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Cutting Edge: Rapid Accumulation of Epidermal CCL27 in Skin-Draining Lymph Nodes following Topical Application of a Contact Sensitizer Recruits CCR10-Expressing T Cells

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Abstract

CC chemokine receptor 10 and its ligand, CCL27, are important components of T cell-mediated cutaneous immunity, but whether they influence lymph node (LN) homing by T cells is unknown. In this study, CCL27 protein was detected in skin-draining LN by Western blotting and ELISA although CCL27 mRNA transcripts were low. CCL27 protein was present at higher levels in skin-draining LN compared with gut-draining LN and spleen. A single topical treatment of mouse skin with the contact sensitizer 2,4-dinitro-1-fluorobenzene (DNFB) resulted in a 13-fold increase in CCL27 protein accumulation in skin-draining LN within 1 h and a 5-fold elevation in CCR10 mRNA (normalized to the T cell marker CD2) within 6h. DNFB treatment also resulted in rapid depletion of ~75% of CCL27 from the epidermis. In summary, we describe a novel mechanism for the recruitment of CCR10-positive T cells to skin-draining LN following the rapid release of preformed CCL27 from the epidermis.

For all mammals, the epidermis forms the first major barrier against external pathogens and toxins. Besides providing a physical barrier, keratinocytes express molecules such as the defensins (1) that kill a variety of bacterial and viral pathogens, cytokines (e.g., IL-1α), and pathogen-associated molecular pattern receptors (e.g., TLRs) (2), which trigger innate immune responses and facilitate the initiation of adaptive immune responses. β-Defensin molecules clearly have chemotactic activity and bind to the CCR6 chemokine receptor (1), but the epidermis produces at least 10 additional CC and CXC chemokines (3).

CCL27 is selectively produced in skin and is one of two known ligands for CCR10, a chemokine receptor expressed by a subset of skin-homing memory T cells (4–6), activated melanocytes, and melanoma cells (7). Unlike many inflammatory chemokines, CCL27 is abundant in resting human and murine epidermal keratinocytes and appears to be only

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Disclosures

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moderately increased in inflamed skin (4, 8). Injection of recombinant CCL27 into the dermis increases the number of T cells in murine skin (8). In mouse models, CCR10 and/or CCR4 play major roles in T cell-mediated models of inflammatory skin disease depending on the particular model used (8, 9). CCL27 and CCR10 may also be important mediators of T cell homing in cutaneous graft-vs-host disease (10) and atopic dermatitis (11).

The physiologic importance of constitutively produced CCL27 in uninflamed epidermis has not been investigated. We hypothesized that this store of epidermal CCL27 might regulate T cell trafficking in the skin and, potentially, in skin-draining lymph nodes (LN)[3] as well. Indeed, other chemokines injected into skin alter the recruitment and motility of immune cells in draining LN (12, 13). To address this hypothesis, we explored the kinetics of CCL27 depletion from skin and its subsequent accumulation in skin-draining LN following the topical application of the experimental contact sensitizing agent, 2,4-dinitro-1-fluorobenzene (DNFB). Our results indicate that preformed epidermal CCL27 is rapidly released upon DNFB stimulation and accumulates in skin-draining LN, where it acts to alter T cell trafficking.

Materials and Methods

Animals

Female C57BL/6 mice (6–9 wk old) were used in all experiments, which were approved by the National Cancer Institute Animal Use and Care Committee (Bethesda, MD).

Skin irritation model

Hair was removed from the back skin of mice by shaving with an electric clipper followed by application of a topical depilatory agent. After 2–3 days, DNFB (0.5% (w/v); Fluka Biochemica) in 1:4 olive oil:acetone was applied (50 μl) to the upper back of shaved mice.

Preparation of LN and skin cell lysates

Skin-draining LN were harvested and then mechanically disrupted with a rubber-tipped syringe plunger in 500 μl of PBS containing 0.05% (v/v) Tween 20. The crude cell suspension was then passed through a 40-μm spleen filter. Following sonication and centrifugation, soluble protein in buffer was removed for further analysis. Care was taken throughout this process to retain as much of the original buffer as possible to ensure the accuracy of the ELISA measurements that followed.

For the determination of chemokine content in skin, shaved dorsal skin samples (~1 cm^2) were treated with Liberase (330 μg/ml; Roche) in 1 ml of DMEM (Invitrogen) for 45 min at 37°C. The skin cells were then mechanically disaggregated using a Medimachine (BD Biosciences) for 6 min in 0.5 mg/ml deoxyribonuclease I (Sigma) (total volume 1 ml of DMEM with 10% FCS). The cell suspension was then passed through a 100-μm spleen filter and centrifuged at 1200 rpm for 5 min. A cell lysate was made by resuspension of the cell pellet in PBS containing 0.05% Tween 20 followed by brief sonication. The lysate was then

[3]Abbreviations used in this paper: LN, lymph node; DNFB, 2,4-dinitro-1-fluorobenzene.

subjected to centrifugation to remove cellular debris after which the supernatant was collected for ELISA.

