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Dominant Th1 and minimal Th17 skewing in discoid lupus revealed by transcriptomic comparison with psoriasis

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Abstract

Discoid lupus erythematosus (DLE) is the most common skin manifestation of lupus. Despite its high frequency in systemic lupus in addition to cases without extracutaneous manifestations, targeted treatments for DLE are lacking, likely due to a dearth of knowledge of the molecular landscape of DLE skin. Here, we profiled the transcriptome of DLE skin in order to identify signaling pathways and cellular signatures that may be targeted for treatment purposes. Further comparison of the DLE transcriptome to that of psoriasis, a useful reference given our extensive knowledge of molecular pathways in this disease, provided a framework to identify potential therapeutic targets. Although a growing body of data supports a role for IL-17 and Th17 cells in systemic lupus, we show a relative enrichment of IFN-γ-associated genes without that for IL-17-associated genes in DLE. Extraction of T cells from the skin of DLE patients identified a predominance of IFN-γ-producing Th1 cells and an absence of IL-17-producing Th17 cells, complementing the results from whole skin transcriptomic analyses. These data therefore support investigations into treatments for DLE that target Th1 cells or the IFN-γ signaling pathway.

Introduction

Lupus erythematosus (LE) is a heterogeneous disease whose hallmark is the formation of circulating autoantibodies. Systemic LE (SLE) is a multiorgan form of LE with

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Conflict of Interest

The authors state no conflict of interests.

manifestations that can variably affect the skin, joints, kidneys and CNS, among other organ systems. The etiology of SLE is multifactorial, and both genetic and environmental influences are believed to contribute.

The most common specific skin manifestation of lupus is discoid LE (DLE), and its presence is one of the eleven American College of Rheumatology diagnostic criteria for SLE (Tan *et al.*, 1982). DLE represents up to 80% of all cutaneous lupus cases (Gronhagen *et al.*, 2011), and this skin manifestation is notable for its tendency to cause disfigurement, alopecia, and scarring (Rothfield *et al.*, 1963). Approximately 1 in 4 patients with SLE have DLE (Sanchez *et al.*, 2011; Uramoto *et al.*, 1999), and, in some DLE cases, the skin can be the only end-organ affected without extracutaneous involvement. Interestingly, a recent observational, population-based study of over 1000 cutaneous lupus patients noted an overall rate of progression to SLE of 18% within a three year follow-up period (Gronhagen *et al.*, 2011). The ease of sampling affected skin potentially makes DLE a convenient and accessible model to study end-organ pathology in SLE.

Currently, antimalarials are the most commonly used systemic treatment for cutaneous lupus, despite the fact that the mechanism of action of this class of drugs is not completely understood (Wallace *et al.*, 2012). Further, a need exists for new treatments because many patients do not respond to antimalarials or other available immunosuppressants. Insights into the pathophysiology of damage to this end-organ have the potential to identify therapeutic targets and new treatments.

Initial studies have focused on the types of cells present in DLE lesions. Histopathologically, a dense perivascular, periadnexal lymphocytic infiltrate with interface dermatitis can be appreciated, with most analyses indicating CD4 T cells are the predominant inflammatory cell type. A growing body of evidence supports the importance of Th17 CD4 T cells in SLE pathogenesis (Alunno *et al.*, 2012; Brajac *et al.*, 2010; Chen *et al.*, 2010; Henriques *et al.*, 2010; Mok *et al.*, 2010; Pan *et al.*, 2008; Shah *et al.*, 2010), although comparisons of the relative frequencies of T helper subtypes in DLE have not been rigorously undertaken. Targeting the specific subset of T cells or their effector cytokines that are critical to the pathogenesis of DLE is a strategy for the design of new therapeutic agents, as has been done for other cutaneous autoimmune diseases. Psoriasis, now known to be a Th17-mediated disease, is a clear example; ustekinumab, a monoclonal antibody that blocks Th17- and Th1-related pathways (Leonardi *et al.*, 2008; Papp *et al.*, 2008) was recently approved for the treatment of psoriasis, and two IL-17/Th17-pathway inhibitors were recently shown to be efficacious in clinical trials (Leonardi *et al.*, 2012; Papp *et al.*, 2012).

We were interested in identifying global perturbations in immune related pathways or immune cell signatures that were present in skin lesions of DLE. Here, we provide a molecular description of the transcriptome of DLE. Analysis of activated gene expression pathways was performed, identifying a preponderance of immune-related pathway activation. Next, we compared the transcriptional profile of DLE skin with psoriasis, the most well-characterized autoimmune skin disease to date and therefore a useful basis for comparison. Our interest was to characterize the type of T cells infiltrating DLE lesions. Our reference skin disease, psoriasis, is caused by interactions between skin keratinocytes and T

cells that make up the Th17, Th22, and Th1 subsets. In contrast, we report relatively minimal Th17 signatures, as ascertained by Gene Set Enrichment Analysis, gene expression and T cell protein production, but rather skewing towards a predominantly Th1 signature in whole skin and infiltrating T cells in DLE. We thus provide a rationale to exploring targeting cells and molecular pathways associated with the formation and function of Th1 cells.

