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The features of skin inflammation induced by lupus serum

Lena Liu [b], Guangqion Xu [a], Hui Dou [a], Guo-Min Deng [a,b,c,*]

[a] Key Lab of Antibody Techniques of Ministry of Health, Nanjing Medical University, Nanjing, China
[b] Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA
[c] State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, China

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ABSTRACT

We recently developed a model of lupus serum-induced skin inflammation, which was used to study the pathogenesis of skin injury in systemic lupus erythematosus (SLE). We further characterized the features of lupus serum-induced skin inflammation. This skin inflammation was evident within 3 h and lasted for at least two weeks. The skin inflammation was characterized by an influx of monocytic, CD11b + cells and by a scarcity of T and B lymphocytes. Depletion of IgG from the serum abrogated the skin inflammatory response. The skin inflammation was related to lupus patients' skin history but not to SLE disease activity and type of autoantibody. The expression of TNFR1, NF-kB and MCP-1 was increased locally in skin lesions. The TLR9 ligand and lupus serum act synergistically to trigger skin inflammation. These findings suggest that this novel model is valuable for the study of the pathogenesis and therapy of skin injury in SLE.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by high levels of autoantibody and multi-organ tissue damage, including kidney and skin damage [1,2]. Skin injury is second most common clinical manifestation in patients with SLE [3,4]. The molecular and cellular mechanisms involved in the expression of cutaneous LE remain unclear [5].

The skin injury of SLE can be divided into lupus erythematous (LE)-specific or LE-nonspecific manifestations by histologic analysis of biopsy specimens [6]. The LE-nonspecific manifestations are commonly associated with systemic organ manifestations or other autoimmune diseases [6]. The LE-specific cutaneous manifestations may present without or with less severe systemic organ involvement and are further classified into several subtypes, including acute (A) CLE, subacute (S) CLE, chronic (C) CLE and intermittent (I) CLE, which was proposed to be a separate category for LE tumidus in 2004 and ICLE is not universally accepted [7–9]. ACLE, SCLE and CCLE are three common clinical subtypes of cutaneous LE. Localized ACLE is often referred to as the 'malar rash' or 'butterfly rash' of SLE, whereas generalized ACLE is frequently referred to as the 'SLE rash' [9]. The skin injuries share the following features of a lichenoid tissue reaction: hyperkeratosis; epidermal atrophy; liquefactive degeneration of the epidermal basal-cell layer; mononuclear cell infiltrates focused at the dermo-epidermal junction, perivascular areas and perifollicular areas; thickening of the basal membrane; and melanin pigment incontinence. ACLE typically presents abruptly in the context of a systemic disease, and almost all patients develop SLE [5]. ICLE seems to be a purely dermatological disease [10,11]. The deposition of immune complexes containing IgG, IgM and complement (C) 3 is typically observed at the dermo-epidermal junction and is defined as a positive 'lupus band' test [12,13].

The production of autoantibodies directed against nuclear antigens and a myriad of other autoantigens characterizes SLE. In the skin, the invariably observed keratinocyte apoptosis may result in the cell surface expression and release of self-antigens, including DNA, Ro/SSA, La/SSB and histones [2,5]. Although exposure to UV and other environmental triggers may contribute to the initiation of cutaneous LE by triggering the apoptosis of keratinocytes, it is still unclear how the inflammatory process begins and is sustained [2,5]. IFN-α plays an important role in the pathogenesis of lupus erythematosus skin lesions [14]. Large numbers of IFN-producing plasmacytoid dendritic cells are also found in SLE skin lesions [15,16].

Tissue damage in SLE is associated with autoantibody production and immune complex formation and deposition [2]. An anti-double-stranded DNA antibody has been linked to kidney [2] and brain pathology [17]. Anti-acidic ribosomal protein P0 (anti-RPLP0) and anti-galectin 3 antibodies are related to the development of skin lesions in SLE [18]. Ro52 is a common target of circulating autoantibodies in SLE. Ro52 is highly expressed in spontaneous and UV-induced cutaneous inflammation in CLE; approximately 80% of cells within infiltrates of CLE lesions are positively stained for Ro52 in the dermis [19]. The loss of autoantigen Ro52 induces skin inflammation and systemic autoimmunity [20]. This result indicates that Ro52 exerts an important regulatory role in lupus skin lesions. Apparently, Autoantigens bind to keratinocytes undergoing apoptosis and contribute to the inflammatory process [2]. MRL/lpr mice are a common animal model of SLE and spontaneously develop lupus-like clinical manifestations characterized by

* Corresponding author at: Nanjing Medical University, China.
E-mail address: gmdeng@njmu.edu.cn (G.-M. Deng).

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high levels of autoantibodies and multiorgan tissue damage, including skin injury [21,22]. These observations indicate that autoantibodies may play an important role in the expression of cutaneous lesions in SLE and led us to hypothesize that lupus serum induces skin inflammation.

Recently, we have confirmed the hypothesis that serum from lupus patients and lupus-prone mice induced skin inflammation [23]. We also found that an animal model of lupus serum-induced skin inflammation is a valuable tool to investigate the pathogenesis and therapy of skin injuries in SLE [24,25]. In this paper, we further defined the features of skin inflammation induced by lupus serum.

2. Materials and methods

2.1. Mice and sera

C57BL/6, BALB/c, Swiss and MRL/lpr mice were purchased from JAX Labs (Cold Harbor, USA). All mice were housed in the animal facility of the Beth Israel Deaconess Medical Center and Nanjing Medical University. Sera were collected from patients with SLE and MRL/lpr mice at different ages and normal C57BL/6 mice. Animal and human use protocols were approved by appropriate Beth Israel Deaconess Medical Center committees and by Nanjing Medical University committees.