Detection of mouse and human chemokines by ELISA

ELISA for murine and human CCL27 involved species-specific capture and biotinylated detection of Ab pairs (R&D Systems). ABTS was used as a chromogen and was detected at 405 nm. CCL21, CCL27, and vascular endothelial growth factor C concentrations were measured in undiluted lymphatic fluid and serum from healthy volunteers and patients with chronic obstructive lymphedema (see below) using human ELISA kits (R&D Systems).

Immunofluorescence microscopy

Staining for CCL27 was accomplished using frozen sections of mouse skin. Sections were air dried after 5 min of fixation in ice-cold acetone and blocked for 1 h in 5% goat serum in PBS containing 1% BSA at room temperature. A rat anti-mouse CCL27 mAb (20 μg/ml; MAB7251, R&D Systems) was applied for 2 h at room temperature after which sections were washed (2×) for 5 min in PBS. Alexa-586-conjugated goat anti-rat Ig Abs (5 μg/ml) were then applied for 45 min followed by washing (2×) and visualization.

Quantitative RT-PCR

Quantitative RT-PCR was performed as previously described (14) using the following murine-specific primer pairs: CCR10, 5′-GGGGATGAAGAGGACGCATACT-3′ (forward) and 5′-CCTGGACATCGGCCTTGT-3′ (reverse); CCL27, 5′-CTGCTGAGGAGGATTGTCCAC-3′ (forward) and 5′-CACGACAGCCTGGAGGTGA-3′ (reverse). Results were normalized to the expression of GAPDH (5′-ACCCACTCCTCCACCTTTGA-3′ (forward) and 5′-CATACCAGGAAATGAGCTTGACAA-3′ (reverse)) or CD2, (5′-CGTATGAGGTCTTAGCAAACG-3′ (forward) and 5′-CAAGAGCACCAAGAGGAGTCC-3′ (reverse)).

Measurement of CCL27 in human lymphatic fluid

Human CCL27 was measured by ELISA in lymphatic fluid obtained from healthy volunteers (age: 22–26 years) and from patients with a history of chronic, obstructive lymphedema (age: 25–60 years), who had a history of inflammatory dermatolymphadenitis and lower limb swelling (without cutaneous inflammation). By lymphoscintigram, these patients showed dilated lymphatic vessels and slow lymphatic flow.

For lymphatic fluid collection, a superficial lymphatic vessel was surgically exposed at the medial aspect of calf (15). This vessel was cannulated and lymph was collected by retrograde flow over 24 h (to minimize differences in capillary filtration rate due to positioning effects) into heparinized tubes at 2-h intervals as described (15, 16). All human subject experiments were performed with the approval of institutional authorities by two of the authors (W.L.O. and M.Z.).

Results

Expression of CCL27 mRNA in skin and LN

Constitutive CCL27 expression in skin has been documented by immunohistochemical staining and mRNA analysis (4). To determine the relative level of expression of CCL27 in the skin-draining LN vs skin, we performed quantitative RT-PCR of cell suspensions prepared from those tissues. The skin suspension yielded 100-fold greater relative mRNA expression of CCL27 than did LN (Fig. 1A). Interestingly, CCL27 protein was readily detected in skin-draining LN of wild-type mice by Western blotting (Fig. 1B) and by ELISA (Fig. 2A). These paradoxical results might be explained by the accumulation of skin-derived CCL27 via the afferent lymphatic vessels. Cannulation of murine lymphatic vessels is not possible because of the small size of these vessels. Analysis of human lymphatic fluid, however, by ELISA demonstrated that CCL27 (as well as CCL21) was detectable in human lymphatic fluid (Table 1). Of note, CCL27 was markedly elevated in the lymphatic fluid of patients with chronic obstructive lymphedema. Moreover, in these same patients serum CCL27 was substantially lower, suggesting that CCL27 may be produced primarily in skin and that it may access the systemic circulation via the lymphatics.

Measurement of CCL27 by ELISA in murine tissues, excluding skin, revealed that CCL27 expression (normalized to total protein in the tissue) was highest in the skin-draining peripheral LN that were tested (i.e., brachial/axillary, cervical, and popliteal), 2- to 8-fold lower in mesenteric LN (depending on which skin-draining LN were sampled), and > 10-fold lower in spleen, liver, and lung. In aggregate, these data suggested that CCL27 protein preferentially accumulated in skin-draining peripheral LN and that the lymphatic system was likely required for skin-derived CCL27 to enter the systemic circulation.

Regulation of CCL27 protein content in skin-draining LN by topical application of DNFB

To determine whether CCL27 present in the epidermis could be released from keratinocytes and whether the released CCL27 was recoverable in skin-draining LN, we applied a commonly used contact sensitizer, DNFB, in a vehicle consisting of olive oil and acetone to shaved mouse skin, assuming that the priming of T cells to DNFB may be accompanied by the homing of CCR10-positive T cells to skin-draining LN. CCL27 protein content in draining LN increased by ~ 10-fold within 1 h of treatment, remained elevated at 6 h, and returned to baseline levels within 24 h (Fig. 2A). By contrast, minimal changes in CCL27 mRNA levels in skin-draining LN were detected following the application of DNFB (Fig. 2B). To determine whether known skin irritants had the same effect as DNFB, we applied 10% SDS (17) in water topically to skin under similar conditions and found no increase in the level of CCL27 in draining LN (data not shown). Moreover, CCL27 protein levels in mesenteric LN did not substantially change with the application of DNFB, 10% SDS, or PBS compared with no treatment at all (i.e., naive mice), suggesting that increases in CCL27 were specific to the skin-draining LN. Thus, despite low levels of CCL27 mRNA expression in skin-draining LN, CCL27 protein increases dramatically in skin-draining LN upon topical exposure of skin to DNFB.

