage in a freezer at –80°C.

Immunohistochemistry. Skin specimens were cut into 5- μ m thick sections and stained for immunohistochemical markers using monoclonal antibodies to CD4 (clone MT310; 1:50; Dako, Copenhagen, Denmark), CD40 (clone B-B20; 1:50; Cymbus Biotechnology, Chandlers Fords, UK), and CD40L (clone 24-31; 1:50; Cymbus Biotechnology).

Before staining, frozen sections were air-dried and fixed in acetone (5 min). Immunolabeling was performed using rabbit anti-mouse bridging antibodies conjugated with alkaline phosphatase (1:10 dilution, 30 min; Dako) followed by incubation with murine alkaline phosphatase/anti-alkaline phosphatase (APAAP) complexes (1:30 dilution, 30 min; Dako). Negative control sections were incubated with nonimmune mouse sera.

Two independent, “blind” observers evaluated serial sections. For quantitative analysis of the dermal infiltrate, positive cells were counted in 3 consecutive microscopic fields (250 \times).

RESULTS

CD4+ cells strongly infiltrated the perivascular and interstitial dermis of all lesional specimens (Figure 1A). Only DLE lesions featured a far more intense infiltration (Figure 2). Sparse dermal CD40+ cells were observed in the healthy sunprotected skin specimens (Figure 1B).

The CD40 was intensely expressed by the basal and suprabasal layers of follicular and interfollicular epidermis in all SCLE (Figure 1C), DLE, DM, LP, and EM lesions, and only focally on basal keratinocytes of healthy sunprotected skin specimens (Figure 1D). CD40+ cells infiltrated the perivascular, periadnexal, and interstitial dermis of all lesional specimens, while only few dermal CD40+ cells were detected in the healthy sunprotected skin. The number of positive cells in SCLE dermis was lower than in DLE and similar to that in DM, LP, and EM, while higher than in SCLE sunprotected skin. Healthy specimens of healthy controls showed a weak epidermal staining for CD40, while only a few positive cells were observed in the dermis.

No immunostaining for CD40L was seen in the epidermis of all the sections examined. Anti-CD40L monoclonal antibodies stained cells infiltrating the perivascular, periadnexal, and interstitial dermis in all SCLE (Figure 1E), DLE, DM, LP, and EM lesions; only a few differences emerged between CD40L+ cell numbers of SCLE and DLE and of SCLE and DM. Instead, CD40L+ cells were more numerous in SCLE lesional skin than in LP, EM, and SCLE healthy sunprotected skin (Figure 1F). Healthy specimens of healthy controls were negative for CD40L immunostaining.

DISCUSSION

The induction of tissue injury in SCLE lesions probably results from various autoimmune effector mechanisms. The injury mediated by autoantibodies directed towards Ro/SSA antigens, displayed on the cell surface of UVB-induced apoptotic keratinocytes^{2,8}, remains only in the field of hypotheses to date. Instead, recent studies seem to indicate that intralesional T cells may represent an important pathomechanism within SCLE lesions. Such population is mainly composed of CD4+ CD45RO+ (memory helper/inducer) T cells^{3,4} that, once recruited into the lesional tissue, are capable of inducing and enhancing a Th1-biased immunoinflammation via the secretion of several cytokines and chemokines^{9,10}.

This tissue pattern has already been demonstrated in another subtype of CLE, i.e., DLE, as well as in the cutaneous lesions of another autoimmune connective tissue disease, DM⁷. We showed recently that the CD40 and CD40L, of which cognate interaction is an important costimulatory system, are strongly expressed in DLE and DM lesions⁷.

As described previously⁴, in our study we showed that many CD4+ T lymphocytes infiltrate lesional and also nonlesional SCLE skin, although mildly in the latter. Such T cells may promote the tissue damage either via the production of proinflammatory cytokines or via the expression of membrane receptors, including CD40 and CD40L.

We then showed that CD40 was strongly expressed in the lesional epidermis of SCLE lesions, almost overlapping with the DM, DLE, LP¹¹, and EM¹² lesional controls. Instead, the number of CD40+ dermal cells, mainly represented by T lymphocytes, macrophages, and dendritic cells^{3,4,13}, was similar in SCLE and DM lesions, while DLE lesions showed a higher amount with respect to the other series⁷.

The immunostaining for the CD40L revealed that several positive cells infiltrated the lesional SCLE dermis, as well as the DM, DLE⁷, LP¹¹, and EM¹² dermis with overlapping distribution and numbers. The topographic contiguity between CD40L+ and CD40+ cells suggests that the CD40/CD40L ligation may trigger the intracellular signaling within CD40+ cells.

