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

Ali Jabbari¹,², Mayte Suárez-Fariñas²,³, Judilyn Fuentes-Duculan², Juana Gonzalez⁴, Inna Cueto², Andrew G. Franks Jr.⁵,⁶, and James G. Krueger²,³
¹Department of Dermatology, College of Physicians & Surgeons, Columbia University, New York, NY, USA
²Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY, USA
³Center for Clinical and Translational Science, The Rockefeller University, New York, NY, USA
⁴Translational Technology Core Laboratory, The Rockefeller University, New York, NY, USA
⁵The Ronald O. Perelman Department of Dermatology, New York University School of Medicine
⁶Division of Rheumatology, Department of Medicine, New York University School of Medicine, New York, NY, USA

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...
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 (Balancescu 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-\(\gamma\) 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-\(\gamma\) 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 manifestations 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 log₂ 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, VA) in RPMI-1640 (Gibco-BRL Life Technologies) with 1 m 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 permeabilized 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.

**Acknowledgements**

We thank Michelle Lowes for her critical review of the manuscript.

A.J. is supported by the Dermatology Foundation Physician Scientist Career Development Award and the Louis V. Gerstner, Jr. Scholars Fund. Research was supported by the Clinical and Translational Science Award (UL1RR024143) from the National Center for Advancing Translational Sciences of the National Institutes of Health.
References

Alunno A, Bartoloni E, Bistoni O, Nocentini G, Ronchetti S, Caterbi S, et al. Balance between Regulatory T and Th17 Cells in Systemic Lupus Erythematosus: The Old and the New. Clin Dev Immunol. 2012; 2012:823085. [PubMed: 22761634]

Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A. 2003; 100:2610–5. [PubMed: 12604793]

Balanescu P, Balanescu E, Tanasescu C, Nicolau A, Tanasescu R, Grancea C, et al. T helper 17 cell population in lupus erythematosus. Rom J Intern Med. 2010; 48:58–9. [PubMed: 21442179]

Bauer JW, Baechler EC, Petri M, Batliwalla FM, Crawford D, Ortmann WA, et al. Elevated serum levels of interferon-regulated chemokines are biomarkers for active human systemic lupus erythematosus. PLoS Med. 2006; 3:e491. [PubMed: 17177599]

Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med. 2010; 197:711–23. [PubMed: 21187315]

Brajac I, Kastelan M, Perisa D, Simonic E, Stojnic-Sosa L, Massari LP. Treatment of alopecia areata: modern principles and perspectives. Lijec Vjesn. 2012; 134:365–9. [PubMed: 21294327]

Braunstein I, Klein R, Okawa J, Werth VP. The interferon-regulated gene signature is elevated in subacute cutaneous lupus erythematosus and discoid lupus erythematosus and correlates with the cutaneous lupus area and severity index score. Br J Dermatol. 2012; 166:971–5. [PubMed: 22242767]

Chang LM, Maheshwari P, Werth S, Schaffer L, Head SR, Kovarik C, et al. Identification and molecular analysis of glycosaminoglycans in cutaneous lupus erythematosus and dermatomyositis. J Histochem Cytochem. 2011; 59:336–45. [PubMed: 21378287]

Chen C, Gennann K, Badner J, Zhang D, Gershon E, Jin L, et al. Removing batch effects in analysis of expression microarray data: an evaluation of six batch adjustment methods. PLoS One. 2011; 6:e17238. [PubMed: 21386892]

Chen XQ, Yu YC, Deng HH, Sun JZ, Dai Z, Wu YW, et al. Plasma IL-17A is increased in new-onset SLE patients and associated with disease activity. J Clin Immunol. 2010; 30:221–5. [PubMed: 20107878]

Dolff S, Quandt D, Wilde B, Feldkamp T, Hua F, Cai X, et al. Increased expression of costimulatory markers CD134 and CD80 on interleukin-17 producing T cells in patients with systemic lupus erythematosus. Arthritis research & therapy. 2010; 12:R150. [PubMed: 20653937]

Fridman JS, Scherle PA, Collins R, Burn T, Neilan CL, Hertel D, et al. Preclinical evaluation of local JAK1 and JAK2 inhibition in cutaneous inflammation. The Journal of investigative dermatology. 2011; 131:1838–44. [PubMed: 21677670]

Gronhagen CM, Fored CM, Granath F, Nyberg F. Cutaneous lupus erythematosus and the association with systemic lupus erythematosus: a population-based cohort of 1088 patients in Sweden. Br J Dermatol. 2011; 164:1335–41. [PubMed: 21574972]

Gudjonsson JE, Ding J, Li X, Nair RP, Tejasvi T, Qin ZS, et al. Global gene expression analysis reveals evidence for decreased lipid biosynthesis and increased innate immunity in uninvolved psoriatic skin. The Journal of investigative dermatology. 2009; 129:2795–804. [PubMed: 19571819]

