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Effects of Overload on Imiquimod-Induced Psoriasis Model Mice: A Basic Experimental Study

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ABSTRACT

Background and Aim: Psoriasis is a skin disorder complicated by arthritis and enthesitis. The cytokines interleukin (IL)-17, IL-23, and tumor necrosis factor (TNF)-α are reportedly key effectors of psoriasis. Additionally, gamma delta (γδ) T cells exacerbate inflammation by producing inflammatory cytokines such as IL-17 and TNF-α. However, details regarding the mechanisms linking pathogenesis and mechanical stress remain unclear. This study aimed to investigate the effect of strenuous exercise on the pathology of psoriasis using mouse models of imiquimod (IMQ)-induced psoriasis.

Methods: Twenty mice were randomly assigned to four groups: IMQ − TRED− (control), IMQ − TRED+ (treadmill running mice), IMQ + TRED− group (IMQ treated mice), and IMQ + TRED+ group (IMQ treated and treadmill running mice). The tissue sections from back skin and thymus were immunostained with antibodies against IL-17, IL-23, and γδ T cells. Shoulder sections were stained using hematoxylin and eosin, and Toluidine Blue and Picrosirius Red. Additionally, the shoulder tissue sections were immunostained with antibodies against TNF-α and matrix metalloproteinase (MMP)-13. Serum cytokine level was measured to evaluate systemic inflammation.

Results: Strenuous exercise exacerbated pathological changes associated with psoriasis, including increased γδ T cell infiltration and upregulated IL-17 and IL-23 expression in the skin, as well as enhanced γδ T cell development and IL-17 expression in the thymus. Although strenuous exercise did not further worsen the modified PASI scores, histological and immunological markers of inflammation were significantly enhanced. Serum levels of TNF-α and IL-17 were significantly elevated in IMQ-induced psoriasis model mice. Moreover, pathological changes induced by strenuous exercise were observed in the enthesis, including angiogenesis and upregulated expression of TNF-α and MMP-13.

Conclusion: This study revealed that strenuous exercise exacerbates pathological changes in IMQ-induced psoriasis model mice.

1 | Introduction

Psoriasis is a chronic inflammatory skin disorder characterized by erythematous and scaly plaques, and affects almost 3% of adults in the United States [1–4]. A number of studies have suggested that genetic, environmental, and immunological factors are involved in the pathology of psoriasis [5]. Recent

studies have highlighted the importance of inflammatory cytokine pathways in the pathogenesis of psoriasis. The cytokines interleukin (IL)-17, IL-23, and tumor necrosis factor (TNF)-α are reportedly the key effectors of psoriasis [6]. Additionally, gamma delta (γδ) T cells that respond to IL-23 augment inflammation by producing inflammatory cytokines such as IL-17 and TNF-α [7]. Interest in new treatments for psoriasis has recently increased following the emergence of effective new classes of drugs, such as biologics.

Approximately 25% of patients with psoriasis also develop psoriatic arthritis, characterized by enthesitis [3, 4]. Enthesitis is inflammation of the entheses, which are the junctions where tendons or ligaments insert into bone, connecting tissues of differing mechanical properties and dispersing mechanical stress [8]. Histologically, entheses are classified as fibrous or fibrocartilaginous. Fibrocartilaginous entheses consist of a four-layered structure composed of tendon, calcified fibrocartilage, uncalcified fibrocartilage, and bone [9]. Enthesitis is a diffuse inflammation affecting adjacent bone and soft tissues, including the synovium. However, details regarding the pathogenesis of enthesitis in psoriatic arthritis remain unclear [10]. Although several treatments are recommended for psoriatic enthesitis, no definitive treatment is available; thus, the final treatment choice is left to the physician and patient. Enthesitis plays a significant role in the reduced quality of life of patients with psoriatic disease, and patient satisfaction remains low even with molecularly targeted drugs [11, 12].

Mechanical stress is a known cause of psoriatic arthritis and enthesitis [6, 13], while details regarding the association between the pathological condition and mechanical stress remain unclear. Imiquimod (IMQ), a topical medication used in the treatment of condyloma acuminatum, was utilized to develop a psoriasis mouse model that is now widely employed in research [14, 15]. This study aims to examine the effects of mechanical stress on the pathology of IMQ-induced psoriasis by applying strenuous exercise and assessing its impact at the physical, biochemical, and histological levels.

