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IL-27 attenuates IL-23 mediated inflammatory arthritis

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ABSTRACT

Interleukin 27 has both pro-inflammatory and anti-inflammatory properties in autoimmunity. The anti-inflammatory effects of IL-27 are linked with inhibition of Th17 differentiation but the IL-27 effect on myeloid cells is less studied. Herein we demonstrate that IL-27 inhibits IL-23-induced inflammation associated not only with Th17 cells but also with myeloid cell infiltration in the joints and splenic myeloid populations of $CD11b^{+}$ $GR1^{+}$ and $CD3^{-}CD11b^{+}CD11c^{-}GR1^{-}$ cells. The IL-27 anti-inflammatory response was associated with reduced levels of myeloid cells in the spleen and bone marrow. Overall, our data demonstrate that IL-27 has an immunosuppressive role that affects IL-23-dependent myelopoiesis in the bone marrow and its progression to inflammatory arthritis and plays a crucial role in controlling IL-23 driven joint inflammation by negatively regulating the expansion of myeloid cell subsets.

1. Introduction

The proinflammatory cytokine IL-23 is involved in many rheumatic diseases including inflammatory arthritis. IL-23 plays a significant role in the differentiation and maturation of conventional $CD4^{+}T_h17$ and non-conventional γδ T cells that contribute to inflammation [1,2]. IL-23 also has direct effects on innate immune cells as it expands myeloid populations in the bone marrow leading to synovial inflammation in murine models of arthritis [3]. IL-23 directly modulates the expansion of osteoclast progenitors via co-stimulatory pathways involving DAP12 and the immunoreceptor CLEC5A/MDL-1 [4,5]. The cumulative effects of IL-23 induced inflammation via IL-17A and direct expansion of osteoclast progenitors contribute to the observed increased osteoclastogenesis and exacerbated bone destruction [4,6]. The induction of T cells that produce IL-17A by IL-23, is widely known as the IL-23/IL-17A axis in inflammation, and plays multiple roles in autoimmunity [7]. However, the activation of myeloid cells by IL-23 is less studied. We previously showed [3] that apart from IL-17A, IL-23 induces IL-27 which is known to have both pro- and anti-inflammatory effects [8]. Activated myeloid cells express IL-27, a pleiotropic member of the IL-6 family of cytokines which is comprised of p28 and EbI3 subunits [9]. IL-27 was shown to have diverse inflammatory roles on T cell subsets and innate cells in autoimmune experimental animal disease models of experimental autoimmune encephalitis and colitis [9–12]. IL-27 was also shown to regulate the activation of macrophages in other pathological conditions [13,14]. In vivo studies utilizing a murine collagen induced arthritis model showed the suppressive effect of IL-27 via IFN-γ induction [15]. In human $CD14^{+}$ cell culture based studies in vitro, addition of IL-27 at the beginning of the culture prior to the addition of macrophage colony-stimulating factor (M-CSF) and RANKL demonstrated a dose dependent negative effect on osteoclast differentiation [16]. Additional data from mouse bone marrow cell cultures further underscored the ability of IL-27 to decrease osteoclastogenesis and not completely abrogate the process [16]. The inhibitory effects of IL-27 on inflammatory arthritis and bone resorption have been previously reviewed [17].

IL-27 induces the proliferation of naïve $CD4^{+}$ cells, polarizes Th1 differentiation and suppresses the development of Th17 cells [18,19]. γδ TCR^{+} cells express both subunits of IL-27R i.e., gp130 and WSX-1 and have recently been proposed as a target for IL-27 activity via STAT1 and STAT3 signalling which is common with IL-23R [20]. While IL-27 is a potent inducer of the anti-inflammatory cytokine IL-10 [21], it also promotes T cell dependent colitis [22].

The effect of IL-27 in inflammatory arthritis is unclear, since numerous animal models of inflammatory arthritis have demonstrated

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that it primarily involves increased proliferation and recruitment of myeloid-lineage precursor cells to the joints, followed by increased differentiation to specialized bone-resorbing multinucleated osteoclasts. T cells and especially IL-17 producing cells are also involved at a later stage of disease progression which maintain and perpetuate the initial inflammatory response.

In this manuscript we explored the possibility of an IL-27 mediated effect in regulating early IL-23 induced inflammatory arthritis and myelopoiesis. We utilized in vivo gene transfer to achieve systemic overexpression of IL-27 and IL-23 and demonstrated that IL-27 attenuates the early events of IL-23-mediated myeloid differentiation and inflammation. Herein we report that prior overexpression of IL-27 significantly reduced IL-23 driven splenic myelopoiesis, myeloid cell infiltration and inflammation in the joints. Overall, our data suggests a significant role of IL-27 in negatively regulating the IL-23 mediated myeloid precursor population.

2. Material and methods

2.1. Mice

C57BL/6 mice were between 8 and 12 weeks at the time of the experiments and were purchased from Jackson Laboratories. Mice were bred and maintained in an IACUC approved animal facility, according to AALAC and institutional guidelines.