Rapid depletion of CCL27 from epidermal keratinocytes following exposure to DNFB

The influx of CCL27 to the draining LN was too rapid to be a result of de novo protein synthesis, particularly given the small changes in CCL27 mRNA observed in the LN. Thus, we reasoned that CCL27 present in keratinocytes in resting skin may be released upon topical application of contact allergens (or irritants) and that this CCL27 then accumulated in dermal lymphatics and skin-draining LN. To test this hypothesis, we probed resting and DNFB-treated skin for CCL27 protein using a specific mAb. As reported by others (4), CCL27 protein was abundant in resting dorsal skin of mice. CCL27 staining, however, was markedly reduced within 30 min (Fig. 3A) and 1 h (not shown) of exposure to DNFB. At both time points we detected focal CCL27 staining in the dermis, some of which was localized next to podoplanin-positive cells (data not shown), indicating that CCL27 might be present in lymphatic vessels. To quantify the loss of CCL27 from the epidermis, cell suspensions were prepared from resting and treated full thickness skin and then analyzed for CCL27 by ELISA. Within 1 h of DNFB treatment, total CCL27 in the skin was reduced by at least 75% (Fig. 3B). Quantitative RT-PCR indicated that no significant reduction in CCL27 mRNA expression had occurred following DNFB treatment for 1 h (Fig. 3C), suggesting that the observed decrease in CCL27 protein (Fig. 3B) had not been due to a decrease in transcription. Thus, both qualitative and quantitative analysis indicated that CCL27 protein was rapidly lost from epidermal keratinocytes (and skin) upon exposure to DNFB.

CCL27 accumulates in skin-draining LN and attracts CCR10-positive T cells

Given the evidence above suggesting that skin-derived CCL27 was being transported to draining LN, we next examined the consequences of the transport of CCL27 to the LN. We measured CCR10 mRNA in skin-draining LN by quantitative RT-PCR and normalized its expression to mRNA levels for CD2, a specific marker for T cells, thus assessing relative CCR10 mRNA changes among CD2-expressing cells. Normalized CCR10 mRNA expression was increased by ~5-fold following DNFB application but decreased to baseline levels by 24 h postinjection (when CCL27 protein also returned to baseline levels; Fig. 2A), suggesting that the transport of recombinant human CCL27 resulted in the transient influx of CCR10-positive cells (presumably T cells) into the LN (Fig. 4). Thus, the transport of CCL27 to the skin-draining LN following exposure to DNFB is also temporally associated with increased numbers of CCR10-expressing T cells within the same LN.

Discussion

Prior work has shown that CCL27 attracts $CCR10^+$ skin-homing (CLA^+) T cells both in vitro (4) and in vivo (8). We have found that CCL27 can be rapidly released from the epidermis following DNFB exposure and that elevated levels of CCL27 in skin-draining LN are detected as early as 1 h following this treatment. Interestingly, accumulation of CCL27 protein in the LN after DNFB treatment was associated with an influx of $CCR10^+$ T cells to the LN that potentially could meet with skin DC that transport Ags. Indeed, treatment of mice with neutralizing CCL27 Abs prevented DNFB-specific contact dermatitis in mice (8).

Most keratinocyte-associated chemokines (e.g., CCL17) appear to be regulated at the transcriptional level following inflammatory stimuli (18). A recent report, however, suggests that CXCL16 is constitutively present in human and mouse epidermis where it may potentially act upon CXCR6-positive T cells (19). In contrast to the rapid release of CCL27 from the epidermis that we observed, CXCL16 is membrane-bound and appears to be released at a much slower rate (occurring over days) (19). The rapidity of CCL27 release from keratinocytes and subsequent accumulation in the LN place it with a class of molecules such as histamine (20) and P-selectin (21), which are rapidly deployed (within seconds or minutes) to quickly recruit inflammatory cells. Together with other chemokines, epidermal CCL27 likely contributes to the influx or composition of T cells and other leukocytes present in skin in inflammatory and neoplastic processes.

Two lines of evidence suggested that CCL27 is released at low levels from keratinocytes in untreated mice and healthy humans. First, we were able to detect and measure CCL27 protein in skin-draining LN, which showed higher CCL27 levels than non-skin-draining LN and tissues. The accumulation of CCL27 in skin-draining LN may also explain the higher rates of nodal metastasis we observed when CCR10-expressing B16 melanoma cells were injected in skin (7). Second, although we were unable to directly measure CCL27 in murine lymphatic fluid due to the small size of mouse lymphatic vessels, we found that afferent lymphatic fluid from healthy volunteers contains readily detectable amounts of CCL27, suggesting that there is baseline secretion of CCL27 from skin under normal homeostatic conditions. We speculate that these low levels of CCL27 from healthy skin may contribute to the recirculation of skin-homing, CCR10-positive T cells into skin-draining LN. Moreover, the dramatically elevated levels of CCL27 in the lymphatic fluid of patients with obstructive lymphedema suggest that entry of CCL27 into the systemic circulation is dependent on patent lymphatic channels through the LN.