Results

In order to define the transcriptional profile of discoid lupus skin lesions, we first performed punch biopsies of the affected skin of discoid lupus patients and healthy control patients. RNA extracted from skin samples was processed and hybridized to GeneChip Human Genome U133 2.0 microarrays. Gene lists of differentially expressed genes between DLE and normal skin were generated using criteria of greater than two-fold change and less than 0.05 false discovery rate and subsequently interrogated using Ingenuity Pathway Analysis. The majority of pathways were indicative of changes in immune-related pathways and immune cell signatures (Figure 1A). Upregulated pathways include that for “SLE signaling” and “Interferon signaling,” consistent with reports of a prominent interferon signature in the peripheral blood of patients with SLE (Baechler *et al.*, 2003; Bauer *et al.*, 2006; Bennett *et al.*, 2003; Kirou *et al.*, 2004) as well as the upregulated interferon genes in DLE lesions reported by others (Wenzel *et al.*, 2005b). Quantitative PCR confirmations supported increased cellular signatures of T cells (CD3D), CD8 T cells (CD8A, GZMB, GNLY), B cells (IGJ), macrophages (CD163), natural killer cells (KLRK1, KLRD1), and dendritic cells (CD86, SIGLEC1, MR1) as well as interferon (MX1, OASL, STAT1), IL-15, and IL-1β signatures. DLE skin, therefore, is marked by potent induction of immune pathways and immune cell signatures.

Because of the high number of pathways and immune cell signatures that were present in our analysis of the DLE transcriptional profile, a well described inflammatory skin disease, psoriasis, was used as a reference for comparison. Although histological and immunohistochemical features of the two diseases are well-known, a comparative tissue-level appraisal of these disease entities has not previously been done and provides context to our molecular analyses. Histological and immunohistochemical comparisons highlighted differences in these skin diseases (Figure 2). In psoriasis, a superficial perivascular infiltrate is appreciated on hematoxylin and eosin staining with overlying hyperplasia of the epidermis, whereas prominent dermal edema and inflammation of the dermo-epidermojunction is appreciated in DLE. DLE was noted to have more inflammatory cell infiltrates of $CD3^+$ and $CD11c^+$ cells. Also, differences were noted in cellular localization; $CD8^+$ infiltrates were predominantly epidermal in psoriasis compared with a dermal preponderance in DLE (Figure 2). It is clear that, despite both entities being autoimmune skin diseases, differences exist in the relatively quantities and tissue localizations of immune cells.

A global comparison of the transcriptional profiles of DLE and psoriasis was performed to identify contrasting elements. DLE and psoriasis samples formed distinct groups on Principal Components Analysis plots, indicative of distinct molecular profiles (Figure 3A).

By comparing 'DLE versus normal skin' and 'psoriasis versus normal skin,' we found that 591 genes were uniquely upregulated and 358 genes were uniquely downregulated in DLE, and, in psoriasis, 770 genes were uniquely upregulated and 989 genes were uniquely downregulated (Figure 3B). A heat map of the most differentially expressed genes between DLE and psoriasis, generated by directly comparing DLE and psoriasis, highlighted the major differences in the transcriptomes of these diseases (Figure 3C). For example, genes that were among the most highly expressed in psoriasis when compared with DLE included DEFB4A, S100A12 and IL8, genes associated with the IL-17-regulated pathway (Guttman-Yassky *et al.*, 2008), and quantitative RT-PCR confirmed this relationship (Supplementary Figure 1). In total, these data indicate DLE has a distinct molecular profile when compared with psoriasis, and indicated that these diseases are the result of distinct pathological mechanisms.

