Published in final edited form as:
J Immunol. 2015 February 1; 194(3): 873–877. doi:10.4049/jimmunol.1402573.

AIM2 and endosomal TLRs differentially regulate arthritis and autoantibody production in DNaseII deficient mice

Rebecca Baum[*], **Shruti Sharma**[*], **Susan Carpenter**[†], **Quan-Zhen Li**[‡], **Patricia Busto**[*], **Katherine A. Fitzgerald**[§], **Ann Marshak-Rothstein**[*,¶], and **Ellen M. Gravallese**[*,¶]

[*]Department of Medicine, Division of Rheumatology, University of Massachusetts Medical School

[†]Diabetes Center, Department of Microbiology and Immunology, University of California San Francisco

[‡]Department of Immunology and Microarray Core Facility, University of Texas Southwestern Medical Center

[§]Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School

Abstract

Innate immune PRRs sense nucleic acids from microbes and orchestrate cytokine production to resolve infection. Inappropriate recognition of host nucleic acids also results in autoimmune disease. Here we utilize a model of inflammation resulting from accrual of self DNA (*DNase II*$^{-/-}$ *Ifnar*$^{-/-}$) to understand the role of PRR sensing pathways in arthritis and autoantibody production. Using mice deficient in DNase II/Ifnar together with deficiency in either STING or AIM2 (TKO), we reveal central roles for the STING and AIM2 pathway in arthritis. AIM2 TKO mice show limited inflammasome activation and, like STING TKO mice, have reduced inflammation in joints. Surprisingly, autoantibody production is maintained in AIM2 and STING TKO mice, while *DNase II*$^{-/-}$ *Ifnar*$^{-/-}$ mice also deficient in Unc93b, a chaperone required for TLR7/9 endosomal localization, fail to produce autoantibodies to nucleic acids. Collectively, these data support distinct roles for cytosolic and endosomal nucleic acid sensing pathways in disease manifestations.

Introduction

Innate immune responses play a critical role in the initiation and perpetuation of several autoimmune disorders in which the sensing of self nucleic acids has become a common theme (1). While attention in this area has focused on the endosomal nucleic acid sensing TLRs, cytosolic DNA sensing receptors can also detect endogenous ligands and promote inflammatory and autoimmune responses (2). DNase II is a lysosomal endonuclease that plays a critical role in the phagosomal degradation of apoptotic debris. In DNase II deficient mice, undigested DNA is sensed by pattern recognition receptors (PRRs) to induce fatal levels of type I IFNs. Deletion of the type I IFN receptor (Ifnar) rescues the embryonic lethality induced by DNase II deficiency (3). However, *DNase II*$^{-/-}$ *Ifnar*$^{-/-}$ double knock

Correspondence to: Ellen M. Gravallese M.D., Tel.: +1 508 856 8730, Fax: +1 508 856 8878, Ellen.Gravallese@umassmed.edu.
[¶]Co-senior authors

out (DKO) mice, eventually succumb to autoimmune disease associated with polyarthritis, autoantibody production and elevated levels of the proinflammatory cytokines TNF, IL-1β, and IL-6 (4, 5).

In this model, DNA from the phagolysosomal compartment gains access to cytosolic nucleic acid sensing receptors. Cytosolic sensing of DNA results in the subsequent engagement of the adaptor protein stimulator of interferon genes (STING) and the downstream transcription factor IRF3, leading to the excessive production of type I IFN. In addition to its role in type I IFN production, STING-dependent pathways play an important role in the IFN-independent inflammatory arthritis that develops in adult DKO mice (6). However, the contribution of additional cytosolic or endosomal nucleic acid sensors to the systemic disease characteristic of DKO mice has not yet been explored. In addition to controlling transcription of interferon responses and NF-κB-driven inflammation, cytosolic DNA is also recognized by absent in melanoma2 (AIM2) (7). AIM2 works independently of STING to form a caspase-1 activating inflammasome that controls the proteolytic maturation of IL-1β and IL-18 and an inflammatory form of cell death called pyroptosis. Here we set out to define the contribution of the STING and AIM2 pathways in the development of arthritis in DKO mice by generating triple knockout mice (TKO) for comparative analysis to DKO mice. Rigorous examination of inflammation and clinical disease in these lines reveals important roles for both the STING and AIM2 pathways in arthritis. Furthermore, we define an additional contribution of endosomal nucleic acid sensors in regulating autoantibody production. Collectively these observations highlight the importance of multiple PRR pathways in controlling autoimmunity. Moreover, they unveil a previously undescribed role for AIM2 as a sensor of endogenous nucleic acids in autoimmunity.