Guttman-Yassky E, Lowe MA, Fuentes-Duculan J, Zaba LC, Cardinale I, Nograles KE, et al. Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. J Immunol. 2008; 181:7420–7. [PubMed: 18981165]

Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012; 366:787–98. [PubMed: 22375970]
Henriques A, Ines L, Couto M, Pedreiro S, Santos C, Magalhaes M, et al. Frequency and functional activity of Th17, Tc17 and other T-cell subsets in Systemic Lupus Erythematosus. Cell Immunol. 2010; 264:97–103. [PubMed: 20553755]

Higgs BW, Zhu W, Richman L, Fiorentino DF, Greenberg SA, Jallal B, et al. Identification of activated cytokine pathways in the blood of systemic lupus erythematosus, myositis, rheumatoid arthritis, and scleroderma patients. Int J Rheum Dis. 2012; 15:25–35. [PubMed: 22324944]

Jabbari A, Johnson-Huang LM, Krueger JG. Role of the immune system and immunological circuits in psoriasis. G Ital Dermatol Venereol. 2011; 146:17–30. [PubMed: 21317854]

Jabbari A, Suarez-Farinas M, Dewell S, Krueger JG. Transcriptional profiling of psoriasis using RNA-seq reveals previously unidentified differentially expressed genes. The Journal of investigative dermatology. 2012; 132:246–60. [PubMed: 22185022]

Johnson-Huang LM, Suarez-Farinas M, Pierson KC, Fuentes-Duculan J, Cueto I, Lentini T, et al. A single intradermal injection of IFN-gamma induces an inflammatory state in both non-lesional psoriatic and healthy skin. The Journal of investigative dermatology. 2012; 132:1177–87. [PubMed: 22277938]

Kirou KA, Lee C, George S, Louka C, Papagiannis IG, Peterson MG, et al. Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus. Arthritis Rheum. 2004; 50:3958–67. [PubMed: 15593221]

Kleczynska W, Jakiela B, Plutecka H, Milewski M, Sanak M, Musial J. Imbalance between Th17 and regulatory T-cells in systemic lupus erythematosus. Folia Histochem Cytobiol. 2011; 49:646–53. [PubMed: 22252759]

Leonardi C, Matheson R, Zachariae C, Cameron G, Li L, Edson-Heredia E, et al. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. N Engl J Med. 2012; 366:1190–9. [PubMed: 22455413]

Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet. 2008; 371:1665–74. [PubMed: 18486739]

Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. The Journal of investigative dermatology. 2008; 128:1207–11. [PubMed: 18200064]

Mee JB, Johnson CM, Morar N, Burslem F, Groves RW. The psoriatic transcriptome closely resembles that induced by interleukin-1 in cultured keratinocytes: dominance of innate immune responses in psoriasis. Am J Pathol. 2007; 171:32–42. [PubMed: 17591951]

Mok MY, Wu HJ, Lo Y, Lau CS. The relation of interleukin 17 (IL-17) and IL-23 to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus. J Rheumatol. 2010; 37:2046–52. [PubMed: 20682672]

Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med. 2009; 361:496–509. [PubMed: 19641206]

Nogales KE, Zaba LC, Gutman Yassky E, Fuentes-Duculan J, Suarez-Farinas M, Cardinale I, et al. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol. 2008; 159:1092–102. [PubMed: 18684158]

Oh SH, Roh HJ, Kwon JE, Lee SH, Kim JY, Choi HJ, et al. Expression of interleukin-17 is correlated with interferon-alpha expression in cutaneous lesions of lupus erythematosus. Clin Exp Dermatol. 2011; 36:512–20. [PubMed: 21631571]

Pan HF, Ye DQ, Li XP. Type 17 T-helper cells might be a promising therapeutic target for systemic lupus erythematosus. Nat Clin Pract Rheumatol. 2008; 4:352–3. [PubMed: 18477998]

Papp KA, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). Lancet. 2008; 371:1675–84. [PubMed: 18486740]

Papp KA, Leonard C, Menter A, Ortonne JP, Krueger JG, Kricorian G, et al. Brodalumab, an anti-interleukin-17-receptor antibody for psoriasis. N Engl J Med. 2012; 366:1181–9. [PubMed: 22455412]
Rothfield N, March CH, Miescher P, McEwen C. Chronic Discoid Lupus Erythematosus. N Engl J Med. 1963; 269:1155–61. [PubMed: 14061123]

Sanchez E, Nadig A, Richardson BC, Freedman BI, Kaufman KM, Kelly JA, et al. Phenotypic associations of genetic susceptibility loci in systemic lupus erythematosus. Ann Rheum Dis. 2011; 70:1752–7. [PubMed: 21719445]

