


Transcription factor Fli-1 impacts the expression of CXCL13 and regulates immune cell infiltration into the kidney in MRL/lpr mouse

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ABSTRACT

Objective Friend leukaemia virus integration 1 (Fli-1) regulates chemokine/cytokine expression and thus plays an important role in the development of lupus nephritis. Chemokine CXC ligand 13 (CXCL13) is a chemokine that promotes the formation of ectopic lymphoid structures and has been reported to be associated with the pathogenesis of lupus nephritis. The relationship between Fli-1 and CXCL13 is unknown. This study aims to elucidate whether Fli-1 impacts CXCL13 expression and contributes to the progression of lupus-like nephritis in adult MRL/lpr mouse.

Methods Serum CXCL13 levels were measured in adult wild-type (WT) MRL/lpr mice and Fli-1 heterozygote knockout (Fli-1^{+/-}) MRL/lpr mice (4 months old or older) using ELISA. Renal mRNA expression (CXCL13 and related molecules) was measured using real-time PCR method. Kidneys were removed, stained and evaluated using a pathology scoring system. The grade of CXCL13 or CXC-chemokine receptor type 5 (CXCR5)-positive immune cell infiltration into the kidney was evaluated using immunostaining with anti-CXCL13 or anti-CXCR5 antibodies. We also used immunofluorescence staining with CXCL13- and CD11b-specific antibodies to detect the infiltration of CXCL13/CD11b double-positive immune cells.

Results Serum CXCL13 levels in Fli-1^{+/-} MRL/lpr mice were significantly lower than that in WT MRL/lpr mice (545.5 and 960.5 pg/mL, *p*=0.02). Renal expression of CXCL13 mRNA and SRY-related HMG box4 (Sox4) (an important factor for B-cell development) levels were significantly lower in Fli-1^{+/-} MRL/lpr mice. Renal histology scores in WT MRL/lpr mice revealed significantly increased glomerular inflammation. Despite similar interstitial immune cell infiltration into the kidney, the number of CXCL13- and CXCR5-positive cells was significantly lower in Fli-1^{+/-} MRL/lpr mice than in WT mice. Furthermore, immunofluorescence staining revealed that Fli-1^{+/-} MRL/lpr mice had significantly fewer CXCL13/CD11b double-positive immune cells.

Conclusion Fli-1 regulates renal Sox4 mRNA expression and infiltration of CXCR5-positive cells as well as CXCL13/CD11b double-positive immune cells into the kidney, which affects CXCL13 expression and lupus-like nephritis.

WHAT IS ALREADY KNOWN ON THIS TOPIC?

⇒ The transcription factor friend leukaemia virus integration 1 (Fli-1) played an important role in the pathogenesis of lupus nephritis. However, the relationship between Fli-1 and chemokine CXC ligand 13 (CXCL13) expression has not been investigated.

WHAT THIS STUDY ADDS?

⇒ Our findings show that Fli-1 impacts CXCL13 expression in the kidneys of MRL/lpr mice with lupus-like nephritis. Fli-1 heterozygote MRL/lpr mice had significantly lower CXCL13 expression and CXC-chemokine receptor type 5 (CXCR5)-positive immune cell infiltration with lower SRY-related HMG box4 (Sox4) mRNA expression.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Fli-1 is associated with CXCL13 expression, and inhibiting Fli-1 could be a novel target for treating lupus nephritis.

INTRODUCTION

SLE is an autoimmune disease with an unknown aetiology that causes chronic inflammation in multiple organs, including the kidney.¹ Lupus nephritis causes diffuse infiltration of inflammatory cells into the kidney, which can lead to renal failure.^{2,3} Many chemokines and cytokines are involved in the development of SLE and lupus nephritis.⁴⁻⁹ The chemokine CXC ligand 13 (CXCL13), also known as B-cell-attracting chemokine-1 or B-lymphocyte chemoattractant, attracts B cells and promotes the formation of ectopic lymphoid structures.¹⁰⁻¹⁴ Follicular dendritic cells (DCs) are the primary source of CXCL13 production in humans; however, epithelial cells, macrophages and T follicular helper (Tfh) cells (but not murine Tfh cells) also produce CXCL13.¹⁵ CXC-chemokine receptor type 5 (CXCR5), formerly known

as Burkitt's lymphoma receptor 1 (BLR1), is CXCL13's sole receptor. CXCR5 is highly expressed in mature B cells and Tfh cells.^{10–11} Previous research found that the CXCL13/CXCR5 axis was essential for B-cell activation, and CXCL13 is required for lymphoid neogenesis and for the maintenance of secondary lymphoid tissue architecture.^{16–17} Therefore, the CXCL13/CXCR5 axis is important for regulating B-cell development and homeostasis.¹⁴

CXCL13 has been reported to be associated with the pathogenesis of SLE and other autoimmune diseases, including inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, type 1 diabetes and systemic sclerosis.¹⁴ Previous research has suggested that the CXCL13/CXCR5 axis plays a role in the development of SLE and lupus nephritis.^{18–22} Lee *et al* have shown that CXCL13 and CXCR5 were found in high concentrations in the renal cortex of patients with lupus nephritis.¹⁸ Ishikawa *et al* discovered increased CXCL13 levels in the kidneys of aged New Zealand Black (NZB)/New Zealand White (NZW) F1 mice. The primary sources of CXCL13 in lupus-prone mice were renal DCs and macrophages.^{12–22} However, the distinct role of CXCL13/CXCR5 axis in the development of lupus nephritis is still to be clarified.