Materials and Methods

Mouse Strains

C57BL/6 *DNase II*$^{+/-}$ embryos were kindly provided by Dr. S. Nagata through the RIKEN Institute, and mice were crossed to *Ifnar*$^{-/-}$ C57BL/6 mice to produce DKO and *DNase II*$^{+/-}$ *Ifnar*$^{-/-}$ heterozygous (Het) mice. DKO mice were bred with STING-deficient mice on a B6/129 background (8) or AIM2-deficient mice (9) to yield STING or AIM2 TKO mice. AIM2-deficient mice on a B6/129 background were generated through the use of a gene-trap embryonic stem cell line and deletion of AIM2 was confirmed by RT-PCR and immunoblot analysis (9). DKO mice were also bred to Unc93b-deficient mice on a B6 background, (Jackson Laboratories), yielding Unc93b TKO mice. All animal procedures were approved by and performed in accordance with the Institutional Animal Care and Use Committee at the University of Massachusetts Medical School.

Clinical and Histologic Inflammation Scores

Clinical arthritis was measured using a previously described scoring system (10). Histologic inflammation was assessed in paraffin-embedded left hind limbs. Blocks from 10 month-old female mice (n=5–8/genotype) were sectioned at 5 μm, deparaffinized, and stained with H&E. 50 sections were cut from each block and sections 10, 20, 30, 40, and 50 were scored using a modification of a previously described system (10) on a scale from 0–4.

K/BxN serum transfer arthritis

KRN T cell-transgenic mice (provided by Drs. Benoist and Mathis, Harvard Medical School and the Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France) (11) were crossed with NOD mice (Jackson Laboratory). Arthritogenic serum was obtained from progeny (10) and transferred to 11 week-old male STING-deficient (STING KO) or 8 week-old male AIM2-deficient (AIM2 KO) mice and controls by intraperitoneal injection of 150μl on days 0, 2, and 7. Clinical inflammation scores and ankle thickness measurements were taken every other day. Histologic inflammation (n=8/genotype) was scored as previously described (10).

Quantitative RT-PCR

Ankle joints from 10–12 month-old mice (n=4–6/genotype) were homogenized in liquid nitrogen using a mortar and pestle. Total RNA was isolated and 500ng was amplified as previously described (12). Gene expression was normalized to expression of the housekeeping gene hydroxymethylbilane synthase (HMBS). All primers were obtained from Qiagen. Data are expressed as the fold-increase in gene expression compared to normalized Het controls, using the 2^-ΔΔCT method.

ELISA and Western Blots

Serum levels of IL-18 were determined by ELISA according to the manufacturer's instructions (Medical & Biological Laboratories). Hind ankles were homogenized and sonicated in RIPA buffer with protease inhibitors for Western blots. Equal amounts of protein were loaded and blots were probed with primary antibody for IL-18 (Biovision, 5180R). Chemiluminescence reagent (ThermoScientific) was used for detection. Equivalent loading of protein was demonstrated by Ponceau S staining.

Autoantigen arrays

Serum samples pretreated with DNAse I were diluted 1:100 and incubated with autoantigen arrays (https://microarray.swmed.edu/products/category/protein-array/) bearing 125 antigens. The autoantibodies binding to the antigens on the arrays were assayed with fluorescent-labeled secondary antibodies (cy3-labeled anti-mouse IgG and cy5-labeled anti-mouse IgM) and the images were generated using Axon 4300A Scanner and analyzed with Genepix Pro 7.0 software (Molecular Devices). Net fluorescence intensities (NFI) were defined as the background subtracted averaged signal intensity normalized to internal controls. The NFI of each autoantibody was used to generate heatmaps using Cluster and Treeview software (13). These data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE63503. (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63503).

Statistical analysis

Statistical significance of differences in mean values was analyzed with the unpaired, two-tailed Student's t test or ANOVA for multiple comparisons. Statistical significance is represented by the following notation in the figures: $p<0.05$ = *, $p<0.01$ = **, $p<0.001$ = ***.