2.2. Production and purification of IL-23, IL-27 or GFP mcDNA

The minicircle-producing vector was obtained from System Biosciences (Mountain View, CA) and reengineered by introducing unique *Pme*I and *Pac*I restriction sites down stream of attB sites of the original plasmid. A FLAG-tagged linked version of cytokine (IL-23/IL-27-Elastikine) encoding sequences was inserted under the control of RSV promoter in the unique *Pme*I and *Pac*I restriction sites. IL-23 mc RSV.FLAG.mIL23.Elasti.bpA or IL-27 mc p2ØC31-RSV-PPT-FLAG-mIL-27.Elasti.bpA were produced as previously described [3]. A single isolated colony from fresh plate was grown for 4 h in 2 ml Luria-Bertani broth with ampicillin. 800 μL of this culture was used to inoculate 1 L of Terrific broth and grown for an additional 17 h. Overnight cultures were centrifuged at 20 °C, 4000 rpm for 20 min. The pellet was resuspended 4:1 (*v*/v) in fresh Luria-Bertani broth containing 1% L-arabinose. The bacteria were incubated at 32 °C with constant shaking at 250 rpm for 2 h. After adding one-half volume of fresh low salt Luria-Bertani broth (pH 8.0) containing 1% L-arabinose, the incubation temperature was increased to 37 °C and the incubation continued for an additional 5 h. Episomal DNA circles were prepared from bacteria using plasmid purification kits from Endofree Qiagen Megaprep (Chatsworth, CA).

2.3. Zymosan-induced arthritis combined with in vivo gene transfer

To induce arthritis in the resistant C57BL/6 strain we combined the zymosan-induced arthritis model with hydrodynamic delivery of minicircle DNA. Briefly, for hydrodynamic delivery in vivo, IL-23 minicircle DNA (IL-23 MC), IL-27 minicircle DNA (IL-27 MC), or control GFP minicircle DNA (GFP MC) were injected in the retro orbital vein plexus of the mice. Mice were bled to validate efficient gene transfer and overexpression of cytokines by serum ELISA. IL-27 MC was injected 3 days prior to IL-23 MC in order to allow recovery and minimize injury-based effect on the mice. Following gene transfer mice were injected with 50 μg of sonicated and autoclaved zymosan (Sigma, MO) in 30 μl of PBS locally on the hind foot paws [23].

2.4. Assessment of Joint Inflammation

The joint inflammation was assessed daily for 8 days and expressed as the clinical score obtained by scoring each limb on a scale of 0–3, where 0 = normal, 1 = swelling of one digit 2 = swelling of two digits and erythema 3 = pronounced edematous swelling of entire paw, yielding a maximum score of 12 per mouse. At the end of the experiment, paws were removed, fixed and decalcified in Formical-4 (Decal Chemical Corp, NY), and paraffin embedded. Sections (7 μM thick) from the joint tissue (n = 3–5 mice per group) were stained with Hematoxylin/Eosin and Safranin-O staining [24] and scored by a board certified pathologist who was blinded to the treatment arms. The histologic scoring of inflammation was noted as previously described [23]. Briefly, joint pathology was scored using the following scales for cumulative score of inflammation: 0 (no signs of cell infiltration) or synovial hyperplasia, 1 (slight infiltration, lining layer hypertrophy within several layers), 2 (mild infiltration and hyperplasia, lining layer hypertrophy, and indistinct layer structure), 3 (moderate infiltration and hyperplasia, no distinct layer structure), and 4 (severe infiltration and hyperplasia, no distinct layer structure); cartilage proteoglycan degradation: 1 (normal staining), 2 (loss of staining at superficial layer), 3 (loss of staining at both superficial and deep layers), and 4 (no staining); and bone marrow hyperplasia: 0 (normal), 1 (slight), 2 (mild), 3 (moderate), and 4 (severe) [23].

2.4.1. Flow cytometry

Animals were sacrificed by CO_2 inhalation on two time points, the next day of zymosan injection (onset phase) and after 7 days (resolution phase) of zymosan injection. Spleens and bone marrow were extracted and homogenized through a 70 μm cell strainer. Cells were surface stained with CD3-Alexa Fluor 700(Clone 17A2), Gr1-PE (Clone RB6-8C5), CD11b-Pacific Blue (Clone M1–70). Acquisition was on a Cyan ADP Analyzer (Beckman-Coulter) or BD FACS Aria II and data was analyzed with FlowJo (Treestar). Gating strategy included gating on live cells followed by singlet identification based on FSC and SSC plots. Singlets were then gated on CD11b and CD3 markers to identify the $CD3^-$ subset that was further gated against GR1 and CD11b markers.

2.4.2. ELISA

Mice were bled on day 3-post gene transfer. Serum levels of soluble IL-27 or IL-23 were determined using Legend Max mouse IL-27 heterodimer pre-coated ELISAMax™ Deluxe sets or mouse IL-23 (p19/p40) ELISA MAX™ Deluxe respectively (BioLegend, CA). BioLegend kits were used according to manufacturer's protocol.

2.5. Statistical analysis

Data were analyzed using unpaired two-tailed *t*-test. Multiple comparisons between different groups were done using one-way ANOVA Tukey's multiple comparisons test where appropriate. A p value <0.05 was considered to be statistically significant (minimum n = 3 experiments unless stated otherwise).

