

Interleukin 17-expressing Innate Synovial Cells Drive K/BxN Serum-induced Arthritis

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ABSTRACT

K/BxN serum can induce arthritis in normal mice because of abundant autoantibodies that trigger an innate inflammatory response in joints. To determine whether IL-17 is involved in the pathogenesis of serum-induced arthritis, we injected wild-type and IL-17^{-/-} mice with K/BxN serum and evaluated them for signs of arthritis. Unlike wild-type mice, IL-17^{-/-} mice did not show any signs of arthritis. IL-17 was produced predominantly by CD3⁺CD4⁺gdTCR⁺NK1.1⁺Sca1^{int}Thy1^{hi} cells residing in the inflamed synovial tissue. When synovial cells extracted from normal joints were stimulated with IL-23 or autoantibody-containing immune complexes, a substantial fraction of Sca1^{int}Thy1^{hi} cells produced IL-17. Thus, we have identified a novel population of IL-17-producing innate synovial cells that play a crucial role in the development of K/BxN serum-induced arthritis.

I. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects the synovial membranes of diarthrodial joints. As a result of the breakdown of self-tolerance, autoantibodies produced during the initiation phase are deposited into the synovial tissue. During the effector phase, these autoantibodies orchestrate diverse innate immune cells and synovial fibroblasts to trigger an inflammatory response, leading to progressive destruction of cartilage and bone. These pathogenic processes are mirrored relatively well in the K/BxN mouse model. Passive transfer of K/BxN serum containing anti-GPI autoantibodies to normal mice can bypass the initiation phase and directly execute effector functioning (1). IL-17 is produced by various cells such as Th17 and gdT cells and innate lymphoid cells, and it elicits IL-17R expressing cells to produce proinflammatory mediators. The observation that patients with RA have higher levels of IL17 in their sera and synovial fluids than healthy controls suggests that IL-17 plays a role in the pathogenesis of arthritis (2). In this study, we evaluated whether IL-17 is essential for the effector phase of inflammatory arthritis in the K/BxN serum transfer model.

II. Materials and methods

Mice

IL-17^{-/-} congenic C57BL/6 mice (here after referred

to as IL-17^{-/-} mice) originally provided by Dr. Iwakura from Tokyo University. C57BL/6 mice were purchased from Orient Bio. K/BxN mice were obtained by crossing KRN TCR transgenic mice on a C57BL/6 background (K/B) with NOD mice (3).

Establishment of K/BxN serum-induced arthritis

Serum was collected from 8-12-week-old arthritic K/BxN mice. Wild-type (WT) and IL-17^{-/-} mice at 7 weeks of age were injected intraperitoneally with K/BxN serum.

Immunohistochemistry

Hind paw tissues were fixed, embedded in paraffin, and sectioned at 7 mm in thickness. Standard immunohistochemical methods were then applied. Goat rabbit anti-mouse IL-17 Ab (eBioscience, San Diego, CA, USA) was used at the appropriate dilution.

Synovial cell extraction and culture

To prepare single cell suspensions from synovial cells of the mice, the synovial tissues around ankle joints were collected. The synovial cells were cultured in RPMI 1640 medium containing 10% FBS (Gibco, USA) in the presence or absence of 10 ng/ml IL-23 (BD Biosciences, USA) or immune complexes for 48h.

FACS

IL-17 expression by synovial cells was detected by FACS.

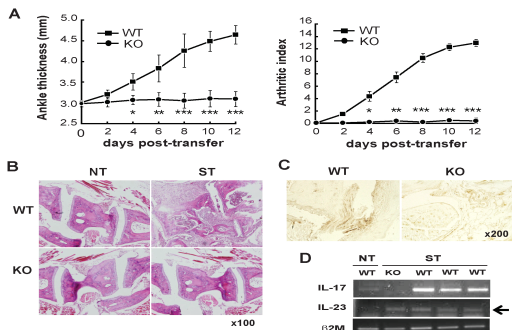
Statistical analysis

Data are presented as means±SEMs. Differences between groups were evaluated by unpaired Student's t-test. p values are indicated when differences between two groups were statistically significant (<0.05).