Shah K, Lee WW, Lee SH, Kim SH, Kang SW, Craft J, et al. Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. Arthritis research & therapy. 2010; 12:R53. [PubMed: 20334681]

Stein LF, Saud GM, Fivenson DP. T-cell cytokine network in cutaneous lupus erythematosus. J Am Acad Dermatol. 1997; 36:191–6. [PubMed: 9039167]

Suarez-Farinas M, Lowes MA, Zaba LC, Krueger JG. Evaluation of the psoriasis transcriptome across different studies by gene set enrichment analysis (GSEA). PLoS One. 2010; 5:e10247. [PubMed: 20422035]

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:15545–50. [PubMed: 16199517]

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis and rheumatism. 1982; 25:1271–7. [PubMed: 7138600]

Tanasescu C, Balanescu E, Balanescu P, Olteanu R, Badea C, Grancea C, et al. IL-17 in cutaneous lupus erythematosus. Eur J Intern Med. 2010; 21:202–7. [PubMed: 20493423]

Toro JR, Finlay D, Dou X, Zheng SC, LeBoit PE, Connolly MK. Detection of type 1 cytokines in discoid lupus erythematosus. Arch Dermatol. 2000; 136:1497–501. [PubMed: 1115160]

Uramoto KM, Michet CJ Jr, Thumboo J, Sunku J, O’Fallon WM, Gabriel SE. Trends in the incidence and mortality of systemic lupus erythematosus, 1950-1992. Arthritis and rheumatism. 1999; 42:46–50. [PubMed: 9920013]

Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med. 2010; 363:1117–27. [PubMed: 20843246]

Wallace DJ, Gudsoorkar VS, Weisman MH, Venuturupalli SR. New insights into mechanisms of therapeutic effects of antimalarial agents in SLE. Nat Rev Rheumatol. 2012; 8:522–33. [PubMed: 22801982]

Wenzel J, Uerlich M, Worrenkamper E, Freulet S, Bieber T, Tuting T. Scarring skin lesions of discoid lupus erythematosus are characterized by high numbers of skin-homing cytotoxic lymphocytes associated with strong expression of the type I interferon-induced protein MxA. Br J Dermatol. 2005a; 153:1011–5. [PubMed: 16225615]

Zhao XF, Pan HF, Yuan H, Zhang WH, Li XP, Wang GH, et al. Increased serum interleukin 17 in patients with systemic lupus erythematosus. Mol Biol Rep. 2010; 37:81–5. [PubMed: 19347604]

Zhou X, Krueger JG, Kao MC, Lee E, Du F, Menter A, et al. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. Physiol Genomics. 2003; 13:69–78. [PubMed: 12644634]
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 × 10^{-4}, **** p < 5 × 10^{-5}, † p < 5 × 10^{-6}, †† p < 5 × 10^{-7}, †††† p < 5 × 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 × 10^{-5}, † p < 5 × 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<sup>a</sup> | NES<sup>b</sup> | p-value | FDR<sup>c</sup> |
|--------------------|------|----------------|----------------|---------|-----------------|
| KC IFNA UP<sup>d</sup> | 28   | 0.70           | 2.23           | 0.000   | 0.000           |
| KC IFNG UP<sup>e</sup> | 965  | 0.29           | 1.46           | 0.000   | 0.047           |

<sup>a</sup>ES: Enrichment score

<sup>b</sup>NES: Normalized enrichment score

<sup>c</sup>FDR: False discovery rate

<sup>d</sup>KC IFNA UP: Keratinocytes cultured with IFN-α (Mee et al., 2007), genes with increased expression

<sup>e</sup>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<sup>a</sup> | NES<sup>b</sup> | p-value | FDR<sup>c</sup> |
|-----------------------|------|----------------|----------------|---------|-----------------|
| KC IL17 UP<sup>d</sup> | 46   | −0.76          | −2.81          | 0.000   | 0.000           |
| KC IL22 UP<sup>e</sup> | 11   | −0.61          | −1.60          | 0.036   | 0.027           |
| KC TNF UP<sup>f</sup>  | 503  | −0.29          | −1.52          | 0.000   | 0.048           |

<sup>a</sup>ES: Enrichment score

<sup>b</sup>NES: Normalized enrichment score

<sup>c</sup>FDR: False discovery rate

<sup>d</sup>KC IL17 UP: Keratinocytes cultured with IL-17A (Nogales et al., 2008), genes with increased expression

<sup>e</sup>KC IL22 UP: Keratinocytes cultured with IL-22 (Nogales et al., 2008), genes with increased expression

<sup>f</sup>KC TNF UP: Keratinocytes cultured with TNF-α (Zaba et al., 2009), genes with increased expression