

Detection of Type 1 Cytokines in Discoid Lupus Erythematosus

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Background: Although multiple studies suggest a dys-regulated T-cell cytokine production in systemic lupus erythematosus, the cytokine profile in discoid lupus erythematosus (DLE) lesions is unknown.

Objectives: To characterize the cytokine profile in DLE by immunohistochemical and molecular methods, and to investigate the role of cytokines in the pathogenesis of DLE.

Design: Patients were evaluated clinically, and biopsy specimens of lesional skin were examined by light microscopy. Reverse transcriptase-polymerase chain reaction and immunohistochemical analysis were performed on 11 biopsy specimens. We investigated the presence of interleukin (IL) 2, interferon γ (IFN- γ), IL-4, tumor necrosis factor α , (TNF- α), and IL-1 β messenger RNA (mRNA) in 8 biopsy specimens of DLE and compared it with 3 biopsy specimens of normal skin.

Setting: Academic referral research hospital.

Patients: Eight consecutive patients with a clinical and histologic diagnosis of DLE.

Results: Localized DLE was found in 7 patients and widespread in 1. During the 4 years of the investigation, none of the patients developed systemic lupus erythematosus. We found significantly elevated levels of IL-2 and IFN- γ mRNA in all 8 biopsy specimens of DLE; in contrast, no transcripts of IL-2 or IFN- γ were detected in 3 biopsy specimens of normal skin ($P < .01$). Similarly, elevated levels of TNF- α mRNA were detected in 8 DLE biopsy specimens, while no TNF- α mRNA was detected in 3 biopsy specimens of normal skin ($P < .01$). No IL-4 or IL-1 β mRNA was detected in 8 biopsy specimens of DLE lesional skin and 3 biopsy specimens of normal patient skin. Immunohistochemical analysis showed increased staining for IL-2 and IFN- γ receptors, while no detectable IL-4 receptor was found. No cytokine mRNA or cytokine receptor protein was detected in biopsy specimens of normal skin.

Conclusions: These findings suggest that DLE is associated with type 1 cytokines characterized by the expression of IL-2 and IFN- γ . Type 1 cytokines may be critical for induction, development, and maintenance of DLE.

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LUPUS ERYTHEMATOSUS (LE) is an autoimmune disease of unknown etiology. Discoid LE (DLE) may occur either as a cutaneous eruption without systemic disease or as part of systemic LE (SLE). Discoid LE is characterized by marked hyperpigmentation, follicular plugging, central depigmentation, and atrophy (most commonly on the face, ears, and scalp). Adults with chronic DLE rarely develop SLE. Approximately 5% of cases of DLE evolve into SLE. One fourth of all patients with SLE have lesions of DLE. Widespread DLE is not common. The more severe and the more widespread the distribution of DLE, the more likely it will be associated with systemic disease.¹

In biopsy specimens of DLE, CD4⁺ T cells are the predominant cells.² Three distinct subsets of T-helper lymphocytes that vary in their cytokine expression pat-

terns have been identified in mice and humans³: T_H1, T_H2, and T_H0 T cells. In response to antigenic stimulation, T_H1 cells secrete interleukin (IL) 2 and interferon (IFN) γ , and promote cell-mediated immunity. T_H2 cells secrete IL-4, IL-5, IL-6, and IL-10, favor humoral immunity, and induce antibody production. T_H0 cells are capable of secreting both T_H1 and T_H2

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cytokines and can be stimulated to differentiate toward either a T_H1 or a T_H2 phenotype. T_H1 and T_H2 subsets are capable of counterregulating each other because IFN- γ secreted by T_H1 cells can inhibit proliferation of T_H2 cells. In contrast, IL-10 produced by T_H2 cells can inhibit proliferation of T_H1 cells.⁴ IL-12 and tumor necrosis factor (TNF) α secreted by antigen-presenting macrophages are strong

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PATIENTS AND METHODS

Histologic examination of biopsy specimens of DLE confirmed the clinical diagnosis in all patients. Cases that met the diagnostic criteria for SLE of the American College of Rheumatology were excluded.⁷ The DLE lesion distribution was classified as either localized (if lesions were only on the head and neck) or widespread (if many lesions were present on the head, neck, and other body parts). Serum antinuclear antibody titers were obtained in all patients. None of patients was treated with chloroquine, hydrochloroquine, thalidomide, or other disease-modifying agents.