Data from several groups suggest that the decoy chemokine receptor D6 may a play critical role in controlling leukocyte infiltration by binding to and sequestering inflammatory chemokines in the skin, particularly those that bind to CCR1–5 (22). D6 is unlikely to sequester CCL27, however, because heterologous radioligand displacement experiments have suggested that D6 does not bind to CCL27 (R. Nibbs and G. Graham, unpublished observations). Thus, CCL27, unlike some other inflammatory chemokines, may freely move from skin to LN to regulate T cell migration.

In summary, our data reveal that the release of CCL27 from keratinocytes (and subsequent accumulation in draining LN) is markedly accelerated following topical application of a contact sensitizer. In the draining LN, CCL27 potentially recruits $CCR10^{+}$ cells to skin-draining LN, which may engage with Ag-bearing dendritic cells from skin or LN dendritic cells that capture soluble Ags entering via afferent lymphatic vessels. Recognition of this pathway provides further impetus to develop small-molecule inhibitors of CCR10 in an effort to improve specific inhibition of T cell-mediated inflammation in the skin.

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References

1. Yang D, Chertox O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schröder JM, Wang JM, Howard OMZ, and Oppenheim J. 1999 β-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286: 525–528. [PubMed: 10521347]
2. Iwasaki A, and Medzhitov R. 2004 Toll-like receptor control of the adaptive immune responses. Nat. Immunol 5: 987–995. [PubMed: 15454922]
3. Schön MP, and Ruzicka T. 2001 Psoriasis: the plot thickens. Nat. Immunol 2:91. [PubMed: 11175798]
4. Morales J, Homey B, Vicari AP, Hudak S, Oldham E, Hedrick J, Orozco R, Copeland NG, Jenkins NA, McEvoy LM, and Zlotnik A. 1999 CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc. Natl. Acad. Sci. USA 96: 14470–14475. [PubMed: 10588729]
5. Baird JW, Nibbs RJ, Komai-Koma M, Connolly JA, Ottersbach K, Clark-Lewis I, Liew FY, and Graham GJ. 1999 ESkine, a novel β-chemokine, is differentially spliced to produce secretable and nuclear targeted isoforms. J. Biol. Chem 274: 33496–33503. [PubMed: 10559234]
6. Jarmin DI, Rits M, Bota D, Gerard N, Graham GJ, Lewis IC, and Gerard C.2000 Cutting Edge: identification of the orphan receptor G-protein-coupled receptor 2 as CCR10, a specific receptor for the chemokine ESkine. J. Immunol 164: 3460–3464. [PubMed: 10725696]
7. Murakami T, Cardones AR, Finkelstein SE, Restifo NP, Klaunberg BA, Nestle FO, Castillo SS, Dennis PA, and Hwang ST. 2003 Immune evasion by murine melanoma mediated through CC chemokine receptor-10. J. Exp. Med 198: 1337–1347. [PubMed: 14581607]
8. Homey B, Alenius H, Muller A, Soto H, Bowman EP, Yuan W, McEvoy L, Lauerma AI, Assmann T, Bunemann E, et al. 2002 CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nat. Med 8: 157–165. [PubMed: 11821900]
9. Reiss Y, Proudfoot AE, Power CA, Campbell JJ, and Butcher EC. 2001 CC chemokine receptor (CCR)4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. J. Exp. Med 194: 1541–1547. [PubMed: 11714760]
10. Faaij CM, Lankester AC, Spierings E, Hoogeboom M, Bowman EP, Bierings M, Revesz T, Egeler RM, van Tol MJ, and Annels NE. 2006 A possible role for CCL27/CTACK-CCR10 interaction in recruiting CD4 T cells to skin in human graft-versus-host disease. Br. J. Haematol 133: 538–549. [PubMed: 16681643]
11. Chen L, Lin SX, Agha-Majzoub R, Overbergh L, Mathieu C, and Chan LS. 2006 CCL27 is a critical factor for the development of atopic dermatitis in the keratin-14 IL-4 transgenic mouse model. Int. Immunol 18: 1233–1242. [PubMed: 16735375]
12. Stein JV, Rot A, Luo Y, Narasimhaswamy M, Nakano H, Gunn MD, Matsuzawa A, Quackenbush EJ, Dorf ME, and von Andrian UH. 2000 The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6Ckine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. J. Exp. Med 191: 61–76. [PubMed: 10620605]
13. Worbs T, Mempel TR, Bolter J, von Andrian UH, and Forster R. 2007 CCR7 ligands stimulate the intranodal motility of T lymphocytes in vivo. J. Exp. Med 204: 489–495. [PubMed: 17325198]
14. Kakinuma T, Nadiminti H, Lonsdorf AS, Murakami T, Perez BA, Kobayashi H, Finkelstein SE, Pothiawala G, Belkaid Y, and Hwang ST. 2007 Small numbers of residual tumor cells at the site of primary inoculation are critical for anti-tumor immunity following challenge at a secondary location. Cancer Immunol. Immunother 56: 1119–1131. [PubMed: 17139493]
15. Olszewski WL, Engeset A, and Sokolowski J. 1977 Lymph flow and protein in the normal male leg during lying, getting up, and walking. Lymphology 10: 178–183. [PubMed: 563502]
16. Olszewski WL, Pazdur J, Kubasiewicz E, Zaleska M, Cooke CJ, and Miller NE. 2001 Lymph draining from foot joints in rheumatoid arthritis provides insight into local cytokine and

chemokine production and transport to lymph nodes. Arthritis Rheum. 44: 541–549. [PubMed: 11263768]