Friend leukaemia virus integration 1 (Fli-1) is a transcription factor that belongs to the Ets family and binds to DNA sequences that contain a consensus GGA(A/T) motif. Fli-1 is a transcriptional activator or repressor that has been found in endothelial cells, fibroblasts and several haematopoietic lineages.^{23–26} We previously reported that Fli-1 plays an important role in the development of lupus nephritis.^{27–29} Zhang *et al*²⁷ have reported that heterozygote Fli-1 (Fli-1^{+/-}) MRL/lpr mice (homozygote Fli-1^{-/-} were lethal in utero) showed significantly less renal inflammation and longer survival than wild-type (WT) littermates. Fli-1 regulates cytokine/chemokine expression and immune cell infiltration into the kidney in a lupus model mouse.^{30–37} Interestingly, peripheral lymphocytes from patients with SLE with active disease exhibited increased expression of Fli-1 transcripts in tandem with disease activity.³⁸ Fli-1 levels were also elevated in patients with newly developed or recurrent lupus nephritis but not in those with SLE who were infected.³⁹ Furthermore, Fli-1 is upregulated in the brains of Alzheimer's disease and Fli-1 inhibition protects 5xFAD mice (transgenic Alzheimer's disease mouse model) from pericyte loss and cognitive deficit.⁴⁰ Taken together, Fli-1 inhibition may present a novel therapeutic target in autoimmune diseases. The relationship between Fli-1 and CXCL13 is currently unknown. This study aims to elucidate whether Fli-1 impacts CXCL13 expression and contributes to the progression of lupus-like nephritis in adult MRL/lpr mice.

MATERIALS AND METHODS

Mice

WT MRL/lpr Fli-1^{+/+} mice and MRL/lpr Fli-1^{+/-} mice were generated as previously reported.²⁷ Back-crossing

with the Fli-1^{+/-} C57BL/6 strain for more than 12 generations produced MRL/lpr Fli-1^{+/-} mice, as described in our previous report. All mice were housed in Fukushima Medical University's animal institute under pathogen-free conditions. We used aged MRL/lpr Fli-1^{+/-} mice and their WT littermates based on the observation of previous studies; more than half of WT MRL/lpr mice showed fatal outcomes due to the progression of lupus-like nephritis (with high serum double-stranded DNA levels, proteinuria, haematuria, etc) in accordance with ageing, which was prominent in 20–35 weeks old compared with MRL/lpr Fli-1^{+/-} mice.^{27–28–31–35}

Genotyping by PCR

Mice were genotyped using PCR to differentiate between WT and mutant Fli-1 alleles, as previously described.²⁷ Briefly, the PCR primers used include the following: Fli-1 exon IX/forward primer (positions 1156–1180), 50-GACCAACGGGGAGTTCAAATGACG-30; Fli-1 exon IX/reverse primer (positions 1441–1465), 50-GGAGGATGGGTGACGGGACAAAG-30; and Pol II/reverse primer, 50-GGAAGTAGCCGTTATTAGTGGAGAGG-30. DNA was isolated from tail snips of 4-week-old mice using the QIAamp Tissue Kit (Qiagen). The PCR analyses were performed using the C1000 Touch Thermal Cycler (Bio-Rad).

Histopathology, immunohistochemistry and immunofluorescence staining

Kidneys of adult MRL/lpr Fli-1^{+/-} mice and their WT littermates (old mice aged 21–33 weeks) were removed, fixed in formalin and embedded in paraffin. The deparaffinised section was stained with H&E. Light microscopy was used to examine kidney sections, and the grade of kidney inflammation was determined using the previously described pathology scoring system.^{27–35} Briefly, blinded H&E-stained kidney sections were graded for glomerular inflammation, proliferation, crescent formation and necrosis. Scores ranging from 0 to 3 (0=*none*, 1=*mild*, 2=*moderate* and 3=*severe*) were assigned to each of these characteristics and then added together to produce a final pathology score. Scores for interstitial inflammation were as follows: 0 for <5% involvement, 1 for 5%–25% involvement, 2 for 25%–50% involvement and 3 for >50% involvement. CXCL13 was detected in paraffin-fixed tissues by anti-CXCL13 antibodies (R&D, Minnesota, USA) and polymer staining reagent using the Histofine Simple Stain Kit (Nichirei, Tokyo, Japan). Similarly, CXCR5+ immune cells were detected using anti-CXCR5 antibodies (R&D). CD11b+ and CXCL13+ cells in tissue were detected using fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD11b antibodies (BioLegend, California, USA) and CXCL13 antibodies (Abcam) with secondary anti-rabbit IgG conjugated to Alexa Fluor 647 (Abcam).³⁵ CXCL13+ and CXCR5+ immune cell infiltrates were detected as previously described.^{2–27} Briefly, 10 randomly selected

Table 1 Primers used for real-time PCR in this study

Primer	Forward primer	Reverse primer
CXCL1	5'-GCTTGAAGGTGTTGCCCTCAG-3'	5'-AAGCCTCGCGACCATTCTTG-3'
CXCL13	5'-CATAGATCGGATTCAAGT-3'	5'-TCTTGGTCCAGATCACAA-3'
CXCR5	5'-TGGCCTTCTACAGTAACAGCA-3'	5'-GCATGAATACCGCCTTAAAGGAC-3'
Sox4	5'-GCCTCCATCTTCGTACAACC-3'	5'-AGTGAAGCGCGTCTACCTGT-3'
Bcl-6	5'-CCGGCACGCTAGTGATGTT-3'	5'-TGTCTTATGGGCTCTAAACTGCT-3'
GAPDH	5'-CCAGTAGACTCCACGACATACTCA-3'	5'-ATCAACGACCCCTTCATTGACC-3'

Bcl-6, B-Cell CLL/Lymphoma 6; CXCL, chemokine CXC ligand; CXCR5, CXC-chemokine receptor type 5; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Sox4, SRY-related HMG box4.

sites were blindly counted to determine the number of CXCL13⁺, CXCR5⁺ and CD11b⁺/CXCL13⁺ cells per high-power field (magnification 200 or 400 if appropriate); cells were photographed using a microscope equipped with a digital camera (BX63; Olympus, Tokyo, Japan).