Results and Discussion

To investigate the contribution of the AIM2 inflammasome to disease in DKO mice, we initially compared the spontaneous arthritis in DKO mice with that of STING TKO and AIM2 TKO mice and controls by clinical joint scoring and histologic evaluation. As reported previously (4), inflammation was significant in the distal joints and paws in DKO mice, but not in the Het control group. STING deficiency completely abrogated clinical arthritis (Figure 1A and B), although we noted minimal but detectable inflammation in STING TKO mice upon histologic evaluation (Figure 1C and D). This is consistent with previously reported findings in DNaseII/STING DKO mice (6). AIM2 TKO mice also demonstrated a significant attenuation of arthritis, as assessed by both clinical and histologic scoring. These results reveal a role for a second, STING-independent, cytosolic DNA sensor in arthritis resulting from the accumulation of DNA in DNase II deficient mice. Of note, unlike *DNase II*$^{-/-}$*/STING*$^{-/-}$ mice that are rescued from lethality, our *DNase II*$^{-/-}$*/AIM2*$^{-/-}$ mice were embryonic lethal, indicating that AIM2 is not a pathway responsible for type I IFN signatures (data not shown).

To better understand how both STING and AIM2 contribute to the development of arthritis, we collected joint tissue from 10–12 month-old mice and compared RNA expression levels for the pro-inflammatory cytokine TNF, as well as expression levels of matrix metalloproteinase 3 (MMP3) as a surrogate marker of inflammation (Figure 2). STING TKO joints had significantly reduced levels of TNF compared with DKO joints, whereas AIM2 TKO joints showed a trend toward decreased TNF expression (Figure 2A) as well as IL-6 expression (not shown). Expression of MMP3 mRNA was significantly decreased in both STING TKO and AIM2 TKO mice compared with DKO mice, consistent with reduced inflammation.

We then determined protein levels of IL-1β and IL-18 in joint extracts as a marker of AIM2 inflammasome activity. Although we were unable to detect IL-1β protein, IL-18 protein expression was markedly diminished in the joints of AIM2 TKO mice compared with DKO mice (Figure 2B). In addition, consistent with defects in AIM2 inflammasome activation, we found that the AIM2 TKO mice had reduced systemic levels of IL-18 (Figure 2C). IL-18 itself, a product of several cell types including macrophages and synovial fibroblasts, has been implicated as an important pro-inflammatory cytokine in autoimmune diseases including rheumatoid arthritis (RA). Inflammation and cartilage destruction are significantly reduced in mice deficient in IL-18 in the collagen-induced arthritis model of RA (14). As noted above, there is also a trend toward decreased TNF expression in the joints of AIM2 TKO compared to DKO mice. This trend could be explained by the presence of known feedback loops among cytokines in inflammatory arthritis.

Our data point to a distinct and prominent role for the AIM2 inflammasome in arthritis pathogenesis. While AIM2 has previously been shown to act in a non-redundant fashion in response to intracellular bacteria and DNA viruses (9), these findings demonstrate that AIM2 also recognizes endogenous DNA, and that the recognition of cytosolic DNA by AIM2 contributes to the pathogenesis of clinical disease.

Arthritis is only one manifestation of the autoimmune disease that results from DNase II deficiency, as these DKO mice also produce autoantibodies (4). Since B cells express both STING and AIM2 (15), it was of interest to determine whether STING and/or AIM2 are required for autoantibody production. As shown by an autoantigen microarray, DKO mice make autoantibodies reactive to an extensive panel of autoantigens by 10 months of age, but not at 3 months of age at a time when arthritis is not present (Figure 3). Surprisingly, despite the significant effect of STING and AIM2 deficiency on arthritis and a prior report that STING is required for the production of anti-DNA autoantibodies (6), the STING TKO and AIM2 TKO mice demonstrate robust autoantibody production. Thus the STING and AIM2 pathways are not required for this process. In murine models of SLE, the production of anti-nuclear antibodies (ANAs) depends on the endosomal TLRs 7 and 9 that detect either RNA or DNA. We therefore evaluated autoantibody production in DKO mice that also failed to express Unc93b, a chaperone protein required for TLR7 and TLR9 endosomal localization. Despite the presence of arthritis in Unc93b TKO mice, autoantibody production to nucleic acid is abrogated (Figure 3), in stark contrast to the DKO mice.

STING TKO and AIM2 TKO mice developed significantly less joint inflammation than DKO mice, despite the presence of high titer autoantibody production. To clarify whether STING or AIM2 play a role in antibody-mediated joint inflammation, we generated arthritis in STING-deficient and AIM2-deficient mice and their respective controls by transfer of arthritogenic serum from K/BxN mice (11). Arthritis in this model is mediated by the deposition of immune complexes within the joint, leading to fixation of complement and ensuing pathology (16). Importantly, we found no differences between the arthritic inflammation generated in STING-deficient (Figure 4 A and B), AIM2-deficient (Figure 4C), and control mice. Thus, antibody-induced inflammation proceeds independently of cytosolic nucleic acid sensors. Furthermore, STING and AIM2 appear to regulate inflammation strictly in settings where accrual of cytosolic DNA is a key pathogenic event.