A CD40L-induced activation of CD40+ cells may represent an important pathomechanism of SCLE lesions. Indeed, CD4+ CD40L+ T lymphocytes, distributed around the dermal vessels and adnexa, as well as below the dermoepidermal junction, might bind to CD40+ antigen-presenting cells^{4,13} and to CD40+ keratinocytes, inducing the secretion of numerous paracrine mediators. In particular, CD40+ dendritic cells may overexpress cell adhesion and costimulatory molecules, and produce several cytokines, such as TNF- α ¹¹, and chemokines¹⁰. CD40+ keratinocytes may secrete interleukin 1 (IL-1)¹⁴, IL-6¹⁵, MCP-1, and nitric oxide (NO)¹⁶, and upregulate adhesion molecules⁴. Many of these mediators are able to exert chemotactic activity on T lymphocytes and monocytes. Moreover, IL-1, IL-6, TNF- α ^{13,14}, and NO may contribute to the apoptotic phenomena that are present in the lesional SCLE epidermis⁸.

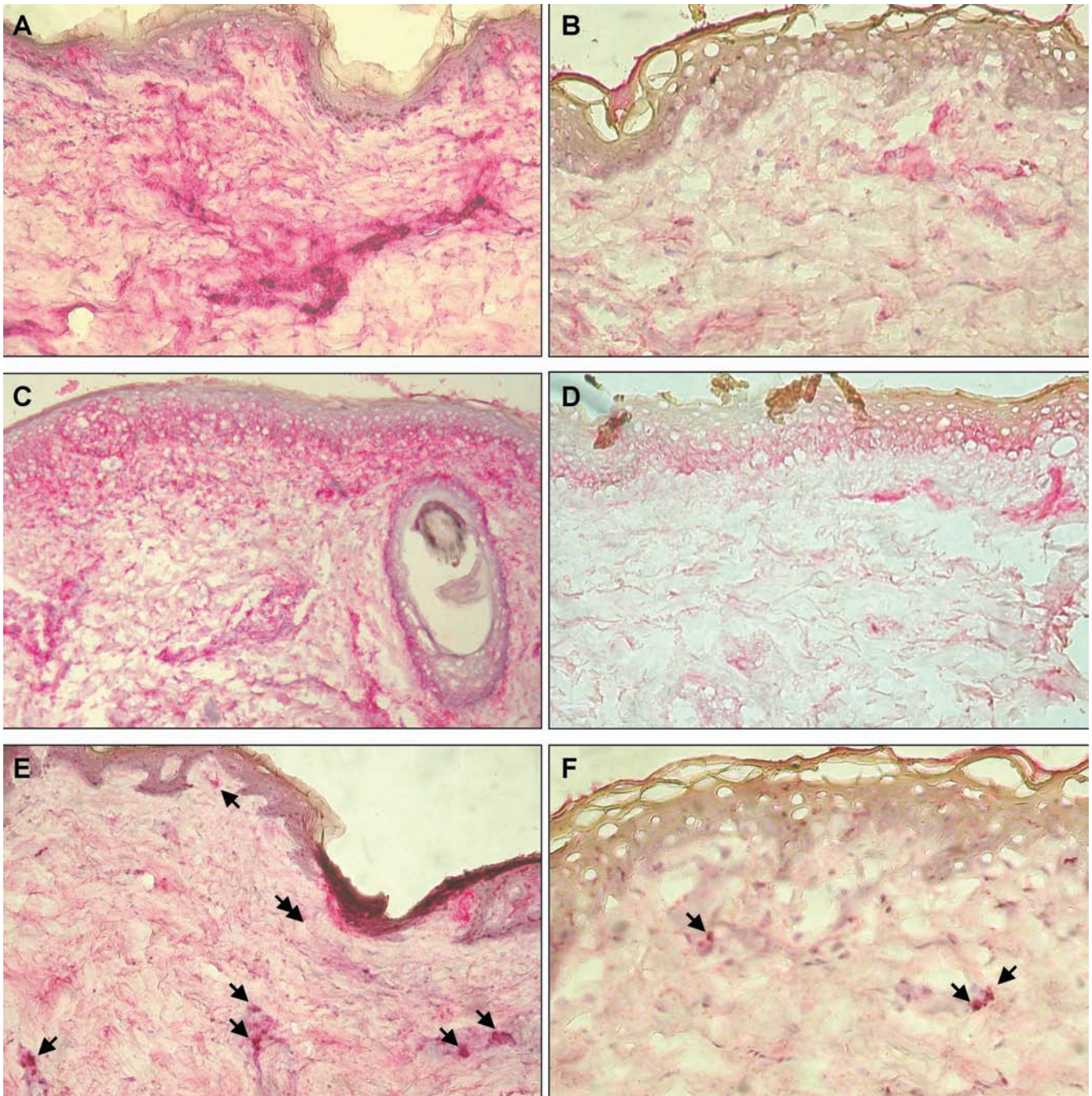


Figure 1. A. SCLE lesional skin: CD4+ cells strongly infiltrate the perivascular and interstitial dermis. B. SCLE, healthy sunprotected skin: sparse dermal CD4+ cells. C. SCLE lesional skin: basal and suprabasal keratinocytes, as well as many cells infiltrating the perivascular, periadnexal, interstitial, and junctional dermis are positive for the CD40 immunostaining. D. SCLE, healthy sunprotected skin: focal epidermal and weak perivascular positivity for CD40. E. SCLE, lesional skin: CD40L+ cells (arrows) infiltrating the perivascular and interstitial dermis. F. SCLE, healthy sunprotected skin: some CD40L+ cells (arrows) in the perivascular dermis. (Immunohistochemistry, original magnification $\times 200$.)