Sera were collected from patients with SLE and controls from Beth Israel Deaconess Medical Center and Jiangsu Province Hospital. Eighteen patients fulfilling ≥4 of 11 revised criteria of the American College of Rheumatology for the classification of SLE were studied. All patients had SLE disease-activity index (SLEDAI) scores ranging from 0 to 12. Medications were discontinued ≥24 h before venipuncture. Ten normal women served as controls in this study. Sera were collected from MRL/lpr mice of different ages and normal C57BL/6 mice.

2.2. Injection protocol procedures

Different doses of lupus serum were injected intradermally in the back of the neck of mice of different strains. All of the intradermal injections were performed on anesthetized mice [24].

2.3. Histopathological examination

Histopathological examination of skin was performed after routine fixation and paraffin embedding of the tissue. Tissue sections from the skin were cut and stained with hematoxylin and eosin. All slides were coded and evaluated in a 'blinded to sample' identity manner. The severity of skin inflammation was scored 0–4 as follows: grade 0, normal; grade 1, hyperplasia of epidermis; and grades 2–4, different amounts of infiltrating inflammatory cells in the skin with or without hyperplasia of epidermis [24].

2.4. Immunohistochemical examination

After deparaffinization and antigen retrieval, samples were stained with antibodies to CD4 + (GK1.5), CD8 + (53.6.7), NF-κB and MCP-1 (Santa Cruz, CA) followed by incubation with biotinylated secondary antibodies, avidin-biotin-peroxidase complexes and 3-amino-9-ethylcarbazole containing H_2O_2. All sections were counterstained with Mayer's hematoxylin [24].

2.5. Immunoglobulins

Serum levels of IgG were measured by an enzyme-linked immunosorbent assay (ELISA). Antisera and immunoglobulin standards specific for IgG were purchased from Sigma.

2.6. Autoantibodies

Serum antibody levels to denatured or single-stranded DNA (ssDNA) and to native or double-stranded DNA (dsDNA), were measured by ELISA using methylated bovine serum albumin (10 μg/ml) to precoat the wells, followed by coating with 50 μg of heat-denatured (boiled for 20 m and then cooled rapidly on ice) calf thymus DNA (Sigma) per milliliter or native calf thymus DNA, as previously described [23].

2.7. Cytokine levels

The cytokine level in mouse serum was determined by ELISA.

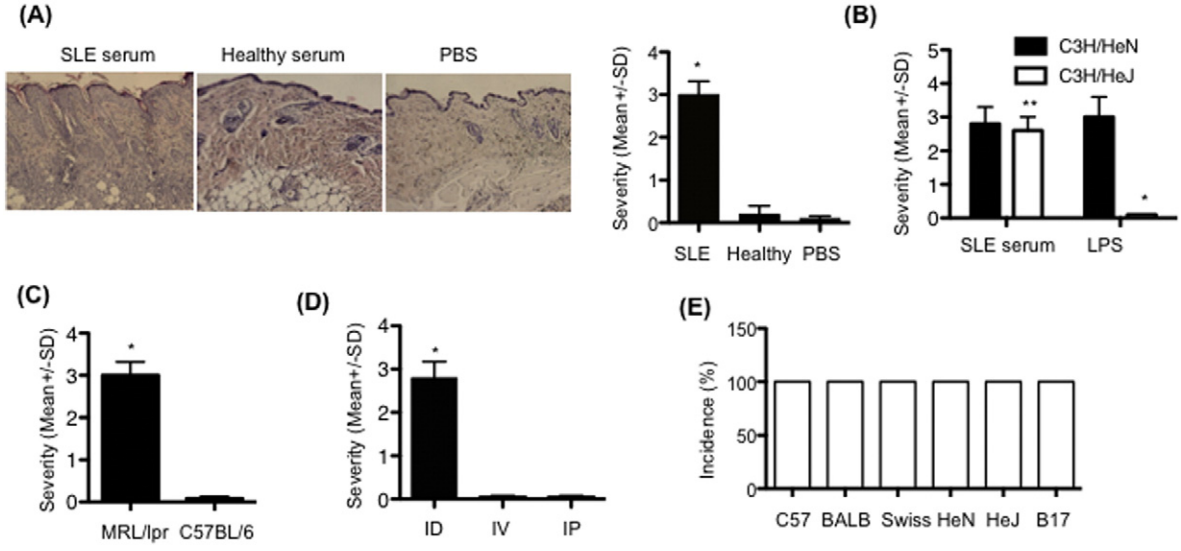

Fig. 1. Lupus serum induces skin inflammation. (A) Representative histopathological photomicrograph. Severity of skin inflammation of C57BL/6 mice sacrificed 3 d after intradermal inoculation of serum (100 μl) from a lupus patient with skin disease, a healthy control and PBS. (B) The severity of skin inflammation of LPS-responding C3H/HeN and LPS-nonresponding C3H/HeJ mice 3 d after intradermal inoculation of lupus serum (100 μl) ($n = 5$ per group). (C) The severity of skin inflammation in C57BL/6 mice ($n = 5$ per group) with intradermal injection of 100 μl of serum from MRL/lpr mice and C57BL/6 mice at 16 weeks of age. (D) The severity of skin inflammation in C57BL/6 mice ($n = 5$ per group) with intradermal injection (ID), intra-venous injection or intraperitoneal injection of 100 μl of lupus serum. (E) Incidence of skin inflammation in various mouse strains with intradermal injection of 100 μl of serum from lupus patients. $^*p < 0.05$; $^{**}p > 0.05$.