There is mounting evidence for a role of cytosolic sensing of DNA during human autoinflammatory conditions. STING has been implicated in a number of type I IFN-driven diseases. For example, patients inheriting mutations in Trex1, and therefore presumably unable to appropriately degrade cytosolic retroelements (17) develop the neuroinflammatory condition Aicardi-Goutieres syndrome (18), while gain of function mutations in STING can lead to pulmonary and vascular inflammation (2). Prior studies of the arthritic phenotype in DKO mice extended the scope of STING-mediated pathologies to the production of type I IFN-independent proinflammatory cytokines (5, 6). We now demonstrate that additional cytosolic and endosomal receptors also contribute to the autoimmune features of DKO mice. AIM2 responds to endogenous ligands in this setting and contributes to the arthritic phenotype through inflammasome activation. Thus deficiency of either STING or AIM2 attenuates arthritis. DKO mice also make anti-nuclear antibodies, but production depends on yet a third type of nucleic acid sensor, endosomal TLRs. This study highlights the complex relationships between multiple innate pathways engaged during autoimmunity, and demonstrates that distinct DNA sensor pathways play unique roles in the development of the various manifestations of autoimmune disease.

Acknowledgements

We would like to thank Kelly Army, Catherine Manning, and Tara Robidoux for animal husbandry and excellent technical assistance.

Grant support: NIH AI093752, AI067497 and AI083713 (to K.A.F), University of Massachusetts Musculoskeletal Center Grant and NIH R21AR067394 (to E.M.G.), Grant # 189143 from the Alliance for Lupus Research (to S.S.), and grants from the Lupus Research Institute and NIAMS/NIH P01 AR050256 (to A.M.R.).

References

1. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med. 2007; 13:543–551. [PubMed: 17479100]
2. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, Tenbrock K, Wittkowski H, Jones OY, Kuehn HS, Lee CC, DiMattia MA, Cowen EW, Gonzalez B, Palmer I, DiGiovanna JJ, Biancotto A, Kim H, Tsai WL, Trier AM, Huang Y, Stone DL, Hill S, Kim HJ, St Hilaire C, Gurprasad S, Plass N, Chapelle D, Horkayne-Szakaly I, Foell D, Barysenka A, Candotti F, Holland SM, Hughes JD, Mehmet H, Issekutz AC, Raffeld M, McElwee J, Fontana JR, Minniti CP, Moir S, Kastner DL, Gadina M, Steven AC, Wingfield PT, Brooks SR, Rosenzweig SD, Fleisher TA, Deng Z, Boehm M, Paller AS, Goldbach-Mansky R. Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 2014; 371:507–518. [PubMed: 25029335]
3. Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S. Lethal anemia caused by interferon-beta produced in mouse embryos carrying undigested DNA. Nat Immunol. 2005; 6:49–56. [PubMed: 15568025]
4. Kawane K, Ohtani M, Miwa K, Kizawa T, Kanbara Y, Yoshioka Y, Yoshikawa H, Nagata S. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature. 2006; 443:998–1002. [PubMed: 17066036]
5. Kawane K, Tanaka H, Kitahara Y, Shimaoka S, Nagata S. Cytokine-dependent but acquired immunity-independent arthritis caused by DNA escaped from degradation. Proc Natl Acad SciU S A. 2010; 107:19432–19437.
6. Ahn J, Gutman D, Saijo S, Barber GN. STING manifests self DNA-dependent inflammatory disease. Proc Natl Acad Sci U S A. 2012; 109:19386–19391. [PubMed: 23132945]
7. Rathinam VA, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. Nat Immunol. 2012; 13:333–342. [PubMed: 22430786]
8. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 2009; 461:788–792. [PubMed: 19776740]
9. Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, Vanaja SK, Monks BG, Ganesan S, Latz E, Hornung V, Vogel SN, Szomolanyi-Tsuda E, Fitzgerald KA. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. Nat Immunol. 2010; 11:395–402. [PubMed: 20351692]
10. Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravallese EM. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol. 2001; 159:1689–1699. [PubMed: 11696430]
11. Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, Degott C, Kikutani H, Rajewsky K, Pasquali JL, Benoist C, Mathis D. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity. 1999; 10:451–461. [PubMed: 10229188]
12. Walsh NC, Reinwald S, Manning CA, Condon KW, Iwata K, Burr DB, Gravallese EM. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. J Bone Miner Res. 2009; 24:1572–1585. [PubMed: 19338457]
13. Li QZ, Zhou J, Wandstrat AE, Carr-Johnson F, Branch V, Karp DR, Mohan C, Wakeland EK, Olsen NJ. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol. 2007; 147:60–70. [PubMed: 17177964]