3. Results

3.1. IL-23 induces pathological features consistent with arthritis

C57BL/6 mice are known to be resistant animal models of arthritis including collagen-induced arthritis and multiple laboratories produce variable results of arthritis induction [25]. Similar observations have been published with IL-23 MC overexpression [26]; thus, we utilized a combination of IL-23 MC with zymosan to develop an animal model that will allow us to study IL-23 biology in C57BL/6 mice and circumvent this issue. In these experiments zymosan was injected in the presence of either IL-23 or GFP MC as previously described **(**Fig. 1A**).** IL-23 gene transfer mice achieved systemic IL-23 expression at the 30-50 ng/ml range **(**Fig. 1B**).** IL-23 in the presence of Zymosan induced an acute paw swelling response characterized by redness and scaling that persisted during the disease course compared to Zymosan treated GFP controls **(**Figs. 1C,D**).** No swelling was observed in the non-Zymosan treated mice

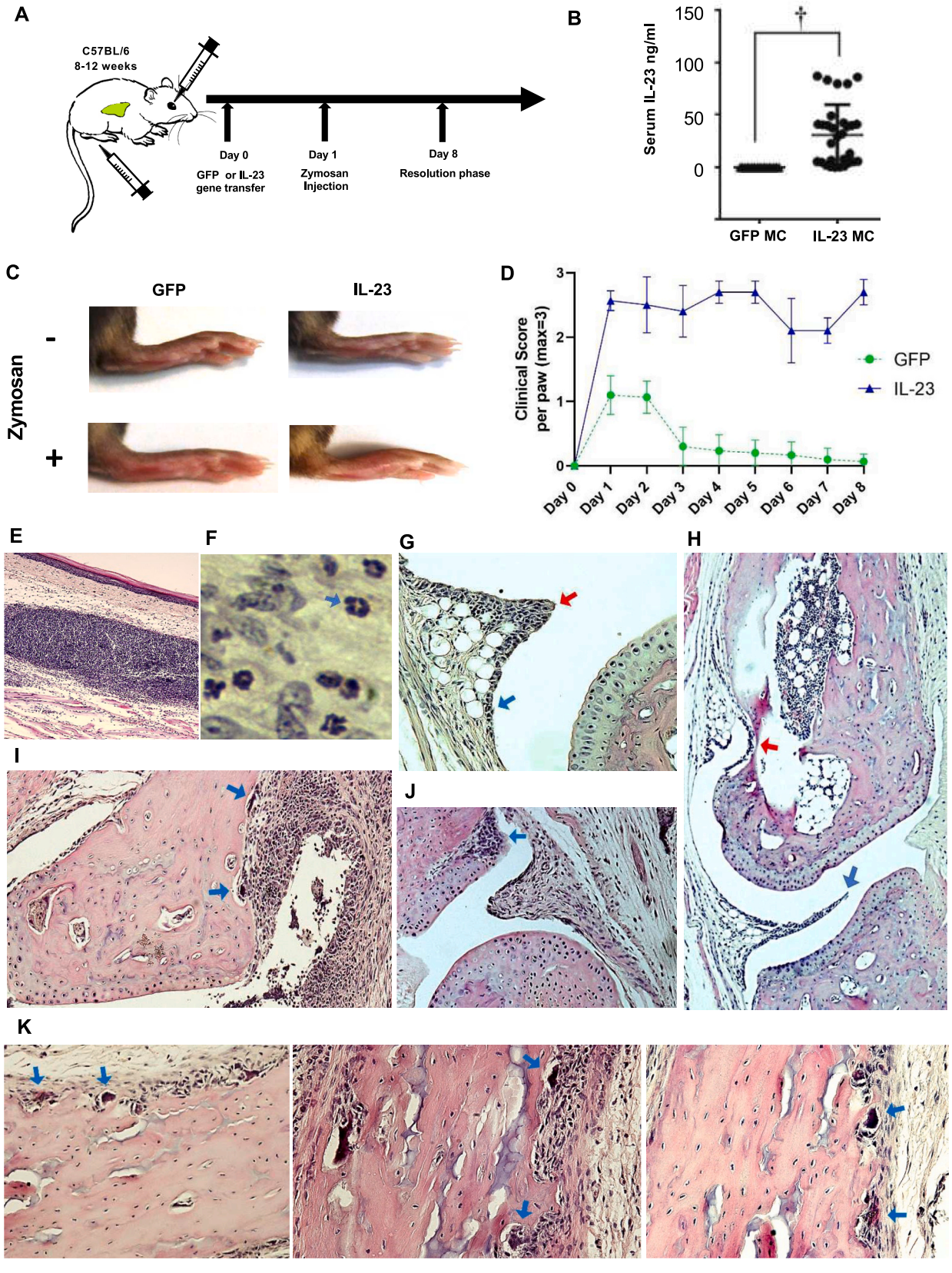

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Fig. 1. IL-23 gene transfer in C57BL/6 mice in combination with Zymosan induces pathological features consistent with arthritis. (A) Schematic illustration of the experimental animal model with timeline and injections: Mice were injected with either GFP MC and/or IL-23 MC on day zero. On day 3 serum was collected to confirm overexpression of IL-23, and overexpressing mice were injected with 10μg zymosan and were sacrificed on day 12. (B) Serum ELISA of IL-23 levels 3d after injection with either relative to GFP control. Data are pooled from three experiments. Statistical analysis was done using two-tailed unpaired *t*-test. $*p < 0.05$; †, $p < 0.0001$. (C) Representative images of hind paws in GFP and IL-23 overexpressing mice injected and not injected with zymosan. (D) Timescale depicting disease severity, mice were scored from 0 to 3, indicative of redness, swelling and scaling. (E) Histological examination of skin in IL-23 MC mice after intra-articular zymosan injection; epidermis is insignificant; however, dermis is infiltrated with dense aggregate of leukocytes resembling an intradermal abscess (x10). (F) Inflammatory aggregate consists of mononuclear and polymorphonuclear leukocytes (blue arrow) (x60). (G) Synovium membrane with normal healthy 1–2 cell lining in the periphery (blue arrow) and proliferation of cells into multi-layered hyperplastic villous (red arrow) (x40). (H) The extension of proliferating synovial membrane into intra-articular space (blue arrow) and eroding cortical bone surface (red arrow) (x4). (I) Metatarsal bone demonstrates dense inflammatory infiltrate in the proliferating synovium (pannus) and the focal cortical bone erosions (arrow) (x10). (J) Subchondral bone erosion (x10). (K) Osteoclast formations and multiple periosteal resorption bays were found responsible for the focal bone erosions at the pannus-bone interface (x40). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

at day 1. Inspection of the sagittal sections of ankle joints revealed arthritic changes as well as diffuse immune cell infiltration throughout the adjacent muscle and subcutaneous tissue, suggesting an extensive inflammatory response following intra-articular injection of zymosan. While skin epidermis was unremarkable, large aggregates of leukocytes consisting of polymorphonuclear and macrophages were present in the skin dermis **(**Figs. 1E,F**).**