Quantitative evaluation of cytokines, chemokines and related cell-signalling molecules by real-time PCR

Transcripts encoding cytokines, chemokines and signalling molecules were evaluated from total RNA extracted from adult mouse renal tissue using the TRIzol reagent (Invitrogen). cDNA was generated using the SuperScript, First-Strand Synthesis System (Bio-Rad). Real-time PCR (RT-PCR) was performed in duplicate using the SYBR Green PCR Master Mix (Thermo Fisher, Waltham, Washington, USA) and two independent RNA preparations, according to the manufacturer's instructions. The primers for the detection of transcript encoding CXCL13 were acquired from OriGene (Rockville, Maryland, USA), and primers for all other transcripts are listed in table 1. Cycling conditions were as per instructions from the company. RT-PCR was carried out using Real-time PCR Detection System (Applied Biosystems) with relative expression analysis determined by reference to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), according to the programme provided by Applied Biosystems. The relative expression of the target genes was calculated using the DDCT method. The RT-PCR expression was 1 for relative quantification in the WT (Fli-1^{+/+}) group.³¹

Measurement of serum chemokine, cytokine (CXCL13, IL-6) and anti-double-stranded DNA antibodies

Immunoreactive CXCL13 and interleukin 6 (IL-6) in sera from adult mice (26–33 weeks old) were quantified using an ELISA (BioLegend and R&D, respectively), according to the manufacturer's instructions. Serum anti-dsDNA antibody levels (100 dilutions) were also evaluated using ELISA, in accordance with the manufacturer's instructions.

Statistics

Results are presented as median±IQR (or mean±SD if appropriate). Quantitative data were analysed using the Student's *t*-test with Welch's correction or Mann-Whitney *U* test according to the characteristics of data. Correlation analysis between CXCL13 and anti-ds DNA levels was conducted using Spearman's correlation test. A *p*-value <0.05 was considered significant. Statistical analyses were conducted using Excel add-ins and Statcel 4 Software (OMS Publishing, Saitama, Japan).

RESULTS

Serum cytokine and chemokine levels

Serum CXCL13 levels and IL-6 levels were measured in MRL/lpr Fli-1^{+/-} mice and compared with age-matched MRL/lpr WT (Fli-1^{+/+}) mice (26–33 weeks old; *n*=6 each). As shown in figure 1A, median serum CXCL13 levels were significantly lower in MRL/lpr Fli-1^{+/-} mice (545.5 pg/mL, IQR 396.5–736.5) than in WT mice (960.5 pg/mL, IQR 795–1103.5) (*p*=0.02; figure 1A). Serum IL-6 levels were also decreased in MRL/lpr Fli-1^{+/-} mice (0 pg/mL, IQR 0–0.9) compared with WT littermates (39.5 pg/mL, IQR 10–70) (*p*=0.01; figure 1B). We also compared serum CXCL13 concentrations between younger (7–9 weeks old) and adult (21–36 weeks old) MRL/lpr mice; as shown in online supplemental figure S2A, younger MRL/lpr mice had significantly lower CXCL13 levels compared with adult MRL/lpr WT mice (*p*<0.05). The median CXCL13 levels were similar between MRL/lpr Fli-1^{+/-} mice and age-matched MRL/lpr WT (Fli-1^{+/+}) littermates (543.5 pg/mL, IQR 458–629.5 and 539.5 pg/mL, IQR 376.8–644.8, respectively).

Serum anti-dsDNA levels and correlation with cytokine/chemokines

Serum anti-dsDNA levels were measured using ELISA and found that anti-dsDNA levels (100 dilutions) were significantly lower in MRL/lpr Fli-1^{+/-} mice than in WT MRL/lpr mice (*p*=0.02; online supplemental figure S1A). Interestingly, serum anti-dsDNA levels were positively correlated with serum IL-6 levels (*r*_s=0.58,

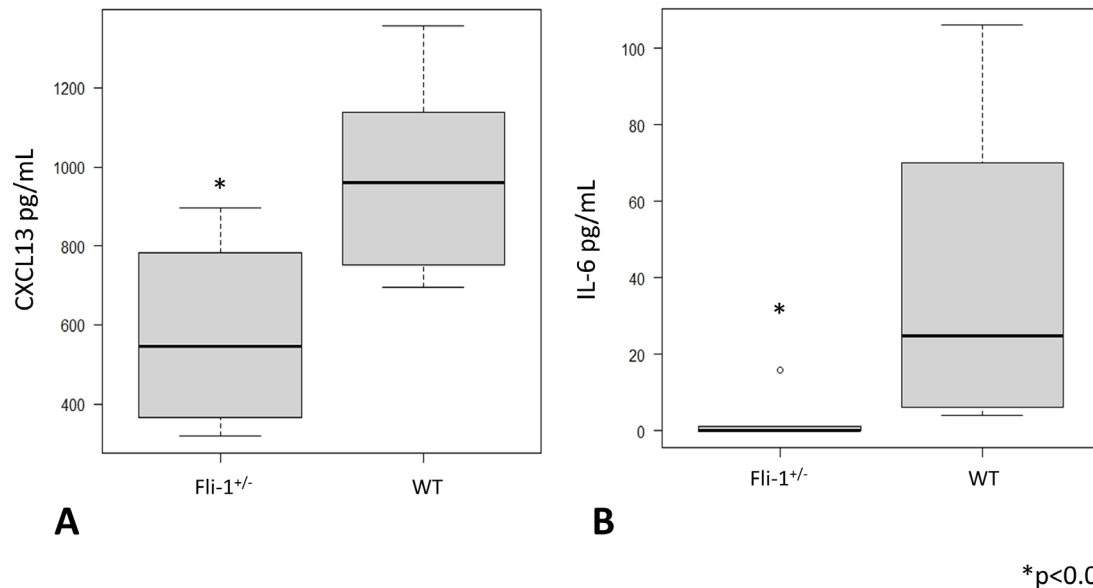


Figure 1 Serum cytokine IL-6 and chemokine CXCL13 levels in adult (26–33 weeks old) MRL WT (Fli-1^{+/+}) and MRL/lpr (Fli-1^{+/-}) mice. (A) Serum CXCL13 and (B) IL-6 levels were significantly lower in MRL/lpr Fli-1^{+/-} mice than in WT MRL/lpr mice (n=6, p<0.05). CXCL13, chemokine CXC ligand 13; Fli-1, friend leukaemia virus integration 1; IL-6, interleukin 16; WT, wild type.

p=0.03) but not serum CXCL13 ($r_s=0.24$, p=0.22; online supplemental figure S1B,C).