III. Results and discussion

IL-17-deficient mice are completely refractory to K/BxN serum-induced arthritis

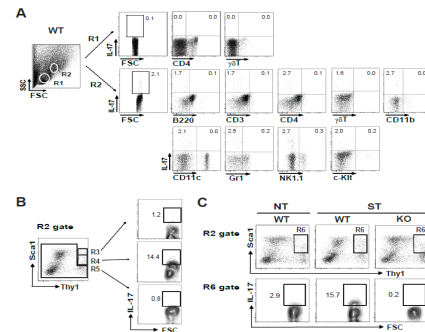
IL-17^{-/-} mice and their WT littermates were injected with K/BxN serum, disease was assessed for 12 days, and then mice were sacrificed for ex vivo assays. (Fig 1A) Ankle thickness of hind-paws and arthritis index (n=15 per group). (Fig 1B) Histopathologic examination of hind-paw sections. Original magnification, 100× (Fig 1C) Immunohistochemical staining of hind-paw sections with anti-IL-17 Ab. Original magnification, 200× (Fig 1D). Synovial tissues were assayed by RT-PCR. The arrow indicates IL-23. Data shown are pooled (A) or representative (B-D) results of three independent experiments. Graphs display means± SEMs. *p<0.05, **p<0.01, and ***p<0.001 by Student's t-test. NT, no treatment; ST, serum transfer. These results indicate that IL-17 is required for the development of arthritis triggered by K/BxN serum and suggest that autoantibodies promote IL-23 expression. IL-23, in turn, activates cells that are residing in or recruited to the synovial tissue to produce IL-17, an essential player in synovitis.



▶▶ Fig 1. IL-17 is required for development of K/BxN serum-induced arthritis

Thy1^{hi}Sca1^{int} synovial cells produce IL-17 in arthritogenic serum-recipient mice

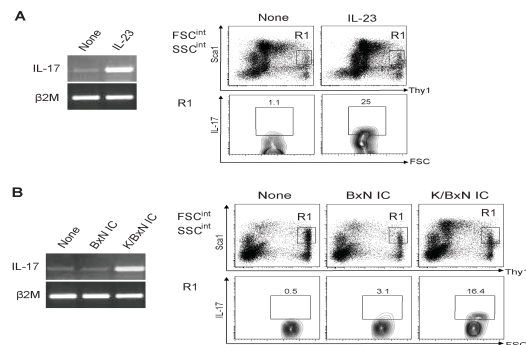
IL-17^{-/-} mice and their WT littermates were administered K/BxN serum. Synovial cells were extracted from mice post-mortem (day 12 post-serum transfer) and analyzed by FACS. (Fig 2A) FACS profiles of cells from WT mice, gated on live lymphocytes (R1) or FSC^{int}SSC^{int} cells (R2). (Fig 2B) The FSC^{int}SSC^{int} cells were divided into three fractions, Sca^{hi}Thy1^{hi} (R3), Sca^{int}Thy1^{hi} (R4), and remaining (R5) cells, and the percentage of IL-17⁺ cells within each fraction is shown. (Fig 2C) FACS profiles displaying Sca1 and Thy1 expression levels of R2-gated cells (the upper panel), and the percentage of IL-17⁺ cells within the R6 gate of upper graphs (the lower panel). Data are representative of three independent experiments. NT, no treatment; ST, serum transfer. Therefore, these results suggest that IL-17 was produced by cells resident in the synovial tissue, rather than by cell infiltrates in response to arthritogenic stimulation, and these cells retained a phenotype distinct from all other lineages known to express IL-17 in the synovium.



▶▶ Fig 2. Phenotypes of IL-17-producing synovial cells.

Thy1^{hi}Sca1^{int} synovial cells produce IL-17 in response to IL-23 or immune complexes

Cells extracted from normal mice were stimulated with 10 ng/ml IL-23 (Fig 3A) or immune complexes (IC) (Fig 3B) for 48h, followed by RT-PCR and FACS analyses. FACS profiles gated on FSC^{int}SSC^{int} (upper panels) and R1 (lower panels) are shown. Data are representative of three independent experiments. These results, taken together, demonstrate that IL-23 and K/BxN immune complexes promote IL-17 production by Thy1^{hi}Sca1^{int} synovial cells in vitro, although it is not clear whether IL-23 and K/BxN immune complexes act directly on Thy1^{hi}Sca1^{int} synovial cells or elicit other synovium-resident cells to activate Thy1^{hi}Sca1^{int} synovial cells. Interestingly, these cells share characteristics such as the Thy1⁺Sca1⁺ phenotype and responsiveness to IL-23 with IL-17-producing innate lymphoid cells identified in inflamed intestines.



▶▶ Fig 3. IL-23 and immune complexes promote IL-17 expression by Sca1^{int}Thy1^{hi} synovial cells.

References

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