Journal of Autoimmunity 65 (2015) 38–48

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Epidermal injury promotes nephritis flare in lupus-prone mice

Kaitlyn L. Clark [a], Tamra J. Reed [a], Sonya J. Wolf [b], Lori Lowe [c, d], Jeffrey B. Hodgin [c], J.Michelle Kahlenberg [a, *]

[a] Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA
[b] University of Michigan Program in Biomedical Sciences, University of Michigan, Ann Arbor, MI, USA
[c] Department of Pathology, University of Michigan, Ann Arbor, MI, USA
[d] Department Dermatology, University of Michigan, Ann Arbor, MI, USA

ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form
7 August 2015
Accepted 12 August 2015
Available online 21 August 2015

Keywords:
Lupus
Flare
Skin
Nephritis
Chemokines

ABSTRACT

Systemic lupus erythematosus is clinically characterized by episodes of flare and remission. In patients, cutaneous exposure to ultraviolet light has been proposed as a flare trigger. However, induction of flare secondary to cutaneous exposure has been difficult to emulate in many murine lupus models. Here, we describe a system in which epidermal injury is able to trigger the development of a lupus nephritis flare in New Zealand Mixed (NZM) 2328 mice. 20-week old NZM2328 female mice underwent removal of the stratum corneum via duct tape, which resulted in rapid onset of proteinuria and death when compared to sham-stripped littermate control NZM2328 mice. This was coupled with a drop in serum C3 concentrations and dsDNA antibody levels and enhanced immune complex deposition in the glomeruli. Recruitment of $CD11b^{+}CD11c^{+}F4/80^{high}$ macrophages and $CD11b^{+}CD11c^{+}F4/80^{low}$ dendritic cells was noted prior to the onset of proteinuria in injured mice. Transcriptional changes within the kidney suggest a burst of type I IFN-mediated and inflammatory signaling which is followed by upregulation of CXCL13 following epidermal injury. Thus, we propose that tape stripping of lupus-prone NZM2328 mice is a novel model of lupus flare induction that will allow for the study of the role of cutaneous inflammation in lupus development and how crosstalk between dermal and systemic immune systems can lead to lupus flare.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Systemic lupus erythematosus (SLE) is characterized by progressive organ damage often exacerbated by acute disease flares. Development of SLE is thought to be secondary to genetic and epigenetic predisposition which promotes autoreactive T and B cell survival following exposure to appropriate environmental stimuli (reviewed in Ref. [1]). Autoantibodies and autoreactive cells can be present in a quiescent state of low disease activity. However, SLE patients will experience episodes of disease flare during which inflammation of various organs results in permanent damage. Knowledge of what drives the onset of flares in a previously stable patient is poorly understood. Viral infection has been linked to exacerbation of disease activity in some patients (reviewed in Ref. [2]). Exposure to ultraviolet (UV) light also may exacerbate cutaneous disease [3] and can accelerate lupus nephritis in certain murine models [4]. The mechanisms promoting flares following these triggers remain poorly understood, yet they serve as important targets for development of preventative therapies for lupus flares.

Murine lupus models have been essential to dissect disease pathogenesis. These models demonstrate an accumulation of autoantibody production and autoreactive B and T cell populations followed by eventual onset of organ damage (reviewed in Ref. [5]). Thus, these models represent essentially a quiescent stage of the disease until organ damage ensues. Acceleration of autoantibody production and acute glomerulonephritis via injection of IFNα-

Abbreviations: AIM2, absent in melanoma 2; CAMP, cathelicidin antimicrobial peptide; CCL, chemokine (c–c) ligand; CCR, chemokine (c–c) receptor; cxcl, chemokine (c-x-c) ligand; IFN, interferon; IRF, interferon regulatory factor; MCP, monocyte chemoattractant protein; MX, myxovirus resistance; NZM, New Zealand Mixed; SLE, systemic lupus erythematosus; TLR, toll-like receptor; TNF, tumor necrosis factor; UV, ultraviolet.

* Corresponding author. University of Michigan Division of Rheumatology, Department of Internal Medicine, 5570A MSRB II, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-5678, USA.
E-mail address: mkahlenb@med.umich.edu (J.Michelle Kahlenberg).

http://dx.doi.org/10.1016/j.jaut.2015.08.005
0896-8411/© 2015 Elsevier Ltd. All rights reserved.

expressing adenovirus has been used as a model to study the onset of acute lupus [6–8], and has been a useful tool for understanding the role of this cytokine in disease onset. Other mechanisms of disease flare have been more difficult to model in murine systems, however. UV induction of systemic disease works only in murine models with increased expression of toll-like receptor (TLR)7 [4]. Importantly, TLR7 agonist stimulation of cutaneous inflammation and glomerulonephritis in wild-type mice requires cutaneous, not systemic application [9], which suggests a role for skin inflammation to promote systemic disease. Epidermal injury through removal of the stratum corneum via tape application in lupus-prone NZB/NZW F_1 mice has been proposed as a valid model for the study of cutaneous lupus [10]. Thus, we chose to use this method of epidermal injury to study the effects of cutaneous inflammation on SLE flare in lupus prone NZM2328 mice.