Numerous regions of subcutaneous soft tissue (reticular dermis layer) and peri-muscular fascia with extensive infiltration of macrophage and polymorphonuclear leukocytes were severe and dense enough to mimic abscess formations. Joint arthritis was evaluated in terms of synovitis, cartilage damage, and bone erosion. Prominent joint space exudate, moderate thickening of the synovial lining, and sublining (multilayered synovial membranes) with enhanced cellular infiltrate and moderately increased cell density is indicative of synovitis **(**Figs. 1G-J**).** Multinucleated bone-resorbing osteoclasts were found in the erosive joints which were responsible for the generation of the synovial osteoclasts and the formation of invasive pannus penetrating into subchondral bone areas **(**Fig. 1K**).** In addition to several superficial bone erosions at the outer surface of the cortical bones, occasionally focal subchondral erosions had progressed resulting in partial penetration of cortical bone causing minor breakthrough of cortical bone into the bone marrow cavity. Collectively the data presented herein are consistent with pathological features of inflammatory arthritis.

3.2. IL-27 gene transfer attenuates IL-23-induced arthritis

In order to investigate the role of IL-27 in IL-23 mediated joint inflammation animals were injected with IL-27 MC DNA 3 days before IL-23 or GFP gene transfer as described in the schematic in (Fig. 2A**).** Mice achieved comparable overexpression of IL-27 as confirmed by quantitative IL-27 ELISA of serum collected from tail bleeds 3 days post-gene transfer **(**Fig. 2B**).** We did not detect serum IL-23 and IL-27 or the biologically active IL-27 subunit, p28 by ELISA in the naïve mice. IL-27 overexpressing mice did not show any clinical signs of inflammation and IL-27 had a protective effect in the Zymosan+IL-23-induced joint inflammation as evidenced by reduced paw swelling, redness and scaling, and only showed mild inflammation between day 1 and day 3 that subsequently subsided by day 7 **(**Figs. 2C,D**).** Zymosan induced inflammation subsided by day 3 which is consistent with previously published data [23] and did not significantly contribute to difference in inflammation between the groups injected with the minicircle DNAs compared to control groups.

Histological examination of joint sections of IL-23 MC mice demonstrated increased bone marrow cellularity compatible with increased myelopoiesis **(**Fig. 2E**).** While GFP MC and IL27 MC mice had normal cellularity a moderate myeloid expansion was occasionally noted in some areas of marrow cavities in the IL23 + IL27 MC mice **(**Fig. 2E**).** Overall IL-27 gene transfer resulted in a decrease in join inflammation and protected from the increased IL-23 induced myelopoiesis in the bone marrow.

3.3. IL-27 mediated protection against synovial inflammation and bone erosion and cartilage destruction

The histologic examination of H&*E*-stained decalcified paw samples from the IL-23 MC injected mice 7 days after zymosan injection revealed extensive joint and marrow myeloid hyperplasia and periostitis compared to GFP injected control group**.** Review of the joint sections from GFP MC mice revealed normal healthy 1–2 cell- layered synovium without significant inflammatory cells. Consistent with reduction in disease severity, prior IL-27 overexpression provided protection against synovial inflammation and bone damage as shown by the reduced myeloid inflammatory infiltrate, bone marrow myeloid hyperplasia, myositis and synovitis in IL-23 overexpressing wild type (WT) mice **(**Fig. 3A**).**