Expression of transcript encoding CXCL13 in the kidney of MRL/lpr Fli-1^{+/-} mice

The mRNA from kidney tissue was isolated and compared between adult MRL/lpr Fli-1^{+/-} mice and control MRL/lpr WT (Fli-1^{+/+}) mice. As a result, the renal relative expression of CXCL13 and SRY-related HMG box4 (Sox4) mRNA levels in Fli-1^{+/-} MRL/lpr mice (21–33 weeks old, n=4–8; **figure 2**) was significantly reduced. CXCR5 and B-Cell CLL/Lymphoma 6 (Bcl-6) mRNA expression did

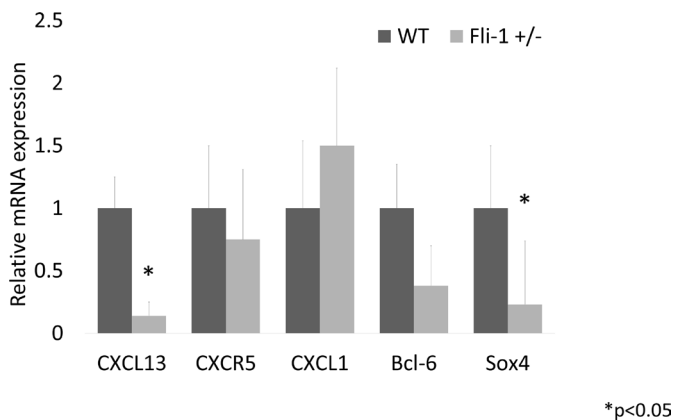


Figure 2 Relative mRNA expression in the kidney of MRL/lpr Fli-1^{+/-} mice compared with MRL/lpr WT control mice (21–33 weeks old, n=4–8). As shown, mRNA expression levels in CXCL13 and Sox4 were significantly lower in MRL/lpr Fli-1^{+/-} mice, whereas CXCR5, CXCL1 and Bcl-6 expression were not significantly lower than the WT control MRL/lpr mice. Bcl-6, B-Cell CLL/Lymphoma 6; CXCL, chemokine CXC ligand; CXCR5, CXC-chemokine receptor type 5; Fli-1, friend leukaemia virus integration 1; Sox4, SRY-related HMG box4; WT, wild type.

not differ significantly between the two groups. Unlike adult MRL/lpr mice, CXCR5 mRNA expression in younger Fli-1^{+/-} MRL/lpr mice was significantly reduced compared with WT MRL/lpr mice (7–9 weeks old, n=4; online supplemental figure S2B).

Reduced CXCL13- and CXCR5-positive immune cell infiltrates in MRL/lpr Fli-1^{+/-} mice

H&E staining revealed severe glomerular inflammation in MRL/lpr WT (Fli-1^{+/+}) mice compared with MRL/lpr Fli-1^{+/-} mice, as shown in **figure 3A,B** (n=5–7). Renal histology scores showed increased glomerular inflammation in WT MRL/lpr mice (p<0.01), but interstitial immune cell infiltrates into the kidney were similar in the two groups, as shown in **figure 3A, B and G**. Immunostaining with anti-CXCL13 and anti-CXCR5 antibodies was performed, and the number of CXCL13- and CXCR5-positive cells in MRL/lpr Fli-1^{+/-} mice was significantly lower than that in WT mice (n=5–7, p<0.05; **figure 3C–F and H**). In contrast, CXCL13 glomerular expression was low in both groups (online supplemental figure S3A,B). Recently, the importance of tertiary lymphoid structure (TLS) lesions has been reported to influence renal prognosis in lupus nephritis; in addition, renal CXCL13 expression has an important role in the infiltration of lymphocytes in TLS.^{41,42} To investigate this, we have reviewed the infiltration of CXCL13-positive immune cells in the kidney between the two groups. Overall, kidneys of WT MRL/lpr mice showed more intense CXCL13-positive cells not only in TLS lesions (observed around arterioles shown in **figure 3C** and online supplemental figure S3C) but also in interstitial inflammatory lesions (online supplemental figure S3C).⁴¹ In contrast, MRL/lpr Fli-1^{+/-} mice showed smaller number of CXCL13-positive cells in TLS lesions,