In order to further define differences in T helper associated cytokine pathways, such as the IL-17 pathway, differentially expressed genes between DLE and psoriasis were then interrogated with Gene Set Enrichment Analysis (GSEA) (Subramanian *et al.*, 2005). The goal of GSEA is to determine whether a defined set of genes (described below) are overrepresented at the extremes (either increased or decreased expression) throughout a reference gene list (in the analysis here, we use the gene list from DLE versus psoriasis). The degree to which a gene set is overrepresented at the extremes of a reference gene list is reflected in an enrichment score. Because we were interested in T helper cell associated cytokine pathways, gene sets from keratinocytes co-cultured with the T helper cytokines IL-17, IFN-γ, and IL-22 (Nograles *et al.*, 2008) were used in this analysis. Additionally, we included gene sets from keratinocytes cocultured with TNF (Zaba *et al.*, 2009), as inhibiting the TNF signal is an efficacious form of treatment in patients with psoriasis, or IFN-α (Mee *et al.*, 2007), a signature cytokine in the peripheral blood of SLE patients and identified in DLE lesions (Baechler *et al.*, 2003; Bauer *et al.*, 2006; Bennett *et al.*, 2003; Kirou *et al.*, 2004). GSEA analysis was conducted for the differentially expressed genes between DLE and psoriasis; the set of genes with increased expression in DLE when compared with psoriasis showed enrichment of the following sets of genes: upregulated genes in keratinocytes cocultured with IFN-α or IFN-γ (Table 1). In contrast, the set of genes with decreased expression in DLE when compared with psoriasis (therefore higher expression in psoriasis compared with DLE) showed enrichment of the upregulated gene sets from keratinocytes cocultured with IL-17, IL-22, or TNF (Table 2). In sum, the GSEA analysis is consistent with prior reports implicating IL-17, IL-22, and TNF pathways in the pathogenesis of psoriasis and type I interferon for DLE, but further supports that activation of the IFN-γ pathway is more characteristic of DLE than is activation of the IL-17 pathway.

One potential caveat of our GSEA analysis is that the IL-17-associated signature, however, may be active in DLE, but not to a sufficient extent to overcome or render statistically insignificant the signal for psoriasis. Because of this possibility, we next examined the expression of the primary T helper associated cytokines. Quantitative RT-PCR for IFN-γ, IL-17A, and IL-22, corresponding to Th1, Th17, and Th22 cells, respectively, revealed substantial mRNA expression of these cytokines in psoriasis skin samples (Figure 4A), consistent with prior reports for psoriasis (Jabbari *et al.*, 2011; Lowes *et al.*, 2008). In

contrast, DLE skin samples expressed relatively low levels of IL-17A and IL-22 and high levels of IFN-γ. IL-13 expression was not detected in psoriasis or DLE samples (data not shown). These data support a Th1/Th17/Th22 mixed signature in psoriasis, but a predominantly Th1-skewed signature, with minimal Th17 activation, for DLE.

In order to assess the relative frequencies of T cells among T helper subsets in DLE lesions, we isolated T cell emigrants from skin samples from patients with DLE and psoriasis for comparison. T cells were stained for intracellular IFN-γ and IL-17A after *in vitro* stimulation with phorbol-12-myristate-13-acetate and ionomycin. A detectable population of CD4 T cells from psoriasis skin samples were able to readily produce both IFN-γ and IL-17A, while only IFN-γ producing CD4 T cells from DLE skin were appreciated in DLE lesions (Figure 4B). All DLE samples had < 1% CD4 T cells expressing IL-17, while psoriasis samples analyzed in parallel and previously published data (Lowes et al., 2008) demonstrated substantial IL-17 production by lesional CD4 T cells (Supplementary Figure 2). These data demonstrating IFN-γ protein elaboration support that the T helper infiltrate of DLE skin samples predominantly consist of Th1 cells.

Discussion

DLE is a cutaneous manifestation of lupus that causes scarring and disfigurement. Treatment usually requires systemic immunosuppressive agents with ill-defined mechanisms of action, the potential for harmful side effects, and a requirement for frequent laboratory surveillance. In many cases, available systemic agents are unable to adequately control the disease. There is therefore a critical need for the development of a targeted therapeutic agent with a favorable side effect profile.

The rational identification of potential therapeutic targets requires a well-developed understanding of the pathogenesis of disease. Psoriasis is an example of an autoimmune skin disease in which our growing understanding of the cytokines and T cells critical to the disease have led to the identification of new treatment targets. Biologics that inhibit IL-17- and IL-22-mediated signaling, recently identified to play critical roles in this disease (Nestle *et al.*, 2009), are at various stages of development (Leonardi *et al.*, 2012; Papp *et al.*, 2012), with some now entering the final phases of clinical trials (http://clinicaltrials.gov).

A molecular characterization of DLE, however, has not been carried out to the same extent as that for psoriasis. The current knowledge of DLE pertains to targeted assessments of specific signaling pathways and has been most well described for type I interferons (Braunstein *et al.*, 2012; Wenzel *et al.*, 2005a; Wenzel *et al.*, 2005b; Wenzel *et al.*, 2009). Cytokines associated with specific T cell subsets have been explored in two separate prior studies (Stein *et al.*, 1997; Toro *et al.*, 2000), although their results conflicted with each other and were published prior to the discovery of the Th17 and Th22 T cell subsets.