In IL-23 + IL-27 MC mice, inflammation was limited to only a small number of affected joints. Mild inflammatory infiltrates of the synovial membrane, occasional pannus formations and minimal bone erosions were found in those few affected tarsal joints. However, the number of inflammatory sites, cellularity and density of infiltrates were markedly lower than those of their counterpart, IL-23 MC mice. The representative image shown for IL-23 + IL-27 MC is merely remarkable for a mild inflammatory infiltration of synovium. These findings suggest that IL-27 plays a protective role against synovial inflammation and bone erosion in IL-23 gene transfer model. (Fig. 3A**).** Interestingly IL-27 injected mice showed mild infiltration of myeloid cells in muscular tissue and very mild bone marrow myeloid hyperplasia but no synovitis or pannus formation (Fig. 3A). Cartilage damage was evaluated using Safranin O staining whereby the absence of redness (de-staining of Safranin O) in the superficial, non-calcified cartilage layer is indicative of the inflammation-mediated proteoglycan loss. Our data demonstrate destruction of cartilage in IL-23 injected mice compared to GFP control, and IL-27-mediated protection of cartilage destruction resulting from IL-23 overexpression (Fig. 3B). Histological score of pannus formation, leucocyte infiltration, and hyperproliferative bone marrow demonstrated that IL-27 overexpression alone can lead to some myeloid infiltration but not pannus formation, cartilage destruction, mild bone marrow hyperplasia, myositis and periostitis **(**Fig. 3C**).** However, IL-27 mediated protection of synovial inflammation, and bone damage, and cartilage destruction in IL-23 induced mice. Cartilage, bone surfaces and articular spaces were also intact. As evidenced by Safranin O staining the articular cartilage maintained smooth surface and all three layers of cartilage consisting of superficial, non-calcified and calcified remained intact. Similarly, no major cartilage erosion or proteoglycan loss was present in the IL-27 MC mice joint sections. However, 3–4 layered synovium with mild cellular aggregates in peri-articular tissue were occasionally detected. Except for some inflammatory sites, the outer surface of cortical bone showed no erosion. Inflammation was present in the most of the tarsal joints on IL-23 MC mice and very few joints were spared of identifiable inflammatory changes. Almost exclusive to the IL-23 MC mice, even in the relatively less affected ankle joints and peri-articular areas without massive inflammatory accumulations, hyperplasia and extension of synovium into the intra-articular space was