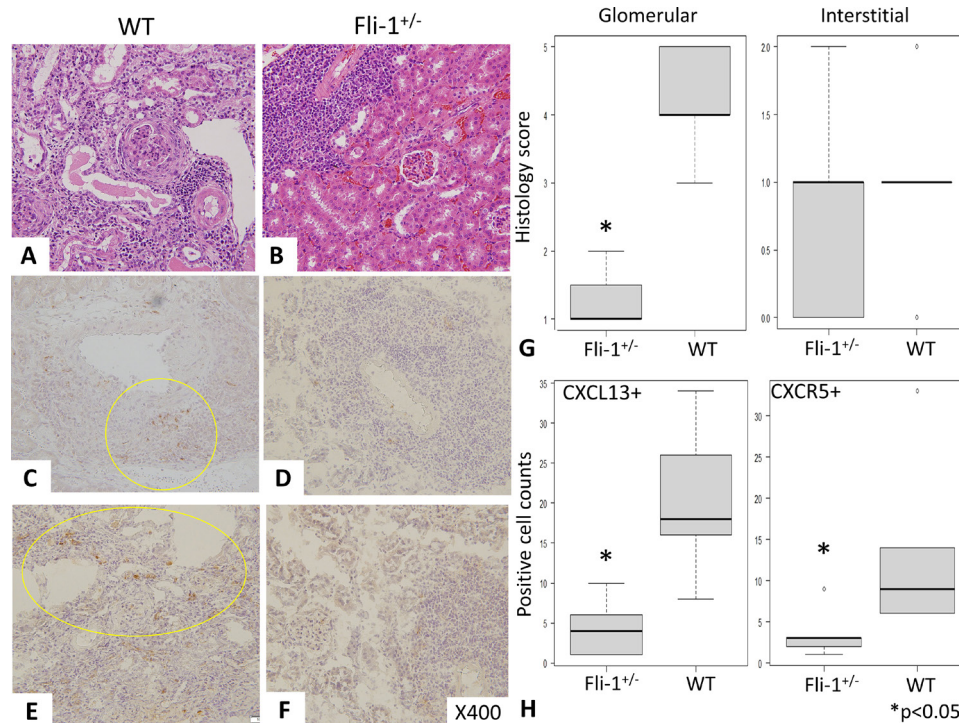


Figure 3 Renal pathology findings (H&E staining and immunostaining) in MRL/lpr wild-type (WT) and MRL/lpr Fli-1^{+/-} mice (21–30 weeks old, n=5–7). (A, B and G) H&E staining showed prominent glomerular inflammation (crescent formation) with higher glomerular scores (Glom) in MRL/lpr WT mice than in MRL/lpr Fli-1^{+/-} mice; in contrast, interstitial inflammation (Int) was similarly observed between the two groups. (C–F) Immunostaining with (C and D) CXCL13 and (E and F) CXCR5 showed decreased positive cell infiltration in the kidney of MRL/lpr Fli-1^{+/-} mice. (H) Pathology scores were significantly decreased in CXCL13- and CXCR5-positive cells in MRL/lpr Fli-1^{+/-} mice. CXCL, chemokine CXC ligand; CXCR5, CXC-chemokine receptor type 5; Fli-1, friend leukaemia virus integration 1.

not spreading surrounding tissues (online supplemental figure S3D).

Detection of CD11b/CXCL13-positive immune cells in the kidney of MRL/lpr WT (Fli-1^{+/-}) and MRL/lpr Fli-1^{+/-} mice

We used immunofluorescence staining with FITC-conjugated anti-CD11b and Alexa Fluor 647-labelled CXCL13 antibodies to detect CXCL13-positive immune cells in the kidneys of MRL/lpr mice. Infiltration of CD11b/CXCL13 double-positive cells was observed in MRL/lpr WT mice (figure 4A, C and E) but not in MRL/lpr Fli-1^{+/-} mice (figure 4B, D and F). Pathology scores also revealed that the number of such cells infiltrating the kidney of MRL/lpr Fli-1^{+/-} mice was significantly reduced ($p < 0.01$, $n = 5-7$; figure 4G). Furthermore, glomerular histology scores were positively correlated with the number of infiltrating CD11b/CXCL13 double-positive cells ($r_s = 0.74$, $p = 0.01$) but not with interstitial inflammation ($r_t = 0.38$, $p = 0.21$; online supplemental figure S3A,B).

DISCUSSION

These results show that Fli-1 impacts CXCL13 expression and immune cell infiltration into the kidney of MRL/lpr mice with lupus-like nephritis. CXCL13/CD11b- and CXCR5-positive cells were significantly reduced in MRL/lpr Fli-1^{+/-} mice. Transcripts encoding CXCL13 and Sox4, a transcription factor required for

B-cell development, were also downregulated. To the best of our knowledge, this is the first study presenting a relationship between CXCL13 expression and Fli-1. The mechanisms by which Fli-1 impacts CXCL13 expression are still unclear, but they can be explained in a direct and/or indirect manner. Previous studies show that Fli-1 directly regulates many chemokines and cytokines, including monocyte chemoattractant protein-1 (MCP-1), Regulated on Activation, Normal T cell Expressed and Secreted (RANTES), interferon-inducible protein 10 (IP-10), CXCL2, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-6.^{30-34 36 37} Additionally, a recent study found that Fli-1 influenced renal IL-17A expression.³⁵ These chemokines/cytokines are required for the progression of renal inflammation in lupus nephritis.^{7 8 43} Lema *et al*⁴³ have shown that chemokine expression (MCP-1 and RANTES) increases concurrently with mononuclear cell infiltration in a lupus model mouse. In terms of cytokine, IL-17A and IL-6 are important for Th17 cell infiltration and subsequent inflammation in the kidney.^{35 44} Reduced Fli-1 expression downregulates these molecules, which may alter the milieu of cytokine/chemokine expression in inflamed kidney tissue, ultimately resulting in decreased CXCL13 expression. Many immune cells, including macrophages and DCs, produce CXCL13. The main