Recent descriptions of the presence of Th17 cells in the blood of DLE patients (Balanescu *et al.*, 2010) as well as a growing literature on the role of Th17 cells in SLE (Balanescu *et al.*, 2010; Chen *et al.*, 2010; Henriques *et al.*, 2010; Kleczynska *et al.*, 2011; Mok *et al.*, 2010; Shah *et al.*, 2010) seemed to support the hypothesis that Th17 cells would be present at

appreciable numbers and may be playing a role in the development of discoid lesions—our data illustrating minimal Th17 involvement in DLE lesions were unexpected. Several reports have described the presence of IL-17 related activity or signals in DLE lesions by immunohistochemistry (Oh *et al.*, 2011; Tanasescu *et al.*, 2010) or in the serum of DLE patients (Balanescu *et al.*, 2010). IL-17-related markers have also been reported as upregulated compared to healthy controls in the serum of SLE patients in some (Shah *et al.*, 2010; Zhao *et al.*, 2010), but not all (Brajac *et al.*, 2010; Higgs *et al.*, 2012)) studies. In a few cases, enhanced numbers of Th17 cells were only significantly increased in those patients with higher SLEDAI scores, a research instrument that integrates clinical data and laboratory results to assess SLE activity and identify SLE flares (Dolff *et al.*, 2010). The results of our analysis, however, indicated that the expected Th17-associated gene set was minimally enriched in our comparative DLE data, and this was further corroborated by the absence of IL-17-producing infiltrating T cells in DLE.

The study presented here provides a global description of gene expression in lesional skin of discoid lupus; although others have used customized microarray technologies in the examination of specific aspects of DLE (Chang *et al.*, 2011), we found no substantial global description of lesional skin in DLE in the literature to date. By comparing our transcriptomic data to that of psoriasis, an autoimmune skin disease whose gene expression profile in skin has been described in dozens of subjects (Bowcock *et al.*, 2001; Gudjonsson *et al.*, 2009; Jabbari *et al.*, 2012; Suarez-Farinas *et al.*, 2010; Zhou *et al.*, 2003), we were able to identify signatures that may be useful in the pursuit of novel therapeutics. Our gene expression and protein data does not support the use of an IL-17 modulating strategy in the treatment of this disease. Rather, these data invite exploring interventions that target IFN-γ or Th1 cell infiltration in the treatment of DLE.

Therapeutic agents that target interferon signaling are currently in development for use in autoimmune diseases. Inhibitors of proximal signaling mediators of IFN-γ signaling, including JAK1 and JAK2, have recently been approved for the treatment of myelofibrosis (Harrison *et al.*, 2012; Verstovsek *et al.*, 2010; Verstovsek *et al.*, 2012). Additionally, some studies have examined the use of these small molecular inhibitors in topical form (Fridman *et al.*, 2011), which would be especially useful in the context of DLE treatment in the absence of SLE.

It is unclear whether similar pathogenic mechanisms are present in other affected end-organs in lupus, and it is possible that systemic inhibition of pathways activated in DLE may be useful in the treatment of SLE. Interestingly, some immunomodulating biologics that are currently in use for the treatment of psoriasis additionally address extracutaneous manifestions of the disease. Another possibility is that DLE is a marker of a global inflammatory profile in a subset of SLE patients, and the presence of DLE identifies those SLE patients that have one particular molecular pattern of end-organ pathology that is distinct from that seen in SLE patients without DLE. In this scenario, molecular profiling of skin lesions may be useful to identify therapeutic targets in a specific subset of SLE patients. Genetic studies have defined SLE-associated single nucleotide polymorphisms that are enriched in SLE patients with DLE, supporting that the presence of DLE may be indicative of a particular “flavor” of SLE (Sanchez *et al.*, 2011). Furthermore, whether the pathogenic

mechanisms in DLE lesional skin is different in the context of systemic disease or cutaneous involvement alone, will require further studies with adequate patient samples to power this form of stratification. While our study does not stratify based on the presence of SLE, our data provide a global description of gene expression in DLE skin and provide a springboard to future work potentially culminating in a specific, efficacious treatment.

Materials & Methods

Skin samples

Eleven patients with active DLE were enrolled in the study (Supplementary Table 2). Punch biopsy and shave biopsy specimens of psoriasis (n=5) and normal (n=3) skin samples were from patients with active moderate to severe disease or healthy subjects, respectively. Additional sample data from prior studies were added as indicated in the text or Supplementary Methods. All procedures were performed under Institutional Review Board-approved protocols at New York University or at the Rockefeller University Hospital. Punch biopsy specimens were frozen in optimal cutting temperature medium for immunohistochemistry or flash frozen with liquid nitrogen for RNA extraction. Shave biopsy specimens were prepared for T cell emigrant isolation and functional cytokine expression (see below) as previously described (Lowes *et al.*, 2008).