14. Sedimbi SK, Hagglof T, Karlsson MC. IL-18 in inflammatory and autoimmune disease. Cell Mol Life Sci. 2013; 70:4795–4808. [PubMed: 23892891]

15. Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW 3rd, Su AI. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol. 2009; 10:R130. [PubMed: 19919682]

16. Mangialaio S, Ji H, Korganow AS, Kouskoff V, Benoist C, Mathis D. The arthritogenic T cell receptor and its ligand in a model of spontaneous arthritis. Arthritis Rheum. 1999; 42:2517–2523. [PubMed: 10615996]

17. Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. Cell. 2008; 134:587–598. [PubMed: 18724932]

18. Crow YJ, Hayward BE, Parmar R, Robins P, Leitch A, Ali M, Black DN, van Bokhoven H, Brunner HG, Hamel BC, Corry PC, Cowan FM, Frints SG, Klepper J, Livingston JH, Lynch SA, Massey RF, Meritet JF, Michaud JL, Ponsot G, Voit T, Lebon P, Bonthron DT, Jackson AP, Barnes DE, Lindahl T. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. Nat Genet. 2006; 38:917–920. [PubMed: 16845398]

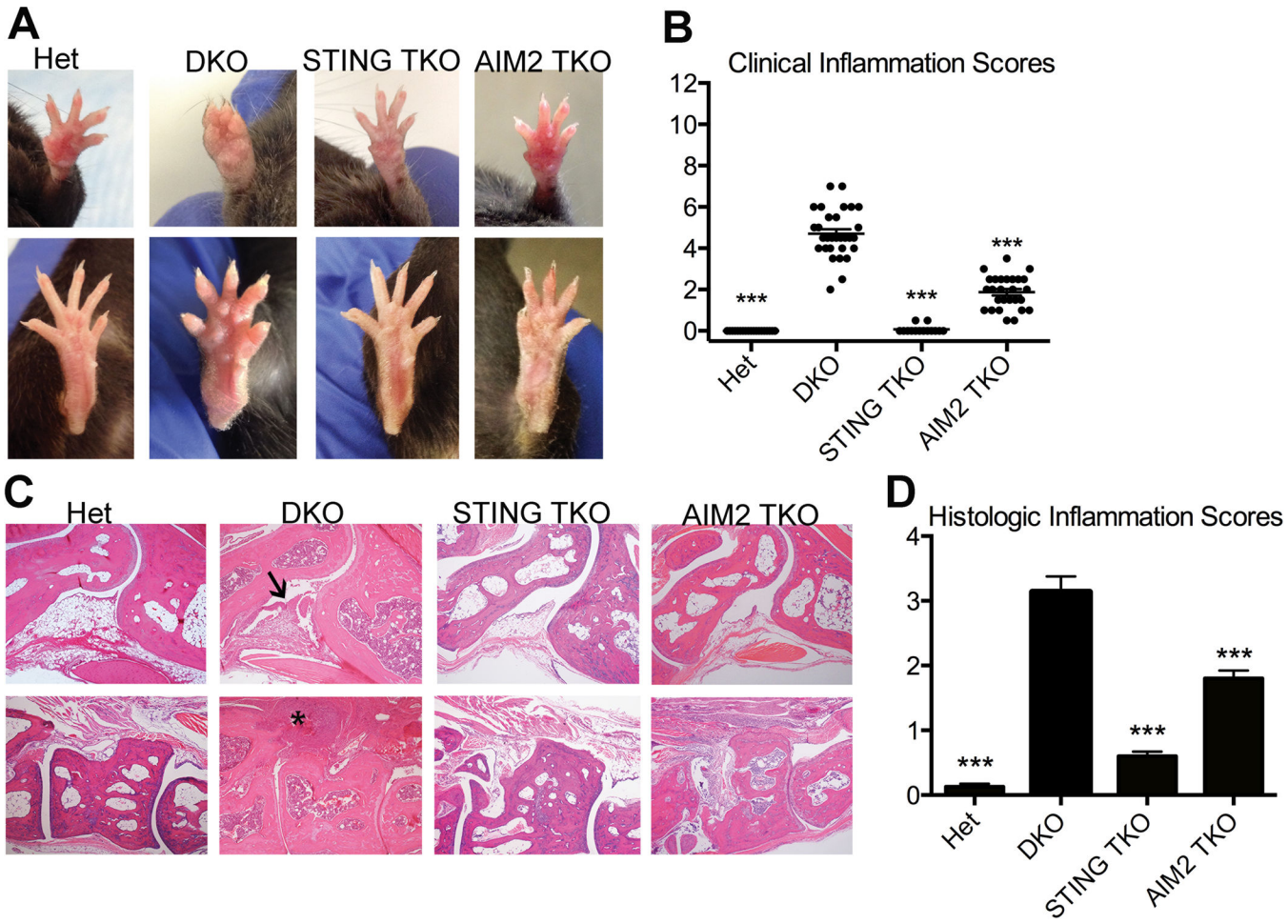

Figure I. Arthritis in DKO mice is regulated by distinct DNA sensing pathways

A) Representative images of clinical arthritis in forepaws (top) and hindpaws (bottom) from 10 month-old female mice demonstrating significant swelling in DKO mice, absence of swelling in STING TKO mice, and an intermediate arthritic phenotype in AIM2 TKO mice. B) Clinical inflammation scores (n=14–24/genotype) showing a statistically different mean inflammation score in STING TKO and AIM2 TKO compared with DKO mice. 10 month-old mice: Het (14 female, 5 male), DKO (11 female, 14 male), STING TKO (9 female, 6 male), AIM2 TKO (9 female, 13 male). C) Histologic inflammation at the ankle (upper panel) and midfoot (lower panel) and D) quantitation of histologic inflammation, confirming differences in inflammation in STING TKO and AIM2 TKO mice compared with DKO mice. Histological analysis performed on 10 month-old female mice. Values are the mean +/− SEM; *** = $p<0.001$ compared to DKO. Arrow and (*) designate two sites of inflammation.