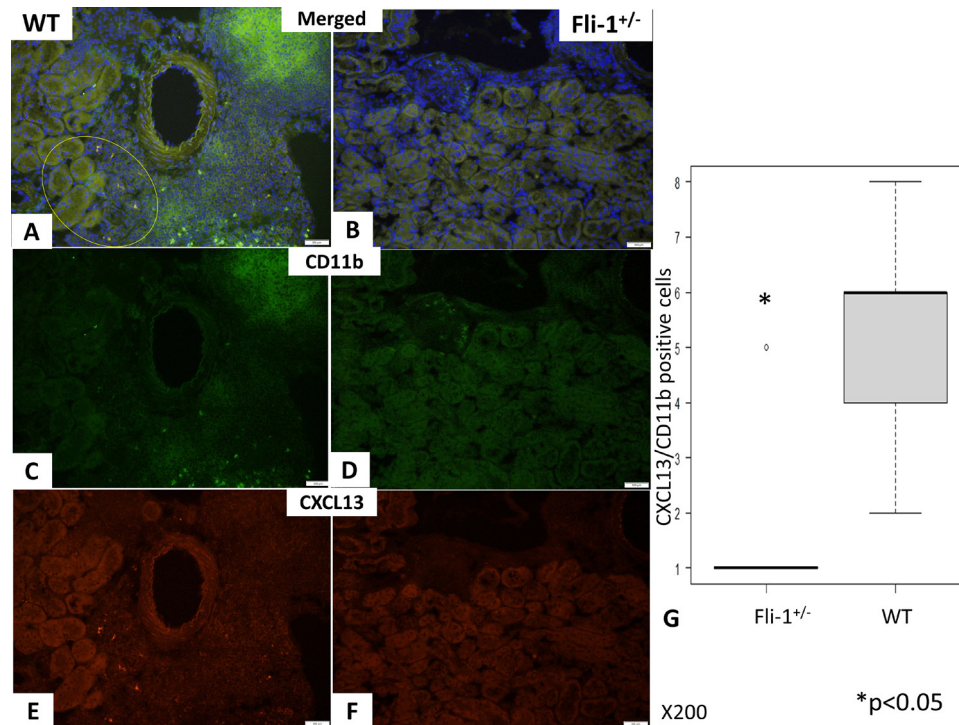


Figure 4 Detection of CD11b/CXCL13-positive immune cells in the kidney of MRL/lpr WT (Fli-1^{+/+}) and MRL/lpr Fli-1^{+/-} mice using FITC-conjugated anti-CD11b and Alexa Fluor 647-labelled CXCL13 antibodies. Infiltration of CD11b/CXCL13-positive cells was observed in (A, C and E) MRL/lpr WT mice compared with (B, D and F) MRL/lpr Fli-1^{+/-} mice. (G) The number of infiltration of these cells was significantly decreased in the kidney of MRL/lpr Fli-1 mice (n=5–7, p<0.05). CXCL13, chemokine CXC ligand 13; FITC, fluorescein isothiocyanate; Fli-1, friend leukaemia virus integration 1; WT, wild type.

sources of CXCL13 production in kidney inflammation were macrophages and DCs.^{12 14} CXCR5 is the CXCL13 receptor that is highly expressed in mature B cells.^{8 14} Our results showed reduced infiltration of CXCL13/CD11b-positive cells (mainly macrophages) and CXCR5-positive cells (mainly mature B cells). Previous research has shown that the grade of inflammatory cell, CD4⁺T cell or CD11b-positive cell infiltration into the kidney was slightly lower (but not significantly) in Fli-1^{+/-} MRL mice than in WT littermates (figure 3).³⁵ These findings could imply that Fli-1 impacts cell migration and regulates local inflammation in the absence of similar interstitial immune cell infiltration of kidney tissue. A decrease in CD19-positive cells in Fli-1^{+/-} lupus-prone mice has previously been described, which may be compatible with the present study (decreased renal CXCR5 expression in early timing and CXCR5-positive immune cell infiltration into the kidney, with B cells being the main source of CXCR5-positive cells).^{18 20} Another possibility is that Fli-1 regulates CXCL13 production directly by binding to the CXCL13 promoter lesion in immune cells/endothelial cells. Because this is an intriguing hypothesis, we are trying to investigate whether Fli-1 directly binds to the promoter lesion that encodes CXCL13. At present, putative Fli-1-binding sites in the promoter lesion of CXCL13 have yet to be described in the literature.

Our result shows that Sox4 mRNA expression is significantly reduced in MRL/lpr Fli-1^{+/-} mice. How did Sox4

deficiency affect inflammation or immune cell infiltration in the kidneys of MRL/lpr mice? Sox4 is a member of the SOX (SRY-related HMG box) gene family, which includes transcription factors involved in cell and organ development and differentiation, as well as the initiation and progression of various cancers, including breast cancer.⁴⁵ Previous research has shown that the transcription factor Sox4 is required for the survival of pro-B cells in mice.⁴⁶ On the other hand, Sox4 is involved in transforming growth factor-beta signalling and suppresses the Th2 immune response by regulating GATA3.⁴⁷ Therefore, skewing the Th2 condition by lowering Sox4 can suppress the Th1 and Th17 immune responses, resulting in lower CXCL13 expression in the kidney. It is also interesting that whether Fli-1 directly regulates Sox4 expression and influences CXCL13 production; however, at present, there are no reports describing the direct regulation of Sox4 via Fli-1. Further investigation is required.

The other possibility is that unrevealed strong CXCL13-producing cells were reduced in MRL/lpr Fli-1^{+/-} mice. In humans, Tfh cells were one of the main sources of CXCL13 production in lymphoid organs; however, murine Tfh cells do not secrete CXCL13.⁴⁸ On the other hand, a recent study has reported that PD1hiCXCR5-CD4⁺T cells, called T peripheral helper (Tph) cells, produce CXCL13 in the ectopic lymphoid-like structures in humans, and these cells were regulated by Sox4.⁴⁹ The existence of Tph cells in lupus-prone mice is unknown; however, unrevealed CXCL13-producing immune cells

as mentioned above may be influenced by Fli-1 reduction. Recent reports have shown that TLS in the kidney has an important role in the disease progression of lupus nephritis. Furthermore, CXCL13 expression in the site of TLS is crucial to recruit inflammatory cells in the kidney.^{41 42} In our observation, the CXCL13-positive immune cells were seen mainly within the immune cell infiltrates in TLS lesions (around arterioles and medulla in the kidney). In addition, the kidney of WT MRL/lpr mice showed wider distribution of CXCL13-positive immune cells, outside of TLS such as periglomerular lesions and kidney interstitium, in contrast to the limited number of CXCL13-positive cells within TLS lesions in MRL/lpr Fli-1^{+/-} mice (online supplemental figure S3C,D). These findings indicate that Fli-1 reduction may impact the infiltration of CXCL13-positive immune cells in TLS lesions and/or surrounding tissues and finally protect kidneys from inflammatory renal damage. Previously, one report has described that glomerular expression of CXCL13 was observed in lupus-prone NZB/NZW F1 mice. However, our results did not show prominent glomerular CXCL13 expression in immunostaining in the kidney of MRL/lpr WT mice (online supplemental figure 3AB). This may imply that inflammatory profiles can be different between NZB/NZW F1 and MRL/lpr mice, even if they show similar phenotypes as lupus-prone mice. Interestingly, serum CXCL13 levels positively correlated with glomerular histology score in our observation (online supplemental figure 4). These facts may indicate that CXCL13 has an important role in the development of lupus-like nephritis in lupus-prone mice. Another previous report described that CXCL13 produced by murine peritoneal macrophages due to Fli-1 deletion contributes to the development of tissue fibrosis, vasculopathy and immune cell activation in systemic sclerosis.⁵⁰ Patients with systemic sclerosis showed increased serum CXCL13 levels, which were correlated with the modified Rodnan total skin thickness score. This is an intriguing observation: in vitro experiment Fli-1 silencing using small interfering RNA (siRNA) in murine peritoneal macrophages successfully initiated CXCL13 expression on lipopolysaccharide (LPS) stimulation. However, human dermal fibroblasts and microvascular endothelial cells with silencing Fli-1 did not show a significant increase in CXCL13 expression.⁵⁰ These results may indicate that Fli-1 can act as either repressor or accelerator for the expression of chemokines in each specific cell type (such as peritoneal macrophages and immune cells) during the development of inflammation in autoimmune diseases. Nonetheless, CXCL13 can be an important chemokine for SLE and lupus nephritis: past reports have indicated that serum CXCL13 levels and renal expression of CXCL13/CXCR5 were significantly increased in patients with SLE. Furthermore, CXCL13 levels were positively correlated with the SLE Disease Activity Index in humans.^{18 19} Blockade of CXCL13 (anti-CXCL13 antibody) attenuates lupus-like nephritis and neuropsychiatric manifestation of MRL/lpr mice.^{51 52} In any case, further investigation regarding