Sample preparation for RT-PCR and gene chip analysis

Total RNA was extracted from flash frozen punch biopsy samples using the RNeasy Mini Kit (Qiagen, Valencia, CA). DNA was removed by on-column DNase digestion by the RNase-free DNase Set (Qiagen).

DNA microarray analysis

Human Genome U133A 2.0 gene chips (Affymetrix, Inc., Santa Clara, CA) were used for this study. The U133A 2.0 array includes more than 22,000 probe sets to analyze expression level of more than 18,400 transcripts, including 12,681 genes with known identity. Descriptions of methods for total RNA preparation for microarray hybridization, microarray data analysis, and Ingenuity Pathway Analysis (www.ingenuity.com) have been included in the Supplementary Materials.

Quantitative RT-PCR analysis

Quantitative PCR was performed using RNA or amplified cDNA using Taqman gene expression assays. Predesigned primer and probe sets were purchased from Applied Biosystems (Carlsbad, CA). Data normalized to human acidic ribosomal protein housekeeping gene were quantified by software provided with Applied Biosystems. Statistical analysis compared $\log_2$ transformed, normalized values from DLE and psoriasis samples by unpaired two-tailed students t-test.

Functional cytokine expression, intracellular cytokine staining, and flow cytometry

Shave biopsy specimens were cultured for two hours in 0.5% dispase (Sigma Aldrich Corp., St Louis, MO) to separate the epidermis and dermis. Dermal T cells were obtained by

culturing the dermis for three days in RPMI 1640 (Gibco-BRL Life Technologies, Carlsbad, CA) supplemented with 10% human pooled serum (Mediatech, Inc., Manassas, VA), and 1% 1 M HEPES buffer (Sigma Aldrich, St. Louis, MO); one DLE sample did not yield a sufficient number of live cells to perform further analyses. T cells were cultured for 4 hours with 10 μg/ml brefeldin A with or without 25 ng/ml phorbol myristate acetate (PMA) and 2 μg/ml ionomycin (all from Sigma Aldrich Corp.). Cells were frozen in 10% DMSO (ATCC, Manassas, VT) in RPMI-1640 (Gibco-BRL Life Technologies) with 1 mM HEPES buffer (Sigma Aldrich), 0.1% gentamicin (Gibco-BRL Life Technologies) and 5% normal human serum (C-Six Diagnostics, Germantown, WI). Frozen cells were thawed, washed, and subjected to an intracellular staining protocol. Briefly, cells were stained for 30 minutes at room temperature with antibodies specific for human CD3 and CD4. After washing with FACS Buffer, cells were permeablized and stained with antibodies specific for human IFN-γ and IL-17A. Samples were acquired using an LSRII (BD Biosciences, Rockville, MO) and analyzed using FlowJo software (Treestar, Ashland, OR).

Immunohistochemistry

Frozen sections were stained with hematoxylin (Fisher Scientific, Pittsburgh, PA, U.S.A.) and eosin (Shandon, Pittsburgh, PA, U.S.A.). As previously described for immunohistochemistry (Jabbari *et al.*, 2012), frozen sections were blocked with 10% normal horse serum, and endogenous peroxidases were quenched by incubation with diluted hydrogen peroxide (1:10 dilution of 3% hydrogen peroxide). Sections were incubated overnight at 4°C with primary monoclonal antibodies. Biotin-labeled horse anti-primary antibodies were used for secondary binding, and thereafter the signals were amplified with avidin-biotin complex (Vector Laboratories, Burlingame, CA, U.S.A.). Subsequently, the sections were developed using chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich, St Louis, MO, U.S.A.).