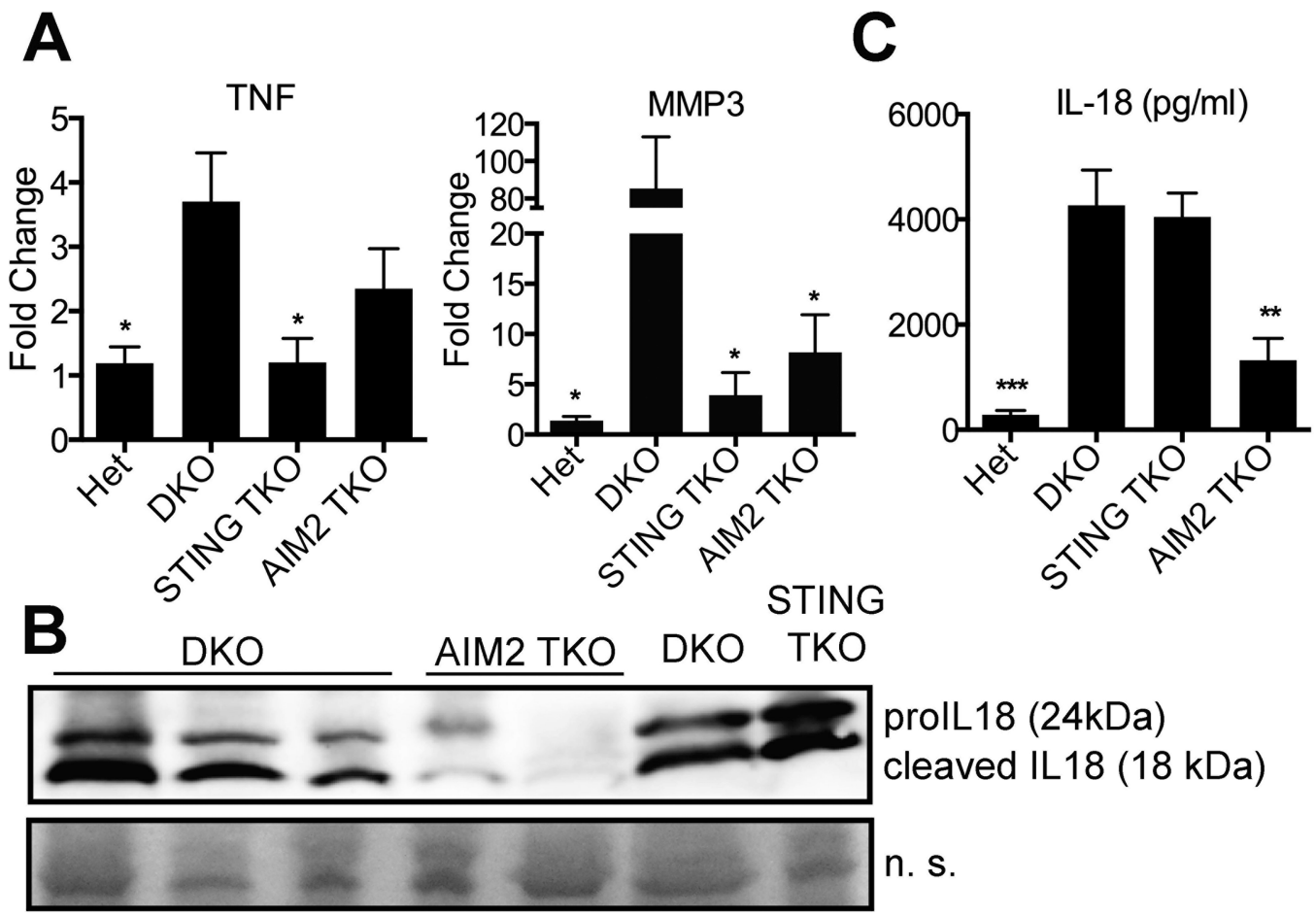

Figure II. AIM2 TKO mice demonstrate a significant decrease in IL-18 expression

A) Joint cytokine mRNA levels show a significant decrease in TNF in STING TKO mice compared with DKO mice, whereas a trend toward a decrease in TNF is seen in AIM2 TKO mice. MMP3 levels are significantly decreased in both STING TKO and AIM2 TKO mice compared with DKO mice, consistent with attenuation of clinical arthritis (n=4–6/genotype). 10 month-old mice: Het (4 female, 2 male), DKO (2 female, 4 male), STING TKO (3 female, 2 male), AIM2 TKO (2 female, 3 male). B) Western blot confirms a decrease in IL-18 protein expression in the joints of AIM2 TKO mice compared with DKO and STING TKO mice (n=1–4/genotype). n.s. designates non-specific protein staining (Ponceau S.). 10 month-old male mice. C) Serum ELISA assay demonstrates that IL-18 levels are significantly decreased in AIM2 TKO mice compared with DKO and