IMMUNOHISTOCHEMICAL ANALYSIS

Antigen retrieval was performed as previously described.⁸ Mouse anti-IFN- γ receptor and anti-IL-4 receptor (Cdw124) were purchased from Genzyme Corporation (Cambridge, Mass) and used at 10 μ g/mL. Mouse anti-IL-2 receptor α chain (CD25) was purchased from Pharmingen (San Diego, Calif) and used at 10 μ g/mL. Biotin-conjugated goat antimouse IgG and fluorescein avidin were purchased from Sigma Company Ltd (St Louis, Mo). Direct immunofluorescence of lesional skin was performed following standard techniques.⁹

SEMIQUANTITATIVE POLYMERASE CHAIN REACTION

Four-millimeter punch biopsy skin specimens were taken from 8 DLE lesions and 3 normal patients and immediately frozen in liquid nitrogen. Complementary DNA was

prepared as previously described.¹⁰ Briefly, total RNA was extracted according to the manufacturer's recommendations using RNeasy (Qiagen, Crawfordsville, Ind); 4 μ g of RNA in 9 μ L of DEPC-treated water were added to 2 μ L of random-hexamer primers (0.5 μ g/mL) (Promega Corporation, Madison, Wis) and 0.5 μ L of RNasin (Promega) and heated to 65°C for 5 minutes; 5.0 μ L of 1.25- mmol/L dNTPs (Boehringer-Mannheim Corporation, Indianapolis, Ind), 4 μ L of 5 \times H-RT buffer, and 1 μ L of Moloney murine leukemia virus–reverse transcriptase (Life Technologies, Rockville, Md) were then added and incubated at 37°C for 1.5 hours. The reaction was stopped by heating the sample to 95°C for 5 minutes and then quenching over ice. The semi-quantitative polymerase chain reaction technique used a polycompetitor construct that contained 9 cytokines and a hypoxanthine phosphoribosyl transferase sequence that had identical primer binding sites to the wild-type cytokines, but with longer intervening sequences allowing for separation on agarose gel electrophoresis. The competitor was constructed as described by Reiner et al.¹⁰ The following complementary DNA primers for human cytokine were used: for IL-2, 5'-ACTCACCAGGATGCTCACAT; for IL-4, 5'-TCCACGGACACAAGTGCATATCACCT; for IFN- γ , 5'-AGTTATATCTTGGCTTTCA; for TNF- α , 5'-GAGTGATCGGCCCCAGAGG; and for IL-1 β , 5'-TACAGTGGCAATGAGGATGACT.

Amplification was done using the following profile: 94°C for 40 seconds, 60°C for 20 seconds, and 72°C for 40 seconds for 35 cycles with a final extension of 72°C for 10 minutes. The products of amplification were electrophoresed on a 0.8% agarose gel (SewaKem LE, FMC) and visualized with ethidium bromide staining under UV light. Data were analyzed using the χ^2 test.¹¹

promoters of T_H1 cytokines.⁵ Elevated IL-10 augments B-cell activation and differentiation, and inhibits the generation of T_H1 responses.⁶ In this study, we characterized the cytokine profile in DLE lesions.

RESULTS

CLINICAL FEATURES

The patient cohort consisted of 6 men and 2 women ranging in age from 25 to 39 years; there were 5 whites, 2 African Americans, and 1 Hispanic patient. During the 4 years of the investigation, none of the patients developed SLE nor did any of them exhibit a serum antinuclear antibody titer higher than 1:40. All patients presented with discoid plaques with an erythematous active border. While 7 patients had localized lesions, only 1 had widespread lesions. One individual exhibited scarring alopecia and 1 had involvement of oral mucosa in addition to skin lesions.