17. Li LF, Fiedler VC, and Kumar R. 1998 Down-regulation of protein kinase C isoforms in irritant contact dermatitis. Contact Dermatitis 38: 319–324. [PubMed: 9687030]
18. Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, and Larsen CG. 2000 A Th2 chemokine, TARC, produced by keratinocytes may recruit $CLA^{+}CCR4^{+}$ lymphocytes into lesional atopic dermatitis skin. J. Invest. Dermatol 115: 640–646. [PubMed: 10998136]
19. Scholz F, Schulte A, Adamski F, Hundhausen C, Mittag J, Schwarz A, Kruse ML, Proksch E, and Ludwig A. 2007 Constitutive expression and regulated release of the transmembrane chemokine CXCL16 in human and murine skin. J. Invest. Dermatol 127: 1444–1455. [PubMed: 17363916]
20. MacGlashan D Jr. 2003 Histamine: a mediator of inflammation. J. Allergy Clin. Immunol 112: S53–S59. [PubMed: 14530789]
21. Wagner DD 1993 The Weibel-Palade body: the storage granule for von Wille-brand Factor and P-selectin. Thromb. Haemost 70: 105–110. [PubMed: 7694385]
22. Martinez de la Torre Y, Buracchi C, Borroni EM, Dupor J, Bonecchi R, Nebuloni M, Pasqualini F, Doni A, Lauri E, Agostinis C, et al. 2007 Protection against inflammation- and autoantibody-caused fetal loss by the chemokine decoy receptor D6. Proc. Natl. Acad. Sci. USA 104: 2319–2324. [PubMed: 17283337]