STING TKO mice (n=6/genotype, 3 males, 3 females; 10 month-old mice). Values are the mean +/− SEM; * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ compared to DKO.

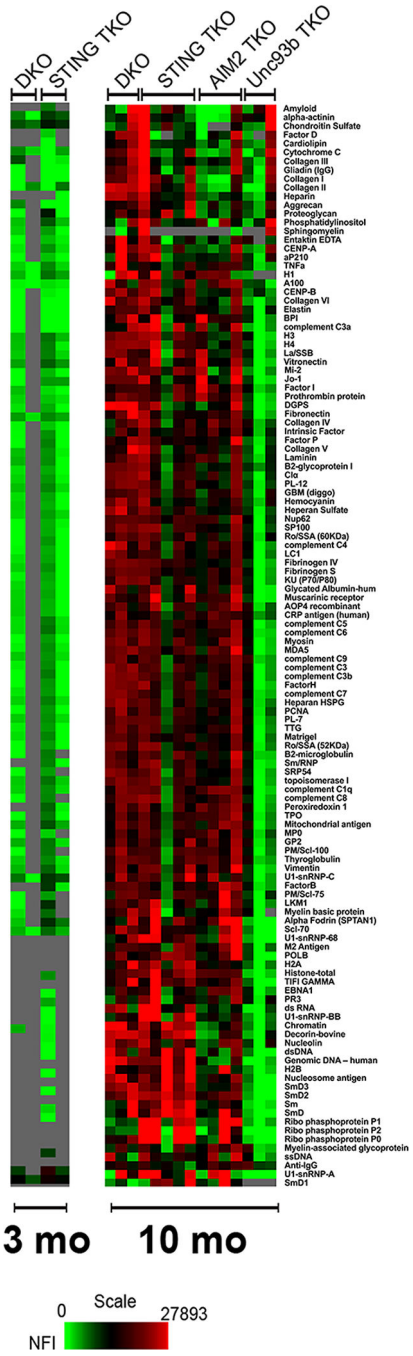

Figure III. The Unc93b pathway uniquely regulates autoantibody production to nucleic acid

Heat map of an array of 125 autoantigens, including DNA- and RNA-associated antigens and common joint associated antigens. Autoantibody production is minimal in DKO and STING TKO mice at 3 months of age (left panel), prior to the onset of arthritis. Despite the significant decrease in arthritic inflammation in STING TKO and AIM2 TKO mice at 10 months, heat maps demonstrate autoantibody production, as in DKO mice. Marked attenuation of autoantibody production is seen in Unc93b TKO mice, confirming the

essential role of endosomal TLRs in sensing nucleic acid for autoantibody production. Female and male mice were used in this analysis.