Human subjects declaration

All studies have been approved by the appropriate Institutional Review Boards and were conducted under the Declaration of Helsinki principles. Informed written consent was received from participants prior to inclusion in the study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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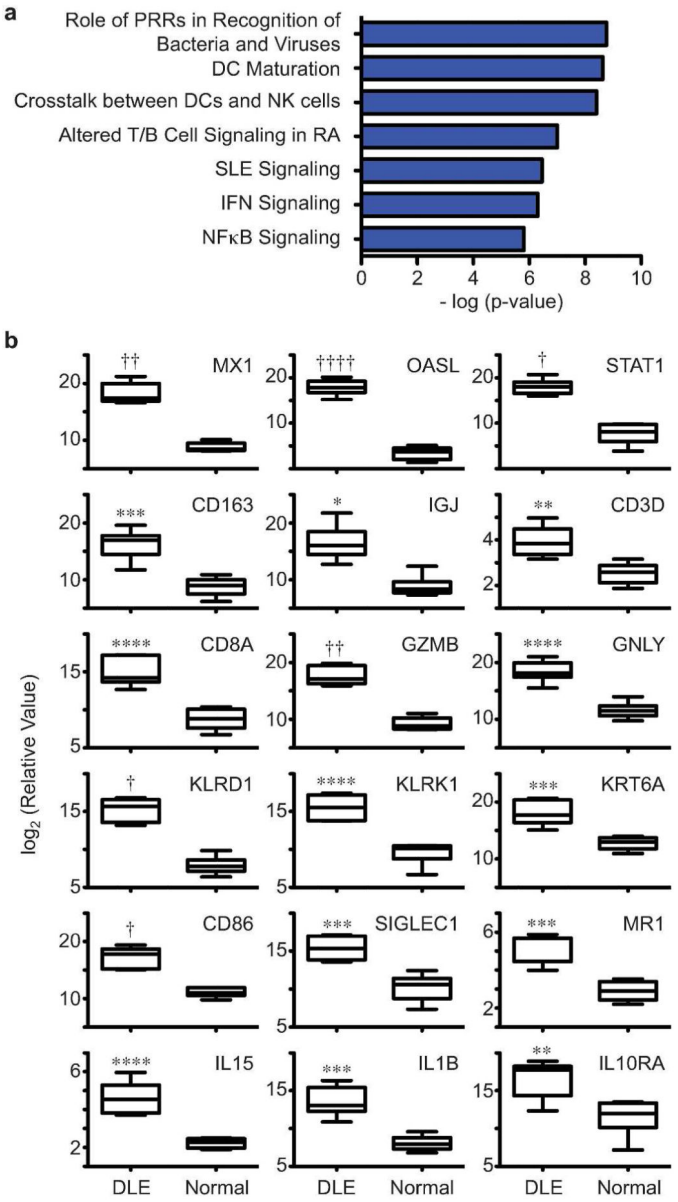

Figure 1.

Transcriptomic analysis of DLE. (A) Selected top canonical pathways from interrogation of the DLE (n=7) versus normal transcriptome (n=13) with Ingenuity Pathway Analysis. (B) Quantitative RT-PCR of selected upregulated genes in the DLE versus normal transcriptome corresponding to interferon-associated genes (MX1, OASL, STAT1), immune cell associated genes, and selected cytokines in DLE (n=7) and normal (n=6) samples. Genes were normalized to human ribosomal acidic protein. Box and whisker plots (middle line: median; box: lower to upper quartile; whiskers: minimum and maximum), * $p < 0.05$, ** $p < 0.005$, *** $p < 5 \times 10^{-4}$, **** $p < 5 \times 10^{-5}$, † $p < 5 \times 10^{-6}$, †† $p < 5 \times 10^{-7}$, †††† $p < 5 \times 10^{-9}$