the role of Fli-1 on CXCL13 regulation in autoimmune diseases is needed.

In conclusion, we found a close relationship between Fli-1 and CXCL13 expression in MRL/lpr mice. Reduced Fli-1 expression affects renal CXCL13 expression as well as CXCL13-positive monocytes/macrophages and CXCR5-positive immune cells in inflamed kidneys of lupus-prone mice. Sox4 expression may be important in regulating CXCL13 expression in the kidney. These results also suggest that Fli-1 might be a novel target for agents that treat or control collagen vascular diseases.

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REFERENCES

- 1 Tsokos GC. Autoimmunity and organ damage in systemic lupus erythematosus. *Nat Immunol* 2020;21:605–14.
- 2 Sato S, Zhang XK. The friend leukaemia virus integration 1 (fli-1) transcription factor affects lupus nephritis development by regulating inflammatory cell infiltration into the kidney. *Clin Exp Immunol* 2014;177:102–9.

- 3 Wilson PC, Kashgarian M, Moeckel G. Interstitial inflammation and interstitial fibrosis and tubular atrophy predict renal survival in lupus nephritis. *Clin Kidney J* 2018;11:207–18.
- 4 Nowling TK, Gilkeson GS. Mechanisms of tissue injury in lupus nephritis. *Arthritis Res Ther* 2011;13:250.
- 5 Adalid-Peralta L, Mathian A, Tran T, et al. Leukocytes and the kidney contribute to interstitial inflammation in lupus nephritis. *Kidney Int* 2008;73:172–80.
- 6 Kulkarni O, Anders HJ. Chemokines in lupus nephritis. *Front Biosci* 2008;13:3312–20.
- 7 Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. *J Biomed Biotechnol* 2011;2011:432595.
- 8 Liao X, Pirapakaran T, Luo XM. Chemokines and chemokine receptors in the development of lupus nephritis. *Mediators Inflamm* 2016;2016:6012715.
- 9 Lech M, Anders HJ. The pathogenesis of lupus nephritis. *J Am Soc Nephrol* 2013;24:1357–66.
- 10 Legler DF, Loetscher M, Roos RS, et al. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* 1998;187:655–60.
- 11 Gunn MD, Ngo VN, Ansel KM, et al. A B-cell-homing chemokine made in lymphoid follicles activates burkitt's lymphoma receptor-1. *Nature* 1998;391:799–803.
- 12 Ishikawa S, Sato T, Abe M, et al. Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by c11b+cd11c+ dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. *J Exp Med* 2001;193:1393–402.
- 13 Schulz O, Hammerschmidt SI, Moschovakis GL, et al. Chemokines and chemokine receptors in lymphoid tissue dynamics. *Annu Rev Immunol* 2016;34:203–42.
- 14 Pan Z, Zhu T, Liu Y, et al. Role of the CXCL13/CXCR5 axis in autoimmune diseases. *Front Immunol* 2022;13:850998.
- 15 Crotty S. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* 2011;29:621–63.
- 16 Ansel KM, Harris RBS, Cyster JG. Cxcl13 is required for B1 cell homing, natural antibody production, and body cavity immunity. *Immunity* 2002;16:67–76.
- 17 Hussain M, Adah D, Tariq M, et al. CXCL13/CXCR5 signaling axis in cancer. *Life Sciences* 2019;227:175–86.
- 18 Lee H-T, Shiao Y-M, Wu T-H, et al. Serum BLC/CXCL13 concentrations and renal expression of CXCL13/CXCR5 in patients with systemic lupus erythematosus and lupus nephritis. *J Rheumatol* 2010;37:45–52.
- 19 Da Z, Li L, Zhu J, et al. Cxcl13 promotes proliferation of mesangial cells by combination with CXCR5 in SLE. *J Immunol Res* 2016;2016:2063985.
- 20 Wiener A, Schippers A, Wagner N, et al. Cxcr5 is critically involved in progression of lupus through regulation of B cell and double-negative T cell trafficking. *Clin Exp Immunol* 2016;185:22–32.
- 21 Steinmetz OM, Velden J, Kneissler U, et al. Analysis and classification of B-cell infiltrates in lupus and ANCA-associated nephritis. *Kidney Int* 2008;74:448–57.
- 22 Schiffer L, Worthmann K, Haller H, et al. CXCL13 as a new biomarker of systemic lupus erythematosus and lupus nephritis - from bench to bedside? *Clin Exp Immunol* 2015;179:85–9.
- 23 Hsu T, Trojanowska M, Watson DK. Ets proteins in biological control and cancer. *J Cell Biochem* 2004;91:896–903.
- 24 Spyropoulos DD, Pharr PN, Lavenburg KR, et al. Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the flil1 transcription factor. *Mol Cell Biol* 2000;20:5643–52.
- 25 Papas TS, Bhat NK, Spyropoulos DD, et al. Functional relationships among ETS gene family members. *Leukemia* 1997;11 Suppl 3:557–66.
- 26 Puztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem* 2006;54:385–95.
- 27 Zhang XK, Gallant S, Molano I, et al. Decreased expression of the Ets family transcription factor Fli-1 markedly prolongs survival and significantly reduces renal disease in MRL/lpr mice. *J Immunol* 2004;173:6481–9.