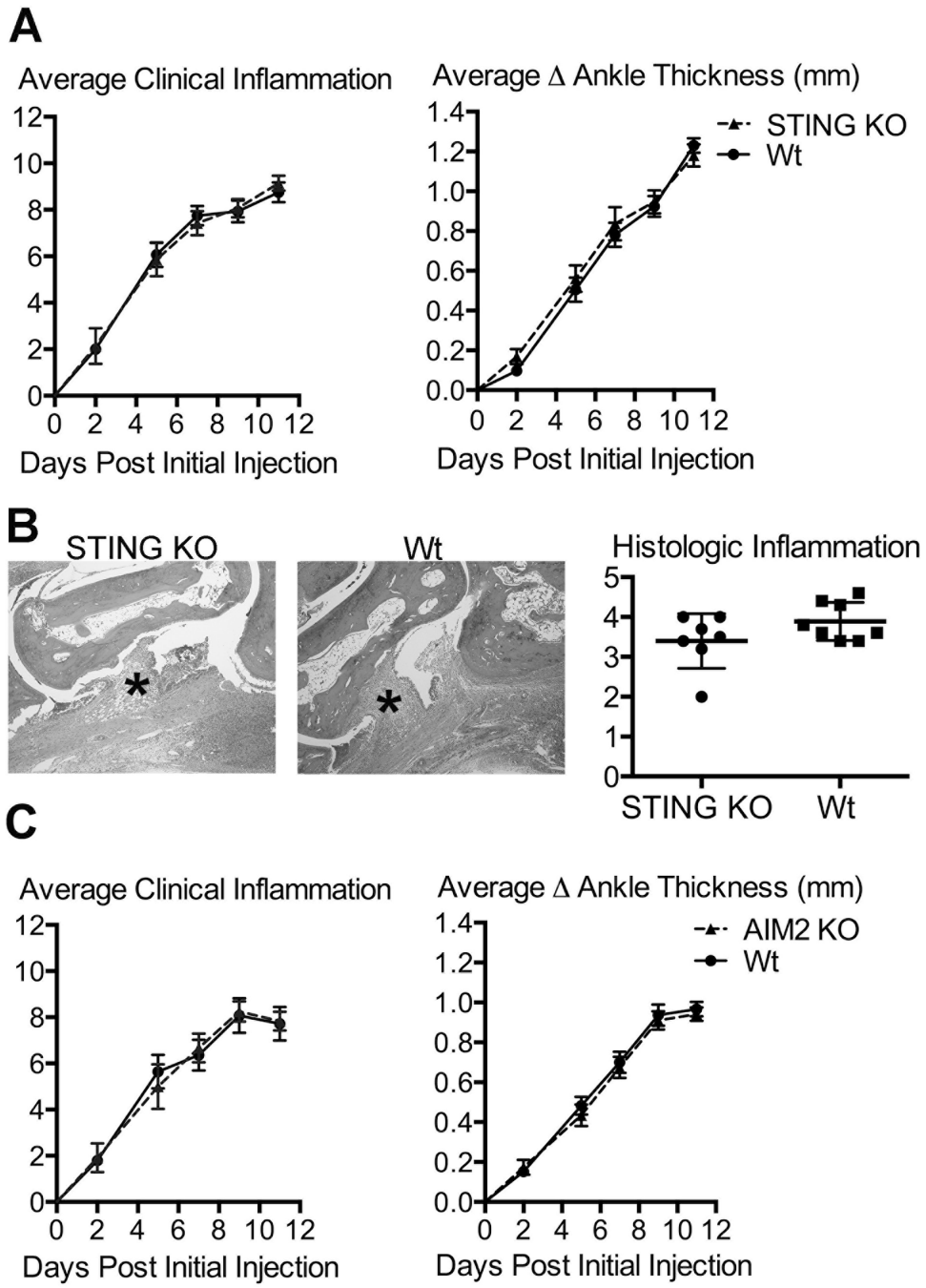

Figure IV. STING and AIM2 do not regulate arthritic inflammation in an immune complex-mediated model

A) Clinical inflammation scores and measurements of change in ankle thickness demonstrate no difference in inflammation in STING deficient (KO) mice compared with controls. 11 week-old male mice. B) H&E images (left) and histologic scoring (right) confirm equivalent inflammation. C) Clinical inflammation scores and measurements of change in ankle thickness demonstrate no difference in inflammation in AIM2 deficient

(KO) mice compared with controls (Wt). 8 week-old male mice. Values are the mean +/− SEM. (*) designates synovial inflammation.