HISTOLOGIC FEATURES

Microscopic examination of DLE biopsy specimens from 8 affected individuals revealed vacuolar degeneration of the dermoepidermal junction, epidermal atrophy,

mucin deposition between collagen bundles, and a dense lymphocytic infiltrate. There was a perifollicular and superficial lymphocytic perivascular infiltrate in 2 biopsy specimens, and a perifollicular, superficial, and deep perivascular lymphocytic infiltrate in 6 biopsy specimens. There were no eosinophils present.

IMMUNOHISTOCHEMICAL ANALYSES

Results of immunohistochemical analysis of 8 DLE biopsy specimens showed increased staining for the IL-2 receptor (**Figure 1B**) and the IFN- γ receptor (Figure 1C) on the cell surface of cells in the dermis. However, no staining for the IL-4 receptor was detected in 8 biopsy specimens of DLE lesional skin (Figure 1C). Receptors for IL-2, IFN- γ , or IL-4 were not identified in 3 biopsy specimens of normal skin (Figure 1A, C, and E). Direct immunofluorescence showed a bandlike granular deposit of IgG, IgM, and C3 along the dermoepidermal junction in all DLE specimens tested.

MOLECULAR STUDIES

We found significantly elevated levels of IL-2 and IFN- γ messenger RNA (mRNA) in all 8 biopsy specimens of DLE. In contrast, no transcripts of IL-2 or