- 28 Mathenia J, Reyes-Cortes E, Williams S, et al. Impact of Fli-1 transcription factor on autoantibody and lupus nephritis in NZM2410 mice. *Clin Exp Immunol* 2010;162:362–71.
- 29 Zhang L, Eddy A, Teng YT, et al. An immunological renal disease in transgenic mice that overexpress Fli-1, a member of the Ets family of transcription factor genes. *Mol Cell Biol* 1995;15:6961–70.
- 30 Suzuki E, Karam E, Williams S, et al. Fli-1 transcription factor affects glomerulonephritis development by regulating expression of monocyte chemoattractant protein-1 in endothelial cells in the kidney. *Clin Immunol* 2012;145:201–8.
- 31 Sato S, Lennard Richard M, Brandon D, et al. A critical role of the transcription factor Fli-1 in murine lupus development by regulation of interleukin-6 expression. *Arthritis Rheumatol* 2014;66:3436–44.
- 32 Lennard Richard ML, Sato S, Suzuki E, et al. The Fli-1 transcription factor regulates the expression of CCL5/RANTES. *J Immunol* 2014;193:2661–8.
- 33 Lennard Richard ML, Brandon D, Lou N, et al. Acetylation impacts flil-1-driven regulation of granulocyte colony stimulating factor. *Eur J Immunol* 2016;46:2322–32.
- 34 Lou N, Lennard Richard ML, Yu J, et al. The Fli-1 transcription factor is a critical regulator for controlling the expression of chemokine C-X-C motif ligand 2 (cxcl2). *Mol Immunol* 2017;81:59–66.
- 35 Sato S, Zhang XK, Temmoku J, et al. Ets family transcription factor Fli-1 promotes leukocyte recruitment and production of IL-17A in the MRL/lpr mouse model of lupus nephritis. *Cells* 2020;9:714.
- 36 Wang X, Lennard Richard M, Li P, et al. Expression of GM-CSF is regulated by Fli-1 transcription factor, a potential drug target. *J Immunol* 2021;206:59–66.
- 37 Wang X, Oates JC, Helke KL, et al. Camptothecin and topotecan, inhibitors of transcription factor flil-1 and topoisomerase, markedly ameliorate lupus nephritis in (NZB x NZW) F1 mice and reduce the production of inflammatory mediators in human renal cells. *Arthritis Rheumatol* 2021;73:1478–88. 10.1002/art.41685 Available: <https://onlinelibrary.wiley.com/doi/10.1002/art.41685>
- 38 Georgiou P, Maroulakou I, Green J, et al. Expression of ets family of genes in systemic lupus erythematosus and sjogren's syndrome. *Int J Oncol* 1996;9:9–18.
- 39 Mackay M, Oswald M, Sanchez-Guerrero J, et al. Molecular signatures in systemic lupus erythematosus: distinction between disease flare and infection. *Lupus Sci Med* 2016;3:e000159.
- 40 Li P, Wu Y, Hamlett ED, et al. Suppression of Fli-1 protects against pericyte loss and cognitive deficits in Alzheimer's disease. *Mol Ther* 2022;30:1451–64.
- 41 Dorradi SE, Kanapathippillai P, Hovd A-M, et al. Kidney tertiary lymphoid structures in lupus nephritis develop into large interconnected networks and resemble lymph nodes in gene signature. *Am J Pathol* 2020;190:2203–25.
- 42 Sato Y, Boor P, Fukuma S, et al. Developmental stages of tertiary lymphoid tissue reflect local injury and inflammation in mouse and human kidneys. *Kidney Int* 2020;98:448–63.
- 43 Lema GPD, Maier H, Nieto E, et al. Chemokine expression precedes inflammatory cell infiltration and chemokine receptor and cytokine expression during the initiation of murine lupus nephritis. *J Am Soc Nephrol* 2001;12:1369–82.
- 44 Shah K, Lee W-W, Lee S-H, et al. Dysregulated balance of th17 and th1 cells in systemic lupus erythematosus. *Arthritis Res Ther* 2010;12:R53.
- 45 Zhang J, Xiao C, Feng Z, et al. Sox4 promotes the growth and metastasis of breast cancer. *Cancer Cell Int* 2020;20:468.
- 46 Sun B, Mallampati S, Gong Y, et al. Sox4 is required for the survival of pro-B cells. *J Immunol* 2013;190:2080–9.
- 47 Kuwahara M, Yamashita M, Shinoda K, et al. The transcription factor Sox4 is a downstream target of signaling by the cytokine TGF- β and suppresses T (H) 2 differentiation. *Nat Immunol* 2012;13:778–86.
- 48 Rao DA. T cells that help B cells in chronically inflamed tissues. *Front Immunol* 2018;9:1924.
- 49 Yoshitomi H, Kobayashi S, Miyagawa-Hayashino A, et al. Human SOX4 facilitates the development of cxcl13-producing helper T cells in inflammatory environments. *Nat Commun* 2018;9:3762.
- 50 Taniguchi T, Miyagawa T, Toyama S, et al. Cxcl13 produced by macrophages due to Fli1 deficiency may contribute to the development of tissue fibrosis, vasculopathy and immune activation in systemic sclerosis. *Exp Dermatol* 2018;27:1030–7.
- 51 Wu X, Guo J, Ding R, et al. Cxcl13 blockade attenuates lupus nephritis of MRL/lpr mice. *Acta Histochem* 2015;117:732–7.
- 52 Huang MW, Stock AD, Putterman C. Cxcl13 neutralization attenuates neuropsychiatric manifestations in lupus-prone mice. *Front Immunol* 2021;12:763065.