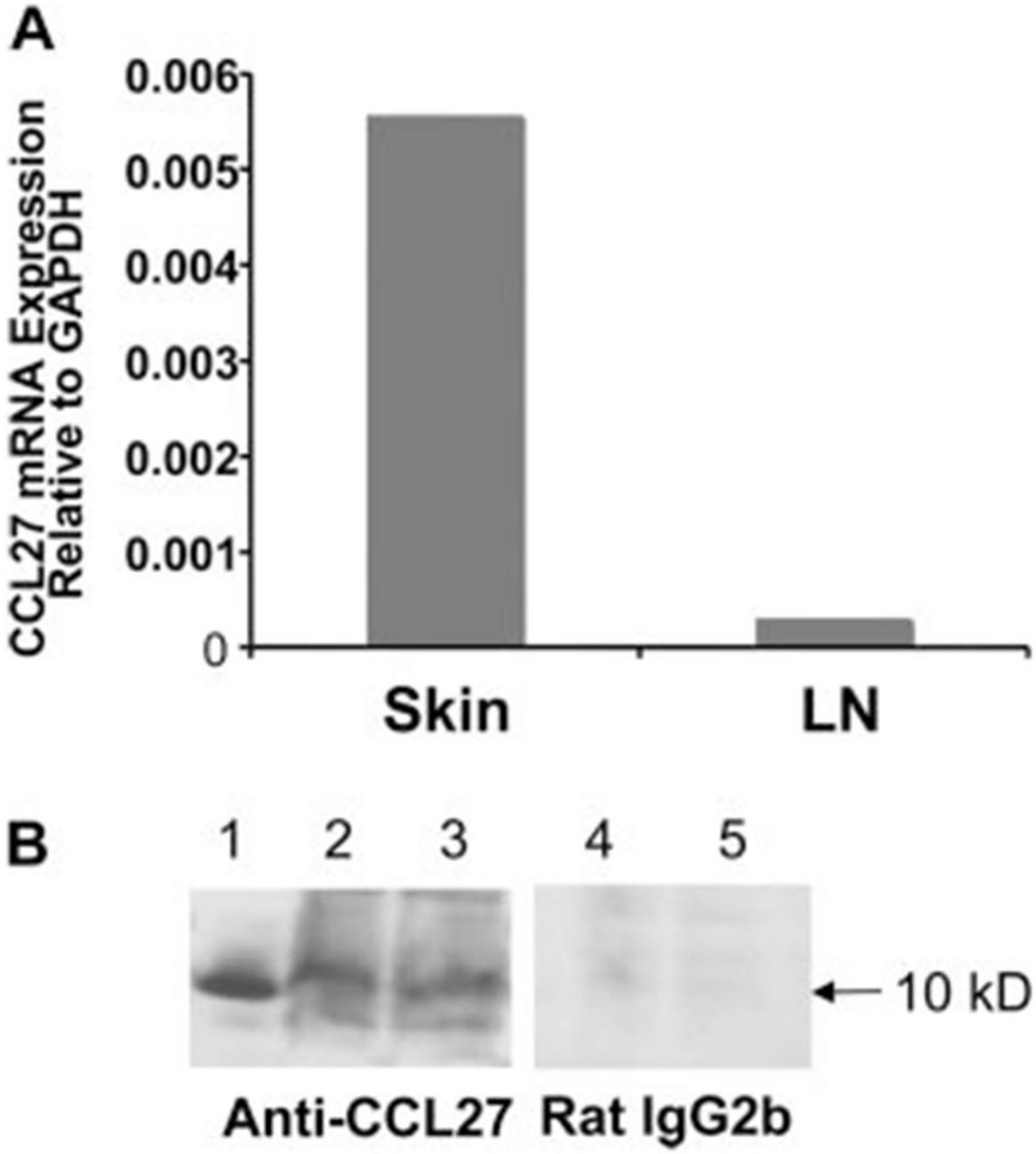

FIGURE 1.

CCL27 protein, but not mRNA, is present in skin-draining LN. *A*, Quantitative RT-PCR shows expression of CCL27 mRNA in skin-draining LN and skin. *B*, Western blotting of recombinant mCCL27 (*lanes 1* and *4*) and either 20 μl (*lane 2*) or 30 μl (*lanes 3* and *5*) of LN lysate was performed with either specific anti-mCCL27 mAb or rat IgG2b isotype control as indicated. Pooled axillary and brachial LN were used in both experiments.

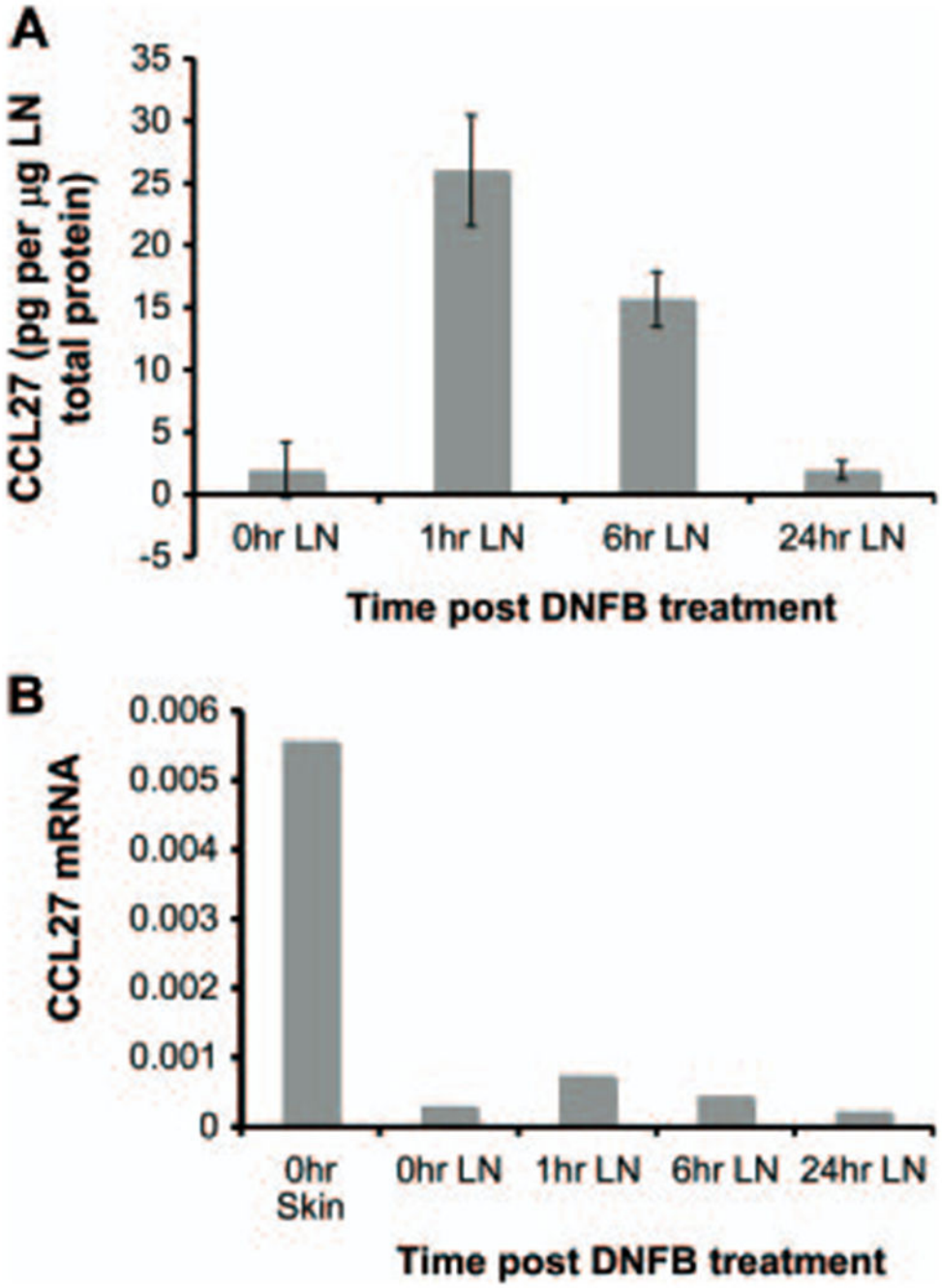

FIGURE 2.