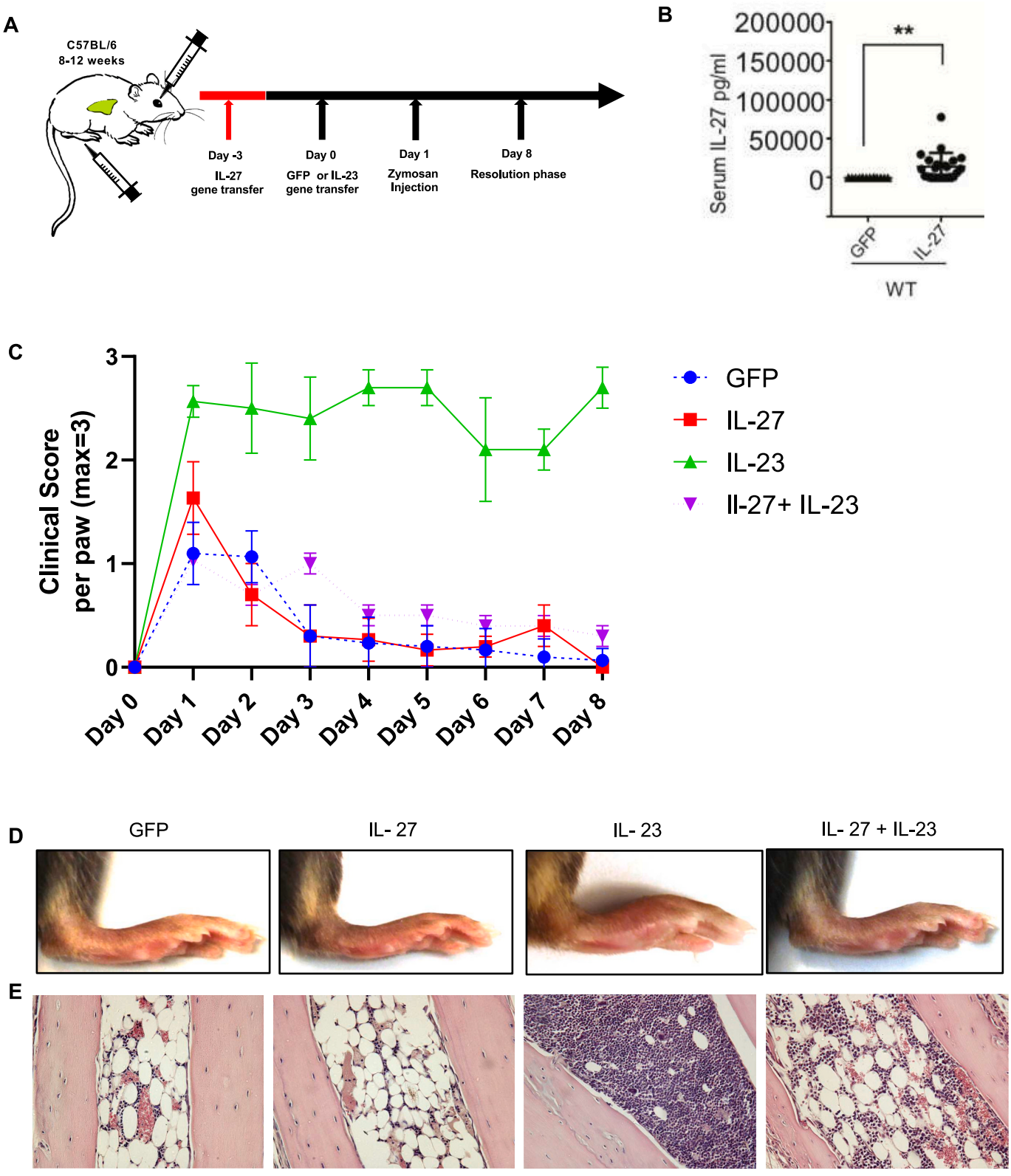

Fig. 2. Systemic overexpression of IL-27 attenuates bone marrow and joint inflammation. (A) Schematic illustration of the timeline and injections: Mice were injected with IL-27 three days prior to either GFP MC and/or IL-23 MC on day zero. On day 0 serum was collected to confirm overexpression of IL-27, and day 3 for IL-23. Overexpressing mice were injected with 10μg zymosan and were sacrificed on day 12 (B) Showing serum IL-27 levels in mice injected hydrodynamically with IL-27 DNA or GFP. Data are pooled from three experiments. Statistical analysis was done using two-tailed unpaired t-test. *$p < 0.05$; †, $p < 0.0001$. (C) Timescale depicting clinical course and disease severity, mice were scored from 0 to 3, indicative of redness, swelling and scaling. (D) Representative images of hind paws injected with respective cytokine combination overexpressing GFP (control), IL-27, IL-23 or IL-23 + IL-27 combo. (E) Hematoxylin/Eosin staining of bone marrow (x20) showing physiological cellularity in GFP MC and IL27 MC mice, and a hypercellular marrow in IL23 MC group. Bone marrow hypercellularity is reduced in IL23 + IL27 MC mice which range from physiological to slightly elevated cellularity.