Although weaker than those observed in SCLE lesions, the level of positivity detected for CD40 and CD40L in healthy sunprotected skin of the same patients overlapped with that detected in the healthy sunprotected skin of patients with DLE and DM. Instead, the healthy skin of healthy controls showed,

as expected, a weak epidermal staining for CD40, and was negative for CD40L. These observations suggest that, in the skin of patients with SCLE, the activation of the CD40/CD40L system is almost in part independent from the UV light and/or stimulatory factors acting within lesional skin.

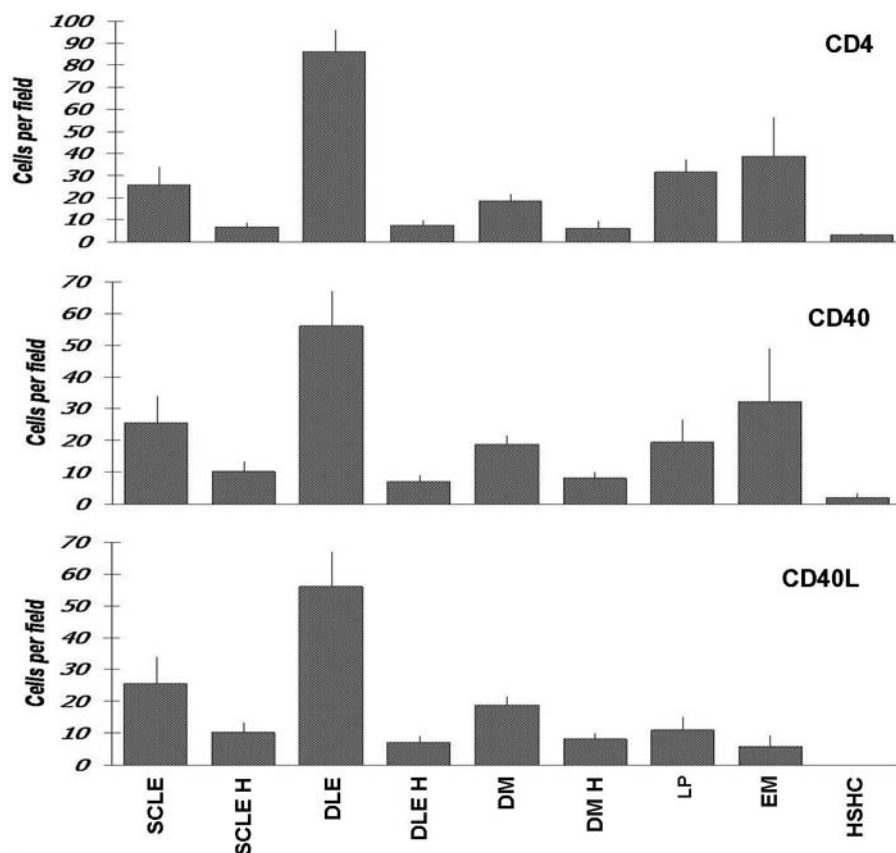


Figure 2. Means + standard deviations and statistical results of CD40-positive and CD40L-positive dermal cells in lesional subacute cutaneous lupus erythematosus (SCLE), compared with controls. H: healthy sun-protected skin; HSHC: healthy skin of healthy controls; DLE: discoid lupus erythematosus; DM: dermatomyositis; LP: lichen planus; EM: erythema multiforme.

Our study represents the first demonstration of the involvement of the CD40/CD40L system in SCLE. We point out that the overexpression of CD40L has been proposed to be a basal feature of patients with SLE, particularly women, and the methylation of the CD40L gene (located on the X chromosome) relates inversely to the level of disease activity¹⁷. It has been shown that CD40L is overexpressed on peripheral lymphocytes of patients with SLE¹⁸, while its soluble form is elevated in SLE sera¹⁹. Since CD40L may contribute to the production of autoantibodies in SLE, e.g., anti-double-stranded DNA antibodies^{19,20}, we hypothesize that an abnormal expression and/or activation of the CD40/CD40L system may also contribute to autoantibody production in SCLE.

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