Clinical Immunology (2014) 154, 66–71

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Interferon alpha on NZM2328.Lc1R27: Enhancing autoimmunity and immune complex-mediated glomerulonephritis without end stage renal failure

Chao Dai[a], Hongyang Wang[a], Sun-Sang J. Sung[a], Rahul Sharma[a], Carol Kannapell[a], Wei Han[a], Qian Wang[a], Anne Davidson[b], Felicia Gaskin[c], Shu Man Fu[a,d,e,*]

[a] *Center for Immunity, Inflammation and Regenerative Medicine, Department of Medicine, University of Virginia, Charlottesville, VA, USA*
[b] *Center for Autoimmunity and Musculoskeletal Diseases, Feinstein Institute for Medical Research, Manhasset, NY, USA*
[c] *Department of Psychiatry and Neurobehavioral Sciences, School of Medicine, University of Virginia, Charlottesville, VA, USA*
[d] *Division of Rheumatology, Department of Medicine, University of Virginia, Charlottesville, VA, USA*
[e] *Department of Microbiology, Immunology and Cancer Biology, School of Medicine, University of Virginia, Charlottesville, VA, USA*

Received 13 June 2014; accepted with revision 17 June 2014
Available online 27 June 2014

KEYWORDS
SLE;
Interferon α;
Mouse model for lupus nephritis

Abstract Interferon alpha (IFNα) may play a significant role in systemic lupus erythematosus (SLE) pathogenesis. Recent literature suggests that IFNα does not correlate with disease activities and blockade of IFNα is not effective in treating SLE. This study aims to delineate further the role of IFNα in SLE. 12-week old NZM2328 and its congenic NZM2328.Lc1R27 (R27) female mice were challenged with adenovirus-IFNα (adeno-IFNα) or adenovirus-LacZ (adeno-LacZ). Only adeno-IFNα treated NZM2328 developed severe proteinuria and died of chronic glomerulonephritis (GN) and end stage renal disease. Adeno-IFNα treated R27 did develop immune complex-mediated GN but had normal renal function. Adeno-LacZ treated NZM2328 showed enlarged glomeruli and increased cellularity without immune complex deposition. Adeno-LacZ treated R27 did not show serological and histological abnormalities. Adeno-IFNα induced anti-dsDNA and anti-kidney autoantibodies in NZM2328 and R27. These

Abbreviations: Adeno-IFNα, adenovirus-IFNα; Adeno-LacZ, adenovirus-LacZ; ANA, anti-nuclear antibody; BUN, blood urea nitrogen; ELISA, enzyme-linked immunosorbent assay; ESRD, end stage renal disease; GN, glomerulonephritis; H&E, hematoxylin and eosin; IFNα, interferon α; IL-1, interleukin 1; LN, lupus nephritis; R27, NZM2328.Lc1R27; SLE, systemic lupus erythematosus.

* Corresponding author at: Box 800133, University of Virginia Health System, Charlottesville, VA 22908-0133, USA. Fax: +1 434 924 9578.
E-mail address: sf2e@virginia.edu (S.M. Fu).

http://dx.doi.org/10.1016/j.clim.2014.06.008
1521-6616/

results suggest that end organ damage is host-dependent and less related to autoimmunity and may have significant implications in SLE pathogenesis.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting multiple organs with complex pathogenesis [1]. The clinical presentation of SLE at the initial diagnosis and at relapse is variable. Among the implicated cytokines, interferon α (IFNα) was first reported to be elevated in the plasma of patients with SLE and other autoimmune diseases in 1979 [2]. With the advent of the concept that an "interferon signature" is present in patients' peripheral blood [3,4], considerable interest in this cytokine has been rekindled (reviewed in [5,6]). The findings that ANA and anti-dsDNA antibodies are induced in patients treated with IFNα and that SLE develops in a small proportion of these ANA positive patients further reinforce the notion that IFNα may play a crucial pathogenic role (reviewed in [7]). Despite this initial enthusiasm, longitudinal studies of lupus patients did not show a correlation of IFNα levels with disease activity [8,9]. In addition, blocking IFNα as a therapeutic strategy has resulted in disappointing results (reviewed in [6]). Thus, the exact role of IFNα in SLE remains to be delineated further.