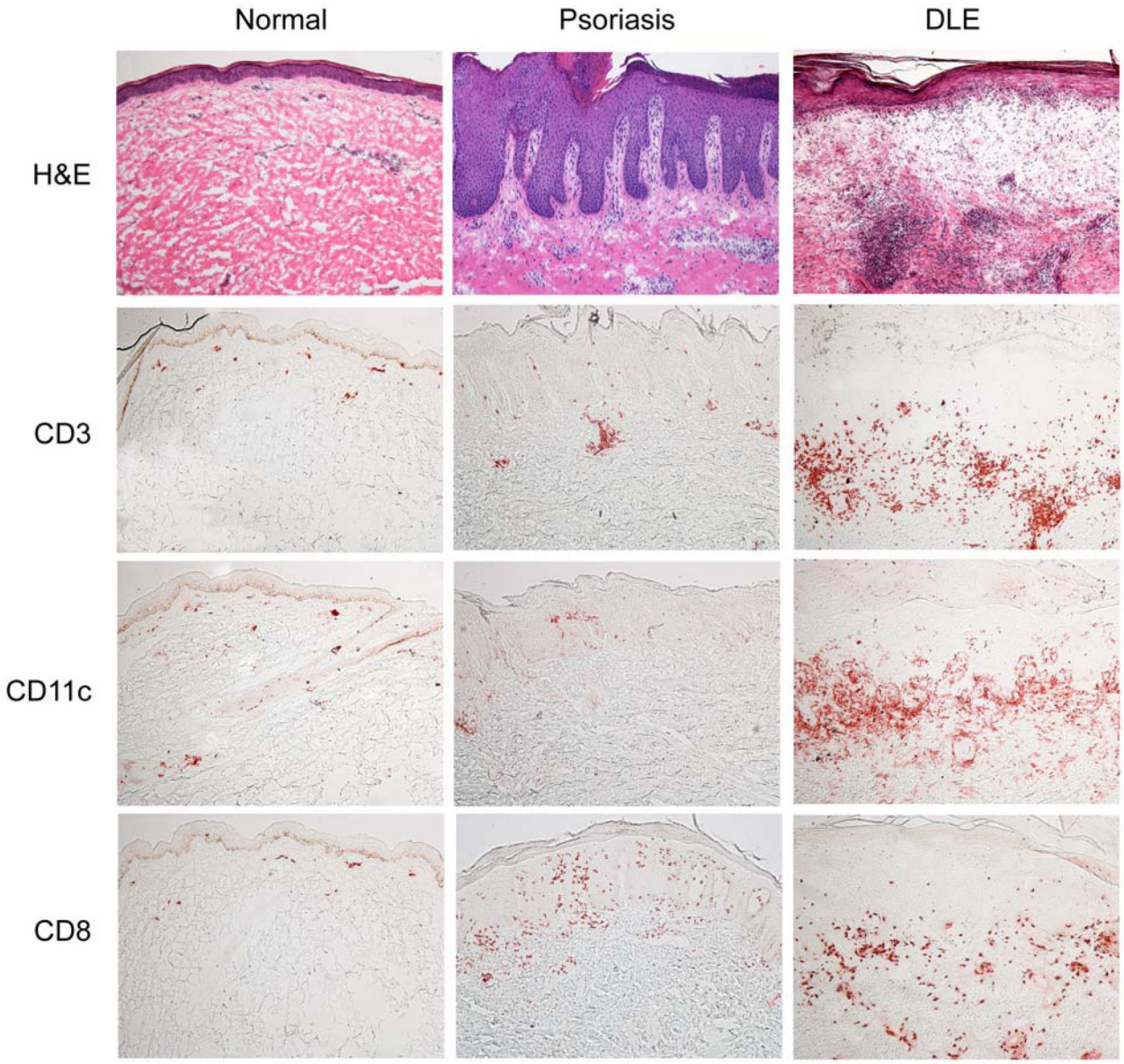

Figure 2.

Microscopic and immunohistologic comparison of DLE and psoriasis. Skin sections from patients with DLE, psoriasis, or healthy control patients were stained with hematoxylin and eosin or were subjected to immunohistochemistry with antibodies specific for CD3, CD11c, or CD8. Scale bar = 100 μm.

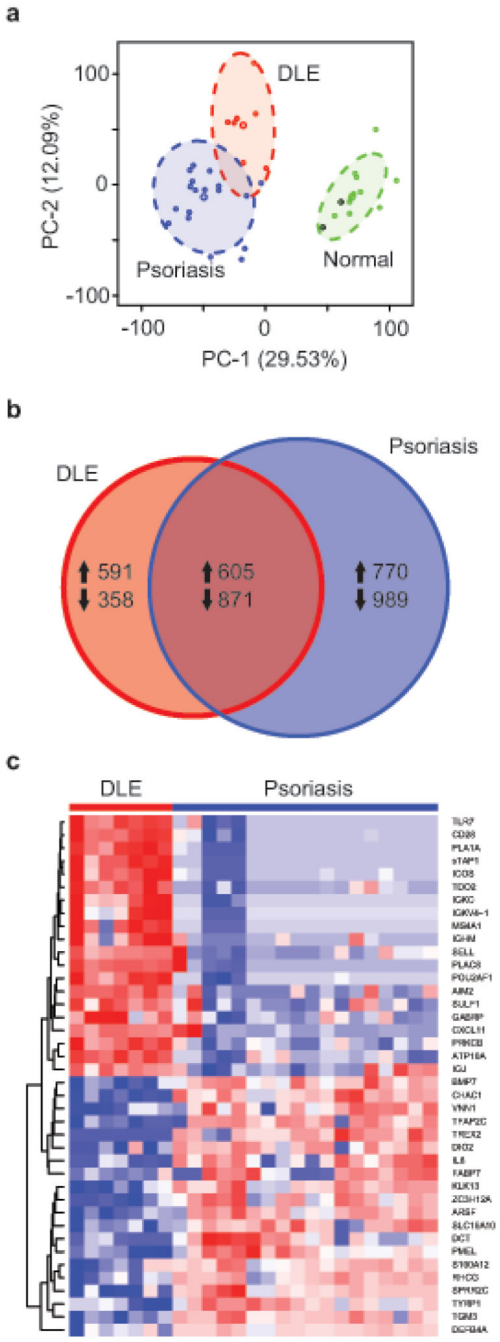

Figure 3.

Transcriptomic comparison between DLE and psoriasis. (A) Principal components analysis plot comparing DLE (n=7), psoriasis (PsO, n=18), and normal control (n=13) samples. (B) Venn diagram comparing upregulated and downregulated genes from DLE versus normal skin (DLE) and psoriasis versus normal skin (PsO). (C) Heat map of the most upregulated and downregulated genes in DLE versus psoriasis comparison.