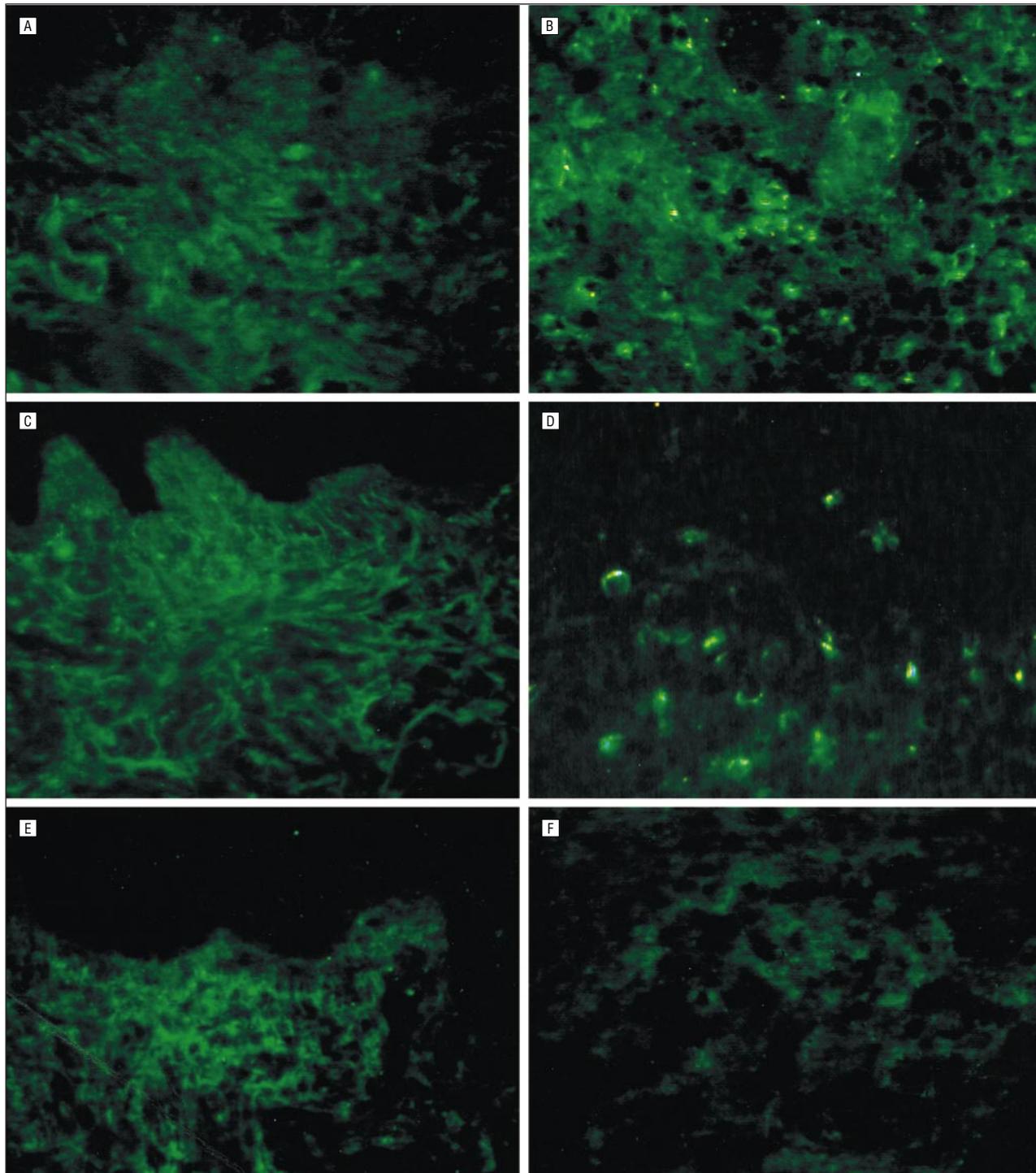


Figure 1. Findings of immunohistochemical analysis for interleukin (IL) 2, interferon (IFN) γ , and IL-4 in biopsy specimens of normal skin and discoid lupus erythematosus (DLE) skin. Antibodies against IL-2 receptors in normal skin (A) and DLE skin (B). Antibodies against IFN- γ receptor in normal (C) skin and DLE skin (D). Antibodies against IL-4 receptors in normal skin (E) and DLE skin (F).

IFN- γ were detected in 3 biopsy specimens of normal skin ($P < .01$) (**Figure 2**). Similarly, significantly elevated levels of TNF- α mRNA were found in 8 DLE biopsy specimens, while no TNF- α mRNA was detected in 3 biopsy specimens of normal skin ($P < .01$) (Figure 2). No IL-4 or IL-1 β mRNA was detected in 8 biopsy specimens of DLE lesional skin and 3 biopsy specimens of normal skin.

COMMENT

In this study we found transcripts for type 1 cytokines IFN- γ and IL-2 in DLE biopsy specimens. Our cohort of patients consisted of a clinically and histologically homogeneous group of individuals with classic DLE, without signs of SLE. These findings suggest that type 1 cytokines predominate in LE localized to the skin and are

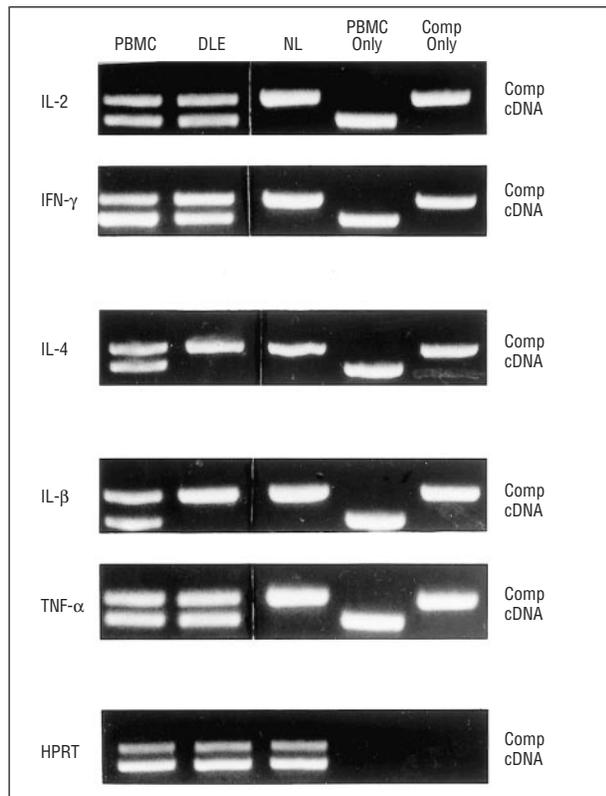


Figure 2. Gel electrophoresis of cytokine WT complementary DNAs (cDNAs) and their competitor (comp) cDNAs. In each lane the competitor is the upper band and the wild type is the lower band. PBMC indicates peripheral blood mononuclear cell; DLE, discoid lupus erythematosus; NL, normal; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; HPRT, hypoxanthine phosphoribosyl transferase.