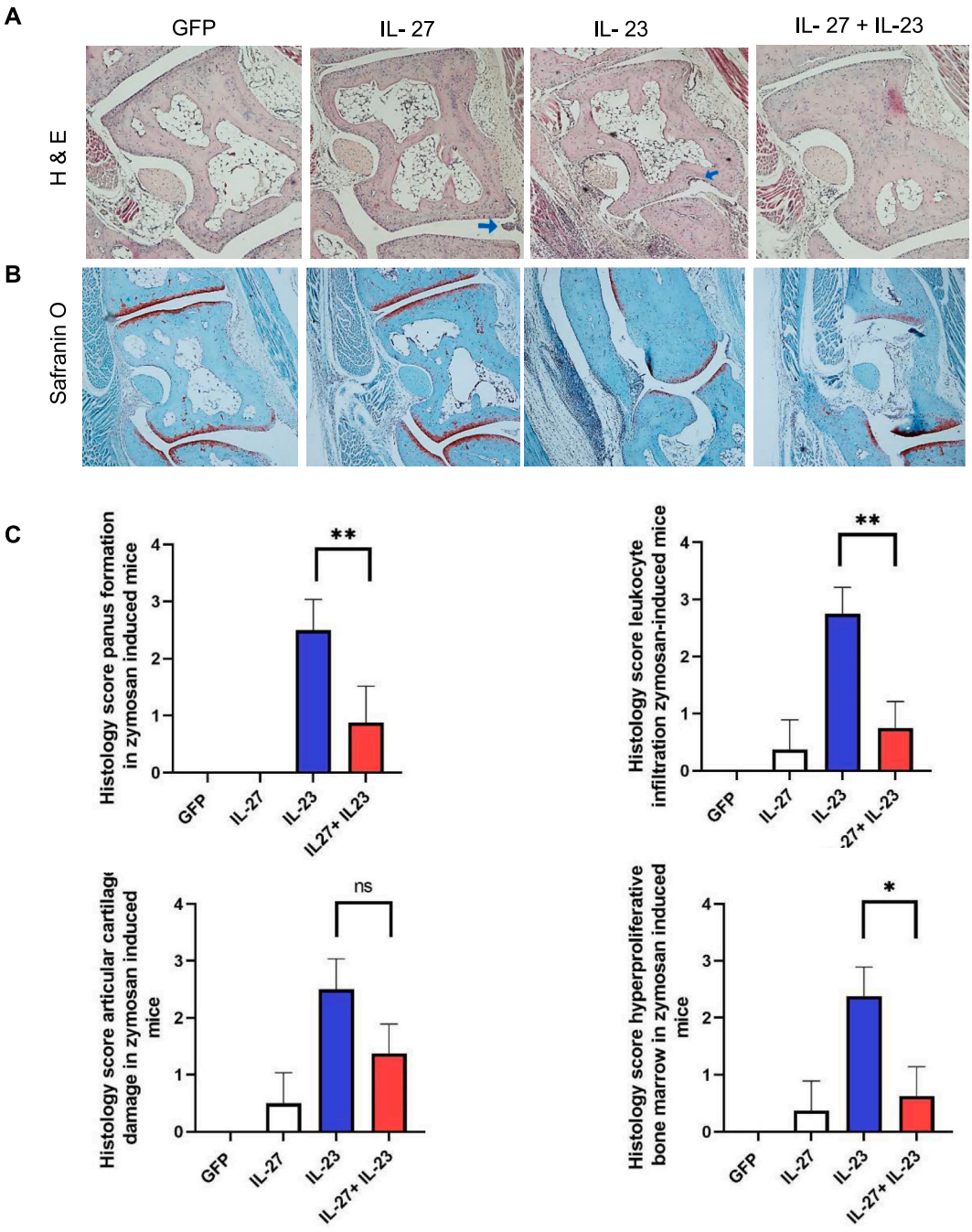

Fig. 3. Systemic overexpression of IL-27 attenuates joint inflammation. (A) Hematoxylin/Eosin staining: GFP MC shows no arthritic features; IL-27 MC mouse demonstrates a relatively clear joint space with minimal inflammatory cell infiltration of the synovium. Articular cartilage and bone were mostly preserved without any remarkable proteoglycan depletion of cartilage or bone erosion or joint architecture disruption; however, small progression of synovium into articular space is noted (blue arrow). IL 23 MC mouse shows features of arthritis including narrowing of joint space, inflammatory infiltrates of synovium and peri-articular connective tissues, as well as the proliferative synovium eroding into adjacent bone surfaces (arrow). IL-27 + IL-23 shows moderate inflammatory infiltrates and extending synovium, suggestive of synovitis, but the joint architecture is perfectly maintained. (B) Hematoxylin/Fast green/Safranin O (pink) staining on paraffined cut sections. IL-23 mouse section shows loss of Safranin O staining in the superficial layer of cartilage suggestive of proteoglycan depletion (x4). (C) Graphs depicting histological score of pannus formation, leucocyte infiltration, and hyperproliferative bone marrow in zymosan induced mice injected with respective MC DNA. Data are representative of two independent experiments $n = 3$–5 mice per group. Statistical analysis was performed using two-way ANOVA $^{*}p < 0.05$; $^{**}p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

noted as a herald of ensuing invasive pannus proliferation. Pannus tissues had occasionally invaded and eroded the superficial cartilage and the underlying calcified layer, leaving behind the subchondral and cortical bone penetrating into the marrow cavity. However, some areas of articular cartilage had maintained the smooth surface or showed only a minor roughening of the non-calcified cartilage layer. Collectively, IL-27 has dual capacity as both a pro-inflammatory and anti-inflammatory cytokine depending on the context of the microenvironment.

3.4. IL-27 mediates inhibitory effect on the IL-23-induced myeloid expansion

We have previously shown that IL-23 induces significant myelopoiesis in the spleen of B10RIII mice [3]. Therefore, we next investigated the role of IL-27 in IL-23-induced myelopoiesis in vivo. Consistent with the clinical score and circulating serum biomarkers above, overexpression of IL-23 alone resulted in prominent myeloid shift. Further as a result of IL-23 overexpression, this increase was limited to only the myeloid subset as we did not observe any significant difference between the $CD11b^{-}CD3^{+}$ populations (data not shown). The observed myeloid shift correlated with an expansion of the $CD3^{-}CD11b^{+}$ (GFP: 1.8 ± 0.32, IL-23: 5.935 ± 0.650 mean ± SD) and CD3-$CD11b^{+}GR1^{hi+}$ (GFP: 0.47 ± 0.12; IL-23: 2.86 ± 0.204) (Figs. 4A-C) splenic populations 3 days post-IL-23 gene transfer compared to GFP controls as evidenced by flow cytometry. This persisted for at least 7 days. Surprisingly, IL-27 overexpression in WT mice resulted in a mild myeloid shift in the total percent of $CD3^{-}CD11b^{+}$ population (IL-27: 3.09 ± 1.07 vs GFP: 1.8 ± 0.32; mean ± SD) and $CD3^{-}CD11b^{+}GR1^{hi}$ (IL-27: 0.68 ± 0.30 vs GFP: 0.47 ± 0.12; mean ± SD)) (Figs. 4A-C). Collectively IL-27 induces anti-inflammatory properties by inhibiting the expansion of total myeloid cells (myelopoiesis) and neutrophil expansion in the spleen and bone marrow.

4. Discussion

In this study we describe the novel role of IL-27 in regulating IL-23 dependent myelopoiesis and joint inflammation. The proinflammatory cytokine IL-23 induces myelopoiesis and bone resorption by exerting its effect directly on myeloid lineage precursor cells as previously described [3,4]. IL-23 induced activation of myeloid cells also results in secretion of IL-27 which exerts anti-inflammatory effect through dendritic cells [10]. Therefore, we explored the possibility of IL-27 mediated regulatory effect on IL-23 induced myelopoiesis and inflammatory arthritis by using gene overexpression-based mice models.