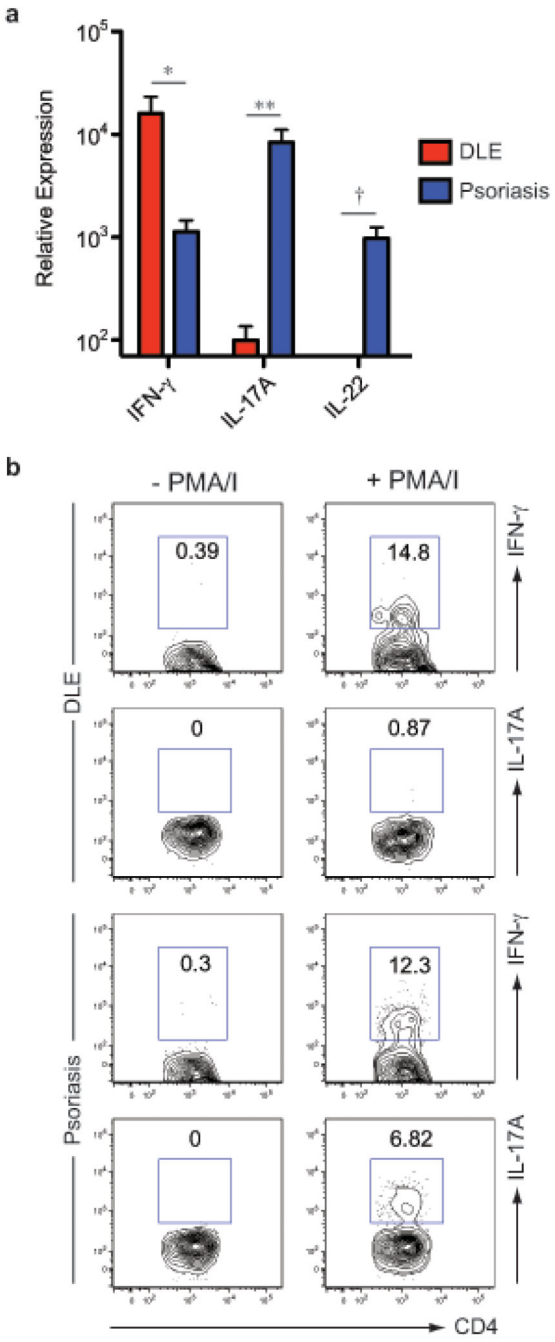

Figure 4.

Comparison of T helper subsets in DLE and psoriasis. (A) Quantitative RT-PCR comparison of T helper subset-associated cytokines in DLE and psoriasis (PsO). * $p < 0.05$, ** $p < 5 \times 10^{-5}$, † $p < 5 \times 10^{-6}$. (B) Intracellular cytokine staining of CD4 T cells extracted from lesions from patients with DLE (n=4) or psoriasis (n=4) for IFN-γ or IL-17A, stimulated with (+ PMA/I, right column) or without (− PMA/I, left column) PMA and ionomycin. Numbers are percentages of $CD3^+CD4^+$ events positive for either IFN-γ or IL-17A.

Table 1

GSEA analysis of upregulated genes in DLE compared with psoriasis.

NAME	SIZE	ES[a]	NES[b]	p-value	FDR[c]
KC IFNA UP[d]	28	0.70	2.23	0.000	0.000
KC IFNG UP[e]	965	0.29	1.46	0.000	0.047

[a] ES: Enrichment score

[b] NES: Normalized enrichment score

[c] FDR: False discovery rate

[d] KC IFNA UP: Keratinocytes cultured with IFN-α (Mee *et al.*, 2007), genes with increased expression

[e] KC IFNG UP: Keratinocytes cultured with IFN-γ (Nograles *et al.*, 2008), genes with increased expression

Table 2

GSEA analysis of downregulated genes in DLE compared with psoriasis.

NAME	SIZE	ES[a]	NES[b]	p-value	FDR[c]
KC IL17 UP[d]	46	−0.76	−2.81	0.000	0.000
KC IL22 UP[e]	11	−0.61	−1.60	0.036	0.027
KC TNF UP[f]	503	−0.29	−1.52	0.000	0.048

[a] ES: Enrichment score

[b] NES: Normalized enrichment score

[c] FDR: False discovery rate

[d] KC IL17 UP: Keratinocytes cultured with IL-17A (Nograles *et al.*, 2008), genes with increased expression

[e] KC IL22 UP: Keratinocytes cultured with IL-22 (Nograles *et al.*, 2008), genes with increased expression

[f] KC TNF UP: Keratinocytes cultured with TNF-α (Zaba *et al.*, 2009), genes with increased expression