pathogenically important in DLE. Interestingly, it has been demonstrated that administration of IFN- γ accelerates LE glomerulonephritis. Treatment with neutralizing antibodies to IFN- γ or soluble IFN- γ receptors results in serologic and renal remission of LE.¹²

We did not identify transcripts for IL-4 in any of the 8 biopsy specimens of DLE, although we used a modified quantitative polymerase chain reaction technique that is ultrasensitive and allows for determinations of genes (mRNA or complementary DNA) in very small amounts of material.¹⁰ Therefore, we can conclude that IL-4 mRNA was absent in our DLE biopsy specimens. Interferon γ may be responsible for the inhibition of IL-4 mRNA in DLE. The inhibitory effect of IFN- γ on IL-4 transcription is well supported in the literature.¹³ Based on our results and in accordance with previous reports in the literature, IL-4 does not play a major role in induction and development of cutaneous lupus.

We found elevated TNF- α mRNA in biopsy specimens of DLE lesional skin. Tumor necrosis factor α is a potent inducer of local inflammatory responses and may play an important role in the pathogenesis of DLE: effective therapies for DLE such as chloroquine, glucocorticoid steroids, and thalidomide have been shown to down-regulate TNF- α expression.¹⁴⁻¹⁶ Recently, thalidomide was proven effective in the treatment of cutaneous lupus refractory to other treatments. In the study by Ordí-Ros et al,¹⁶ 12 (75%) of 16 patients achieved complete

remission. A topical formulation of thalidomide may be the ideal treatment for patients with localized DLE because it may avoid systemic adverse effects. Recently, elevated transcription of TNF- α in bone marrow cells from patients with SLE¹⁷ and elevated TNF- α gene expression in lupus nephritis tissue have been reported.¹⁸

Indeed, cytokine abnormalities in SLE were first documented in studies of IFN- α ,¹⁹ levels of which have now been shown to be elevated in serum samples from most patients with SLE.²⁰ However, the cellular source and stimulus for production is unknown; IFN- α potentiates monocyte activation and favors a type 1 cytokine response, promoting IL-2 and IFN- γ cytokine production.²¹ This could explain our finding of elevated IL-2 and IFN- γ transcripts in biopsy specimens of DLE.

Type 2 cytokines have been studied in SLE; IL-6 and IL-10 have been found in high levels in serum samples from individuals affected with SLE.²² Initially, these observations suggested that SLE should be considered a type 2 cytokine disease, a proposal supported by the prominent role for B-cell hypersensitivity and hypergammaglobulinemia. This proposal has been challenged. Some studies have shown that IL-4 mRNA, the prototype type 2 cytokine, is decreased in mononuclear cells from patients with active SLE compared with controls.²³ Peripheral and tissue eosinophilia present in type 2 cytokine diseases were not found in our patients with DLE. This finding provides indirect evidence that DLE is a type 1 rather than a type 2 cytokine disease.

In conclusion, our findings suggest that DLE is associated with type 1 cytokines characterized by the expression of IL-2 and IFN- γ . Type 1 cytokines may be critically important for the induction and development of DLE. These results further our understanding of the pathogenesis of lupus. T cells are known to produce type 1 cytokines.¹¹ In our study, a predominant CD4⁺ lymphocytic T-cell infiltrate was present in biopsy specimens with high levels of IL-2 and IFN- γ mRNA. This suggests that CD4⁺ T cells are most likely the source of type 1 cytokines in DLE. Future studies using intracellular cytokine analysis by cytofluorography, combined with quantification of cytokine levels, should confirm our findings and identify the source of IL-2 and IFN- γ in DLE.

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