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Invariant natural killer T cells are enriched at the site of cutaneous inflammation in lupus erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is associated with a numerical and functional reduction of peripheral blood (PB) invariant natural killer T (iNKT) cells. Limited information exists on the role of iNKT cells in the pathogenesis of lupus erythematosus.
Objective: To investigate the frequency and phenotype of iNKT cells in PB and dermal infiltrates from patients with SLE, subacute-cutaneous lupus erythematosus (SCLE) and discoid lupus erythematosus (DLE).
Methods: PB was obtained from 23 SLE, 6 SCLE, and 11 DLE patients, and from 30 healthy controls. iNKT cell frequency and CCR4/CCR6 surface expression were assessed by flow cytometry. The frequency and phenotype of skin infiltrating $V\alpha24^{+}V\beta11^{+}$ iNKT cells were investigated by immunofluorescence in lesional biopsies from 20 patients, unaffected skin from 3 patients, and from 6 healthy controls.
Results: Lupus erythematosus patients displayed significantly lower percentages of circulating $CD3^{+}6B11^{+}$ iNKT cells compared to healthy controls. Whereas CCR6 expression on iNKT cells was enhanced in active SLE patients regardless of cutaneous involvement compared to healthy controls, CCR4 was exclusively increased in patients with active cutaneous lesions. Furthermore, iNKT cells were significantly enriched in lesional skin of SLE and DLE patients, but not in unaffected skin of lupus patients. The majority of lesional iNKT cells expressed IFN-γ and CCR4.
Conclusion: The deficiency in circulating iNKT cells in cutaneous lupus erythematosus is associated with an increase of iNKT cells at the site of cutaneous inflammation. These data underscore the importance of analyzing iNKT cells not only in PB, but also in the target tissues.

1. Introduction

Cutaneous lupus erythematosus (CLE) occurs in heterogeneous clinical manifestations [1]. The most frequent form is discoid lupus erythematosus (DLE) and potentially the most severe form systemic lupus erythematosus (SLE) [2]. Mucocutaneous lesions are present in 60–85% of SLE patients and may be associated with life-threatening organ involvement including nephritis, serositis, or central nervous system vasculitis. A third variant of lupus erythematosus, subacute-cutaneous lupus erythematosus (SCLE), is characterized by an annular or psoriasiform photosensitive skin rash and mild systemic symptoms such as arthralgias and fatigue [1]. While patients with SLE and SCLE usually display antinuclear antibodies (ANA), these autoantibodies are only detected in a subset of DLE patients. The pathogenesis of LE involves genetic polymorphisms and alterations of the innate and adaptive immune system [3].

Human invariant natural killer T (iNKT) cells are a subset of T lymphocytes characterized by expression of an invariant T cell receptor (*i*TCR) α chain (Vα24-Jα18) paired with a Vβ11 chain [4]. *i*TCR engagement mediated by CD1d-bound glycolipid antigens results in a prompt release of Th1 (e.g IFN-γ, TNF-α) or Th2-like cytokines (e.g. IL-4, IL-10) leading to transactivation of dendritic cells, B and T lymphocytes [5]. Due to the polarizing cytokines they produce, iNKT cells are functionally versatile and may mediate both pathogenic (allergic inflammation) and regulatory immune functions (maintenance of transplant tolerance, inhibition of autoimmunity) [6]. iNKT cells are implicated to play a role in the pathogenesis of various skin disorders including contact hypersensitivity, atopic dermatitis, psoriasis and skin cancer [7].

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We, and others have previously reported a numerical and functional deficiency of peripheral blood (PB) iNKT cells in active SLE patients and in lupus-prone mice [8–13]. In a hydrocarbon-induced mouse model of lupus, co-stimulation with lipopolysaccharide (LPS) and the synthetic glycolipid α-GalCer induced an increase in iNKT cells and led to a pronounced suppression of autoantibody production [12]. In addition, α-GalCer-mediated expansion of iNKT cells in the MRL-lpr/lpr lupus mouse model was shown to alleviate lupus dermatitis [13]. Taken together, these findings suggest a role for iNKT cells in the prevention or suppression of SLE [14]. Whether the deficiency of circulating iNKT cells in SLE is a primary phenomenon, or secondary due to migration to the site of inflammation remains to be established. It has been previously suggested that iNKT cells are intrinsically defective in patients with SLE since low PB iNKT cell frequencies were observed even in healthy relatives of SLE patients [10]. However, in patients with biliary cirrhosis [15] or Behcet's uveitis [16] significant iNKT cell infiltrates were detected in lesional biopsies despite a deficiency of iNKT cells in PB.

Here, we assessed the frequency and phenotype of iNKT cells in PB and cutaneous lesions from patients with different subtypes of CLE compared to healthy controls in order to investigate the role of iNKT cells in mucocutaneous lupus erythematosus. To our knowledge this is the first study demonstrating enhanced iNKT cell frequencies in a target tissue of lupus erythematosus.