CCL27 protein, but not mRNA, rapidly increases following exposure of skin to DNFB. CCL27 protein (*A*) and mRNA (*B*) was measured by ELISA and RT-PCR, respectively in pooled axillary and brachial LN draining dorsal back skin that had been treated with 0.5% DNFB in olive oil/acetone.

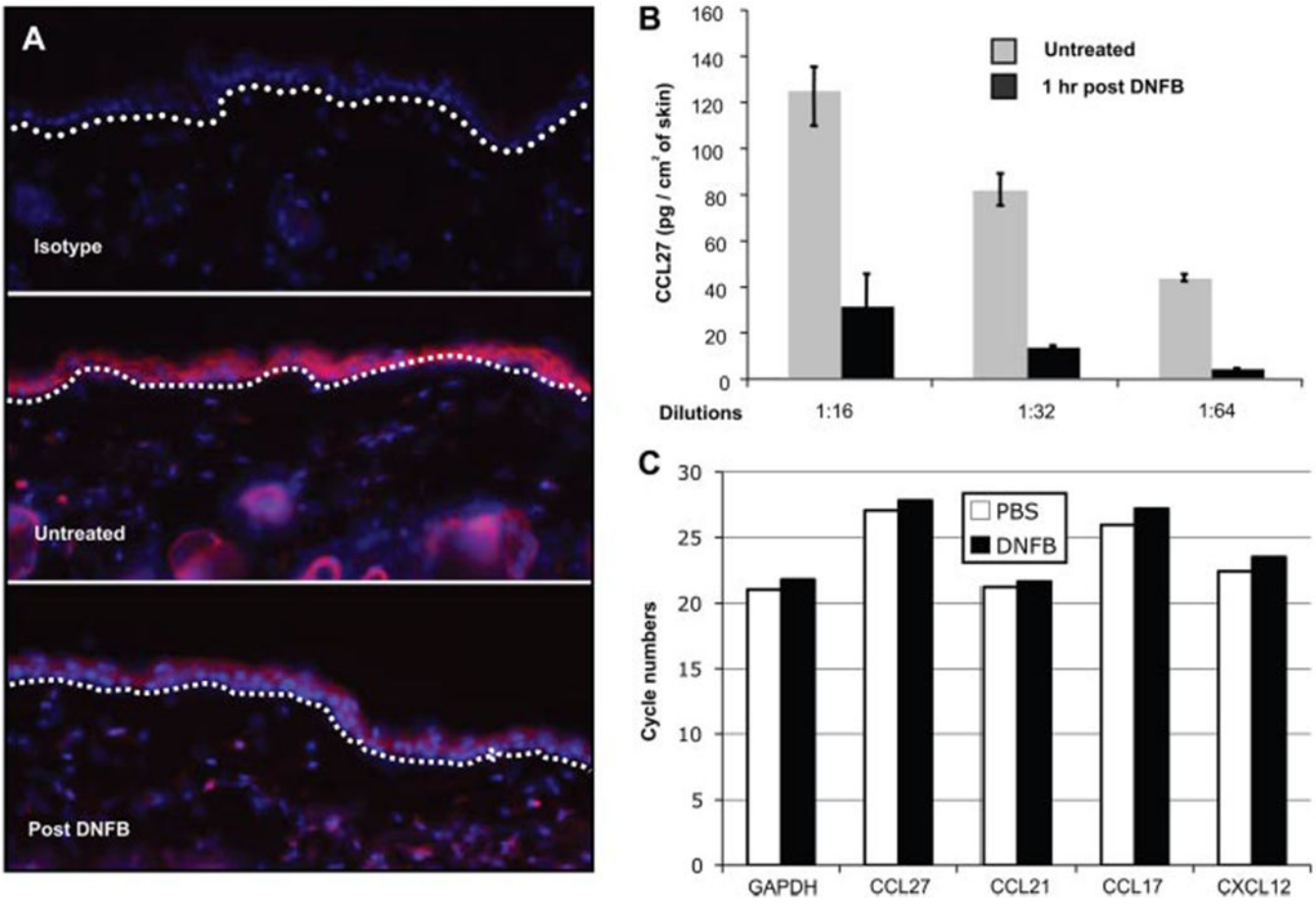

FIGURE 3.

Assessment of skin-localized CCL27 before and after topical application of DNFB. *A*, Eight-micrometer sections were taken from the dorsal skin of C57BL6 mice 30 min after 0.5% DNFB treatment (*bottom panel*) or from untreated control mice (*top* and *middle panels*) and stained with mAb against CCL27 (red) or isotype-matched control mAb (*top panel*). Dotted lines indicate the approximate level of basement membrane separating epidermis (*above*) from dermis (*below*). Nuclei (blue) were counter stained with DAPI. *B*, Quantification of skin-associated CCL27 by ELISA of untreated and DNFB-treated skin was performed at the indicated ratio of skin cell suspension to buffer (see Materials and Methods). *C*, Quantitative mRNA expression (RT-PCR) of the indicated genes in mouse skin was performed following topical treatment with 0.5% DNFB or PBS for 1 h. The cycle number (average of two samples) indicates the point during amplification when consistent linear detection of PCR products was first recorded. Lower cycle numbers reflect greater initial amounts of mRNA for a given gene.