Our laboratory has used NZM2328 as a model for lupus nephritis (LN) [10–12]. LN and end stage renal disease (ESRD) develops preferentially in female mice in this model [10]. Genetic studies showed that autoantibody production and susceptibility to development of ESRD were under separate genetic control [10,11]. The phenotype of NZM2328.Lc4 is the development of LN and ESRD in female mice without ANA and anti-dsDNA antibodies, providing evidence that these antibodies are not required for the development of fatal LN [11]. Recently our laboratory generated a congenic strain NZM2328.Lc1R27 (R27) whose disease phenotype showed that chronic glomerulonephritis (GN) and immune complex-mediated acute GN are under separate genetic control and that immune complex-mediated GN need not progress to chronic GN leading to ESRD [12]. Studies of GN pathogenesis in NZM2328 and its congenic strain R27 now allow us to delineate the role of IFNα in LN. We show here that young NZM2328 treated with IFNα developed accelerated GN and exhibited all the features of spontaneous LN found in older NZM2328 mice, including severe proteinuria, chronic glomerular nephritis, as well as early mortality. In contrast R27 injected with adeno-IFNα are resistant to chronic kidney damage, even though autoantibody-mediated autoimmunity is induced.

2. Materials and methods

2.1. Mice and IFNα

NZM2328 mice were originally purchased from the Jackson Laboratory [10], and R27 were generated by our laboratory [12]. Only female mice were used. Adenovirus containing interferon alpha (adeno-IFNα) and control virus adeno-LacZ were used as described [13]. Adeno-IFNα and adeno-LacZ lysates were amplified in early passage in 293T cells, and the titer was determined by using Adeno Rapid Titer Kit (Clontech).

2.2. Study design

12 week old female NZM2328 or R27 mice were injected with 5×10^7 particles of adeno-IFNα or adeno-LacZ. The mice were followed for 2 months by monitoring weekly for the development of severe proteinuria (>300 mg/dl) with Multistix 10 SG (SIEMENS) and anti-dsDNA antibodies by ELISA [10]. Moribund mice were euthanized and perfused with PBS before collecting kidneys for further studies. Mice in the control groups injected with the vector adeno-LacZ were sacrificed at sixty days after the injection of the virus. Adeno-IFNα treated R27 were sacrificed either 8 weeks after viral injection or followed for nine months after the treatment.

2.3. Methods

Sera and urine were collected weekly or before euthanization. Serum BUN and urinary albumin/creatinine ratios were determined as described [12]. IgG, C3 and IgG2 deposition in kidneys by direct immunofluorescence, splenocyte cell surface marker expression by flow cytometry, serum anti-dsDNA antibodies by ELISA and ANA on 3T3 cells by immunofluorescence were carried out as described [12]. Renal Ig eluates were used in Western blot analysis with kidney lysate from two month old NZM2328 as the substrate as described [12]. Two-tailed unpaired Student t-tests were used to evaluate the significance of the results with assigned p-values.

3. Results

3.1. Acceleration of fatal GN in NZM2328 with adeno-IFNα

In a preliminary experiment, it was determined that 5×10^7 adeno-IFNα viral particles given intravenously induced the development of fatal GN within 6 weeks after they were injected into 12 week old NZM2328 female mice. This dose of adeno-IFNα was used in subsequent experiments. 5×10^7 adeno-LacZ viral particles were used as control virus and injected into a cohort of NZM2328 as the control group. As shown in Fig. 1A, 12 week old NZM2328 mice (n = 7) injected with adeno-IFNα developed severe proteinuria at different time points after the injection. By 5 weeks after the injection, all the treated mice developed severe proteinuria. Shortly after the development of severe proteinuria, the affected mice were sacrificed because they appeared to