2. Materials and methods

2.1. Patients and healthy control subjects

In this monocenter prospective study, circulating iNKT cells were examined in 23 SLE and 6 SCLE patients with active mucocutaneous involvement who were identified from a cohort of patients managed in the lupus clinic at University College London Hospital (UCLH). 11 DLE patients were recruited from the Department of Dermatology at UCLH and from the Department of Dermatology at the Royal Free Hospital, London. Seven SLE patients with no mucocutaneous involvement at the time of the study and 30 healthy individuals served as controls. Demographic and clinical data of the patients and controls is summarized in Table 1. All SLE patients met at least 4 of the revised criteria of the American College of Rheumatology, and had active SLE at the time of the study [17]. Discoid lesions were the most prevalent cutaneous manifestations, present in 11/23 (47.8%) SLE and 11/11 (100%) DLE patients. More precisely, 8 DLE and 4 SLE patients had localized discoid lupus in contrast to 3 DLE and 7 SLE patients with disseminated discoid lesions. Of the SLE patients, 5 had acute cutaneous lupus erythematosus (ACLE), and 7 non-specific manifestations such as cutaneous vasculitis or urticaria (Table 1). Serological parameters of disease activity such as anti-dsDNA antibodies, complement levels (C3), and the intensity of immunosuppressive treatment were examined in all patients at the time when blood and cutaneous biopsies were taken.

For the identification of iNKT cells in the skin, lesional skin biopsies were collected from 8 SLE, 4 SCLE, and 8 DLE patients. In addition, non-lesional biopsies from 3 SLE patients, and normal skin from 6 healthy controls were assessed. Tissue biopsies were snap-frozen in liquid nitrogen in OCT embedding medium and stored at −80 °C until further use. Written informed consent was obtained from all participants. The study was approved by the University College London Hospital ethics committee.

2.2. Flow cytometry

PB mononuclear cells (PBMCs) were obtained by density-gradient centrifugation of heparinized venous blood using Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden). For iNKT cell staining anti-human CD3-APC (eBioscience, San Diego, CA, USA), *i*TCR-PE (clone 6B11 which binds specifically to the conserved CDR3 region of the Vα24-Jα18 TCR; BD Biosciences, Oxford, UK), CCR4-V450 (BD Biosciences), CCR6-FITC (eBioscience) monoclonal antibodies (mAbs), and appropriate isotype controls were used. Cells were stained with combinations of antibodies at 4 °C for 20 min and were fixed with 2% paraformaldehyde. Data were acquired on the LSRII (Beckton-Dickinson) and analyzed using FlowJo (TreeStar, Ashland, OR, USA) as described previously [9].

Table 1
Demographic and clinical characteristics of patients and controls.

	SLE with active skin lesions ($n=23$)	SLE without skin lesions ($n=7$)	SCLE ($n=6$)	DLE ($n=11$)	HC ($n=30$)
Mean age (range)	37 (18–68)	41 (32–72)	42 (27–65)	48 (32–75)	36 (24–60)
Sex (male:female)	3:20	0:7	1:5	3:8	4:26
Ethnicity					
White	14 (60.9%)	4 (57.1%)	3 (50.0%)	5 (45.5%)	24 (80.0%)
Asian	4 (17.4%)	1 (14.3%)	1 (16.7%)	4 (36.4%)	3 (10.0%)
Afro-Caribbean	5 (21.7%)	2 (28.6%)	2 (33.3%)	2 (18.1%)	3 (10.0%)
Mean disease duration in years (range)	9.3 (0.17–26)	6.4 (1–18)	12.5 (1–30)	11.3 (1–28)	n.a.
Type of CLE					
ACLE[a]	5 (21.7%)				
SCLE			6 (100.0%)		
DLE	11 (47.8%)			11 (100.0%)	
Non-specific lesions[b]	7 (30.4%)				
C3[c] < 0.8 g/l	7 (30.4%)	4 (57.1%)	0 (0%)	1 (9.1%)	n.a.
ANA[d] titer ≥ 1:80	23 (100.0%)	7 (100.0%)	6 (100%)	4 (36.4%)	n.a.
dsDNA[e] ≥ 50 IU/ml	13 (56.5%)	5 (71.4%)	0 (0%)	0 (0%)	n.a.
Treatment					
Hydroxychloroquine	15 (65.2%)	7 (100.0%)	3 (50.0%)	9 (81.8%)	n.a.
Prednisolone ≥ 5 mg/d	12 (52.2%)	5 (71.4%)	2 (33.3%)	0 (0%)	n.a.
Immunosuppressants[f]	12 (52.2%)	6 (85.7%)	3 (50.0%)	2 (18.2%)	n.a.

[a] ACLE, acute cutaneous lupus erythematosus.
[b] Vasculitis, panniculitis, urticaria, or urticarial vasculitis.
[c] C3, serum complement component C3 (laser nephelometry).
[d] ANA, antinuclear antibodies (indirect immunofluorescence).
[e] dsDNA, anti-dsDNA antibodies (ELISA, Shield Diagnostics, Dundee, UK).
[f] Azathioprine, mycophenolate mofetil, methotrexate, or cyclophosphamide.