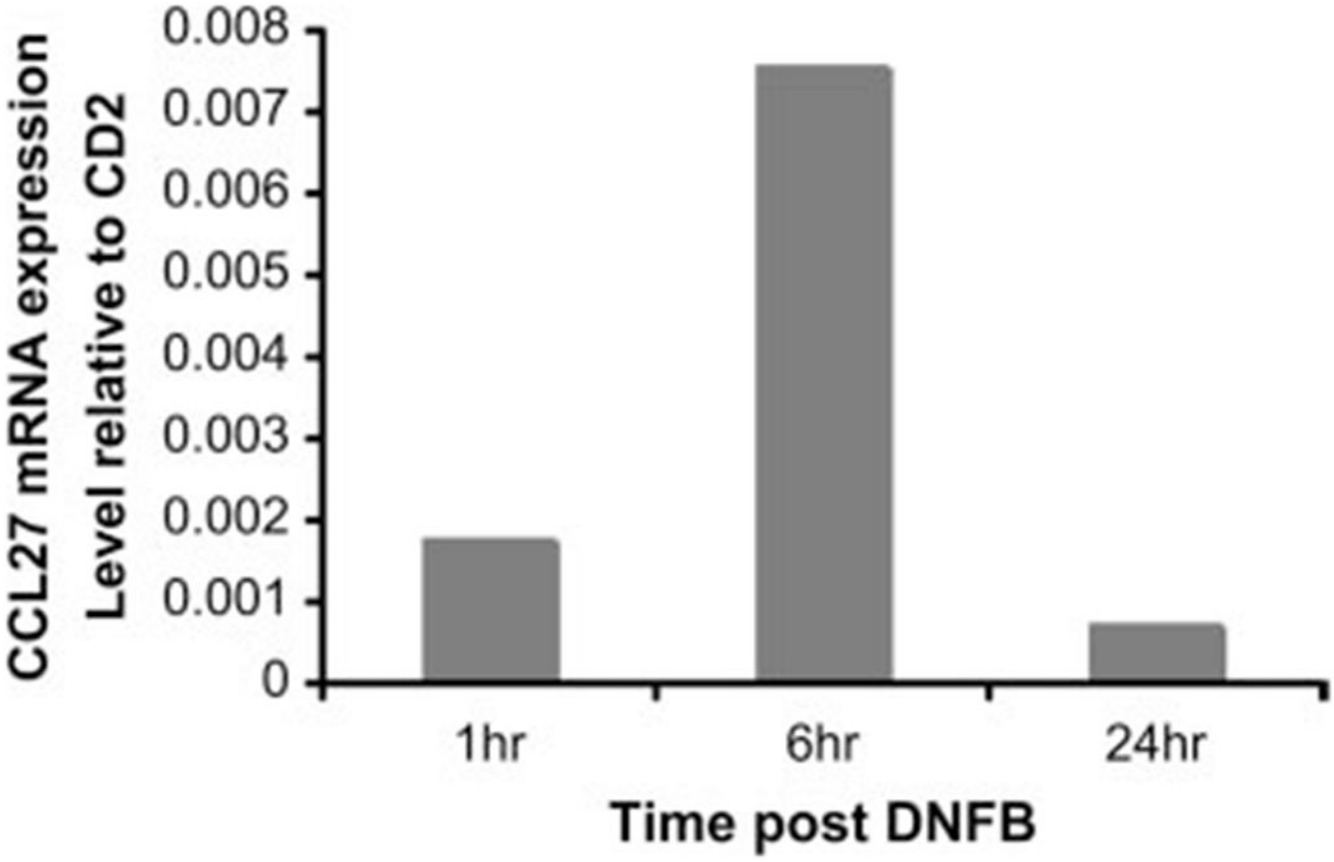

FIGURE 4.

Time course of CCR10 mRNA expression in draining LN following application of DNFB. DNFB was applied to the dorsal back skin of shaved mice. At the indicated time points, pooled draining axillary and brachial LN were harvested for RNA isolation and quantitative RT-PCR. CCL27 mRNA expression was normalized to mRNA expression of the T cell marker CD2.

Table 1.

CCL27 and CCL21 expression in serum and lymphatic fluid of healthy controls and patients with chronic obstructive lymphedema[a]

	CCL27 Serum (*n* = 10)	CCL21 Serum (*n* = 15)	CCL27 Lymph (*n* = 10)	CCL21 Lymph (*n* = 15)
Healthy controls	621 ± 223^	297 ± 137	181 ± 29 *	885 ± 514
Lymphedema patients	33 ± 40^	410 ± 191	732 ± 792 *	643 ± 628

[a]Undiluted sera and lymphatic fluid were collected and assayed by ELISA for human CCL27 and CCL21 as described in Materials and Methods. Units are pg/ml ± SD. Measurements of CCL21 included the same 10 patients used for the assessment of CCL27 plus five additional patients and controls.

^ and * indicate statistical significance for CCL27 serum and lymph, respectively, between the values for healthy controls vs lymphedema patients by Student's *t* test at $p < 0.001$.