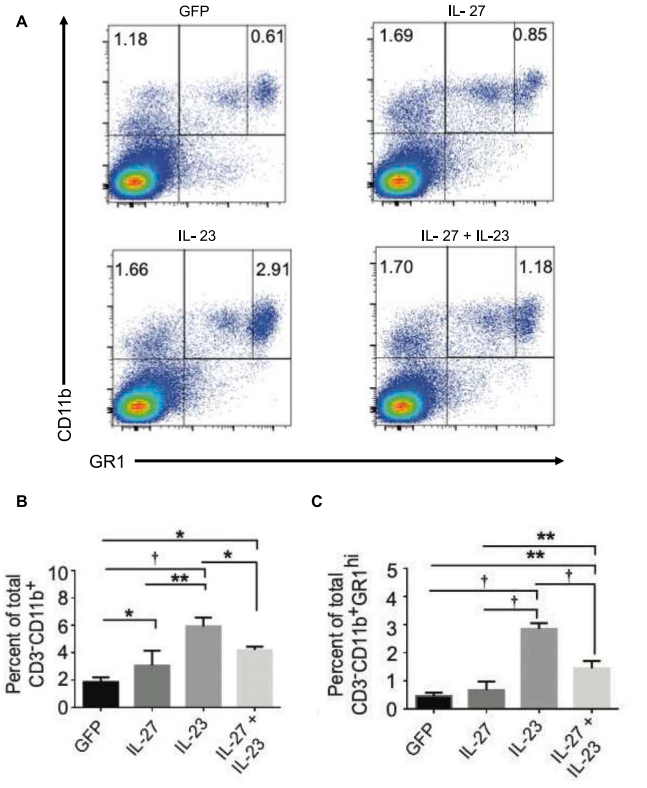

Fig. 4. The IL-27 mediated suppressive effect on IL-23-dependent myelopoiesis. (A) Representative flow plots demonstrating the percentages of $CD3^{-}CD11b^{+}$ myeloid cells in the spleen of WT mice injected with GFP, IL-27, IL-23 and IL-27 + IL-23 MC DNA. Graphs depicting the percentages of total (B) $CD3^{-}CD11b^{+}$ and (C) $CD3^{-}CD11b^{+}GR1^{hi}$ myeloid cells. Representative data from two independent experiments with $n = 4$–6 mice per group. Statistical analysis was done using one-way ANOVA. *p < 0.05; ** p < 0.01; ***$p < 0.001$; †, $p < 0.0001$.

Histology and flow cytometry data showed the presence of myeloid cells upon overexpression of IL-27 alone. We observed less than two-fold myelopoiesis in IL-27 overexpressing mice in contrast to over 2.5-fold in IL-23 overexpressing mice. However, the effect that IL-27 had on joint-inflammation was minimal. Additionally, cytometric bead array analysis demonstrated that IL-27 gene transfer did not induce the expression of TNF, IL-6, G-CSF, IFNγ and IL-17A compared to GFP suggesting that IL-27 does not have pro-inflammatory effects. This could be explained by the fact that while IL-27 might contribute towards myelopoiesis and migration of myeloid cells to some extent, it may generate a different class of myeloid cells incapable of producing proinflammatory cytokines. Consistent with our results a recent study reported the infiltration of immune-regulatory IL-27-producing $CD14^{+}$ cells in the inflamed joints of rheumatoid arthritis patients [27]. The balance between myeloid suppressor cells that inhibit inflammation through the secretion of multiple anti-inflammatory cytokines and pathogenic myelopoiesis that exacerbates inflammation and induces osteoclastogenesis is paramount for the development of arthritis and bone destruction [28]. Indeed, the IL-27 pre-exposed mice had reduced levels of myelopoiesis in the bone marrow which also correlated with reduced joint inflammation. Notably, the amount of osteoclastogenesis that was observed with IL-23 was abrogated with IL-27 gene transfer.

In corroboration with the previously published data by us and others, we also did not observe any significant difference between the $CD11b^{-}CD3^{+}$ populations in both the IL-23 and/or IL-27 gene transfer groups at these early time-points (data not shown) [3,29]. Furthermore, we show that IL-27 is also protective against cartilage destruction. This is of interest as previously published reports have demonstrated independent mechanisms of cartilage destruction to synovial inflammation in collagen induced arthritis model involving IL-1β [30,31]. Our study may provide evidence of yet another uncoupling event between IL-1β dependent cartilage destruction and joint inflammation. This again underscores the interplay of multiple cellular and soluble components at the inflamed joint.

A limitation to the study is that we did not perform any analysis on the myeloid subsets but rather focused on the total $CD11b^{+}$ and the neutrophil population $CD11b^{+}GR1^{+}$ as an early responder in acute inflammation. Follow-up studies should examine the contribution of other myeloid subsets especially those expressing a functional IL-27R. Additionally, as these mice were not housed in specific pathogen free facility, it could be expected to produce small levels of IL-10 even in the absence of experimental challenge. Nevertheless, our findings demonstrate that IL-27 exert anti-inflammatory responses controlling bone marrow and spleen myelopoiesis and the resulting inflammation in IL-23 induced arthritis. This study further indicates the fine-balance between the anti-inflammatory and pro-inflammatory myeloid cells, that control the disease course and how IL-27 which is produced by activated dendritic cells and macrophages may be significant in controlling inflammatory arthritis by tipping the balance towards anti-inflammatory responses and inhibit IL-23 induction of inflammatory arthritis.

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Declaration of Competing Interest

The authors have no financial conflicts of interest.

Data availability

No data was used for the research described in the article.

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