2. Materials and methods

2.1. Mice

The University of Michigan committee on use and care of animals reviewed and approved all animal protocols for this study. NZM2328 mice were a kind gift of Dr. Chaim Jacob, University of Southern California, and were bred at the University of Michigan Breeding core. Mice were housed in specific-pathogen free housing at the University of Michigan. The NZM2328 strain was chosen as it is an excellent model for lupus nephritis in that it develops high titers of dsDNA antibodies, immune complex deposits in the kidney and is the closest congenic strain to the well-studied NZB/NZW (F_1) model [11]. Further, this model does not spontaneously develop cutaneous disease, which allows us to study the effects of cutaneous inflammation without confounders. BALB/c mice were a kind gift from Dr. Mary O'Riordan at the University of Michigan.

2.2. Cutaneous injury via tape stripping

20-week old female NZM2328 mice or 20-week old female BALB/c mice were given 1 mg buprenorphene via subcutaneous injection for analgesia followed by hair removal via shaving and application of Veet to a 3 × 4 cm area of dorsal skin. Urine and serum were collected and stored. The mice were anesthetized in a drop jar via isoflurane (Vet One, Fluriso) and the stratum corneum was removed by 25 applications and removal of 3M duct tape placed to the dorsal skin. Rash development and urinary protein were monitored twice a week. Sham animals were given buprenorphine followed by anesthetization via isoflurane, hair removal via shaving and application of Veet, but they did not undergo duct tape application. See Table 1 for summary of treatments and sample collection.

2.3. Characterization of anti-double stranded DNA and C3 serum levels

Serum anti-dsDNA and C3 levels were quantified via ELISA (Alpha Diagnostic International, San Antonio, TX and Innovative Research Inc.,Novi, MI), according to manufacturer's protocols.

2.4. Proteinuria analysis

Prior to euthanasia, urine samples were collected and assessed for microalbumin using Albuwell kits (Exocell, Philadelphia, PA) and total creatinine (Cr) using a commercial kit (Bioassay Systems, Hayward, CA). Ratios of microalbumin:Cr were calculated to estimate 24 h urinary protein excretion.

2.5. Renal histopathology and immune complex deposition scoring

To score glomerular inflammation (activity index) and scarring (chronicity index), kidneys perfused with 10 U/ml heparin sulfate (Sigma) in PBS were fixed in 10% formalin, paraffin embedded, and section at 3 μm thickness. Periodic Acid Schiff (PAS)-stained sections were examined and graded (JBH) in a blinded manner as previously described by us and others [12,13]. Briefly, a semi-quantitative scoring system assessed 3 different parameters of activity (mesangial hypercellularity, crescents and endocapillary cellular infiltrate) in 30 glomeruli per mouse. Scores were defined as: 0 = no involvement, 0.5 = minimal involvement (<10%), 1 = mild involvement (10–30%), 2 = moderate involvement (31–60%), 3 = severe (>60%). The activity index was generated by combining the scores for mesangial hypercellularity, crescents, and endocapillary cellularity for each glomerulus and calculating the average for each mouse. The chronicity index was likewise generated by combining the scores for mesangial sclerosis, capillary sclerosis, and organized crescents and calculating the average for each mouse.

Glomerular immune complex deposition was quantified on 6 μm frozen kidney sections via staining for C3 and IgG deposition as previously described [13]. Briefly, sections were stained with FITC-conjugated anti-C3 (ICL, Portland, OR) and Texas-red-conjugated anti-mouse IgG (Sigma) for 1 h at 4°C; Hoechst (Invitrogen, Eugene, OR) was used to visualize DNA. Immune complex staining was quantified using Metamorph v7.0. 6 glomeruli per mouse were analyzed. Integrated FITC and Texas Red staining was calculated and presented as staining per area.

Table 1
Summary of treatments and findings in injured mice.

Day	Treatment	Findings
0	Serum and urine collected	
1	Tape Strip or Sham treatment	**Skin**: Increased neutrophilic epidermal inflammation
7	Euthanize for day 7 time point	**Skin**: epidermal hyperplasia and chronic inflammatory changes **Kidney**: Increased macrophage infiltration; rise in renal inflammatory gene expression
14	Serum and urine collected, Tape Strip or Sham treatment	
15	Euthanize for day 15 time point	**Kidney**: increased immune complex deposition, increased DC and macrophage infiltration; resolution of inflammatory gene expression but increase in CXCL13.
30, 60	Serum and urine collection, organ harvest at euthanasia (at the onset of proteinuria)	**Skin**: in surviving mice, rash is persistent and demonstrates lymphocytic and mast cell dermal inflammation. **Serum**: Drop in dsDNA titers, C3 and serum DNA correlates with rapid development of proteinuria and death. **Kidney:** Profound renal inflammation at proteinuria onset

Download English Version:

https://daneshyari.com/en/article/3367696

Download Persian Version:

https://daneshyari.com/article/3367696

Daneshyari.com