2 | Materials and Methods

2.1 | Animals

Seven-week-old BALB/c female mice were purchased from Japan SLC Co. (Shizuoka, Japan). All mice were provided sterilized food and water ad libitum and housed in cages (maximum of five animals per cage) maintained at an air-conditioned temperature of 22°C ± 2°C with constant humidity (40%–60%) under a 12-h light/dark cycle. All animal experiments were performed according to a protocol reviewed by the Institutional Animal Care and Use Committee and approved by the President of Okayama University (OKU-2022573).

2.2 | Experimental Design for the Psoriatic Arthritis–Induced Model

Twenty mice were randomly assigned to four groups: (1) a control group without IMQ treatment or treadmill strenuous exercise (IMQ − TRED−), (2) treadmill strenuous exercise without IMQ treatment (IMQ − TRED+), (3) IMQ treatment without treadmill strenuous exercise (IMQ + TRED−), and (4) IMQ treatment and treadmill strenuous exercise (IMQ + TRED+) (Figure 1a). IMQ cream (5%; 62.5 mg Beselna cream; Mochida Pharmaceutical Co., Tokyo, Japan) was applied topically to the shaved back skin of treated mice 5 days per week for 4 weeks. TRED+ mice were subjected to running on a treadmill machine (MK-680 S, Muromachi, Tokyo, Japan) for 1 h per day, 5 days per week, for 4 weeks. The initial running speed on Day 0 was 15 m/min and increased by 1 m/min/day up to 25 m/min. The inclination angle of the treadmill was set at 15° downhill (Figure 1b,c). After 4 weeks, the mice were euthanized and utilized for experiments.

2.3 | Psoriasis Area and Severity Index Score

The severity of skin inflammation was monitored and graded using a modified clinical Psoriasis Area and Severity Index (PASI) score [15, 16]. This is a modified version of the PASI score, commonly used in evaluating human psoriasis, which we adapted for animal models. Components related to lesion thickness and area calculations were removed. Erythema was scored on a scale ranging from 0 to 3 (0 = no redness, pale pink; 1 = pale pink with < 20% red patches; 2 = pink back with 20%–60% red patches; 3 = pink back with > 60% red patches), and scaling was scored on a scale ranging from 0 to 3 (0 = 0% scaling area; 1 = scaling area < 20%; 2 = 20%–60% scaling area; 3 = scaling area > 60%). The cumulative score for each parameter was calculated as a measure of inflammation severity (scale 0–6). This scoring was conducted at multiple time points: Day 4 (first week), Day 11 (second week), Day 18 (third week), and Day 25 (fourth week).

2.4 | Histological and Immunohistochemical Analyses

After 4 weeks, mice were anesthetized with isoflurane (3% v/v), and blood was obtained by cardiac puncture and subsequently serum was separated. The back skin, spleen, thymus tissues, and shoulder tissues were resected and fixed in 10% formalin for 3 days. After fixation, the back skin, spleen, and thymus tissues were embedded in paraffin. Shoulder specimens were decalcified in 0.3 M ethylenediaminetetraacetic acid (pH 7.5) for 6 weeks. After decalcification, samples were embedded in paraffin blocks. The blocks were sliced at a thickness of 5 μm along the coronal plane. After deparaffinization, the sections were stained using hematoxylin and eosin (HE) to examine the subacromial bursa tissue. The severity of bursa inflammation was evaluated and graded using a modified histological score for angiogenesis developed by Minkwitz et al. [17] Angiogenesis was scored on a scale ranging from 0 to 3 (0 = no blood vessels; 1 = one or a few blood vessels; 2 = several blood vessels; 3 = numerous blood vessels throughout the tissue).

Sections of shoulder tissue were also stained with toluidine blue and picrosirius red to visualize collagen fibers and enthesis. Deparaffinized and hydrated sections were stained with 0.05% toluidine blue solution (pH 4.1) (Muto Pure Chemicals Co. Ltd., Tokyo, Japan) for 30 min, followed by 1% sirius red (Muto Pure Chemicals Co. Ltd.) and picric acid solution (Nacalai Tesque Inc., Kyoto, Japan) for 10 min. Sections were then dehydrated and

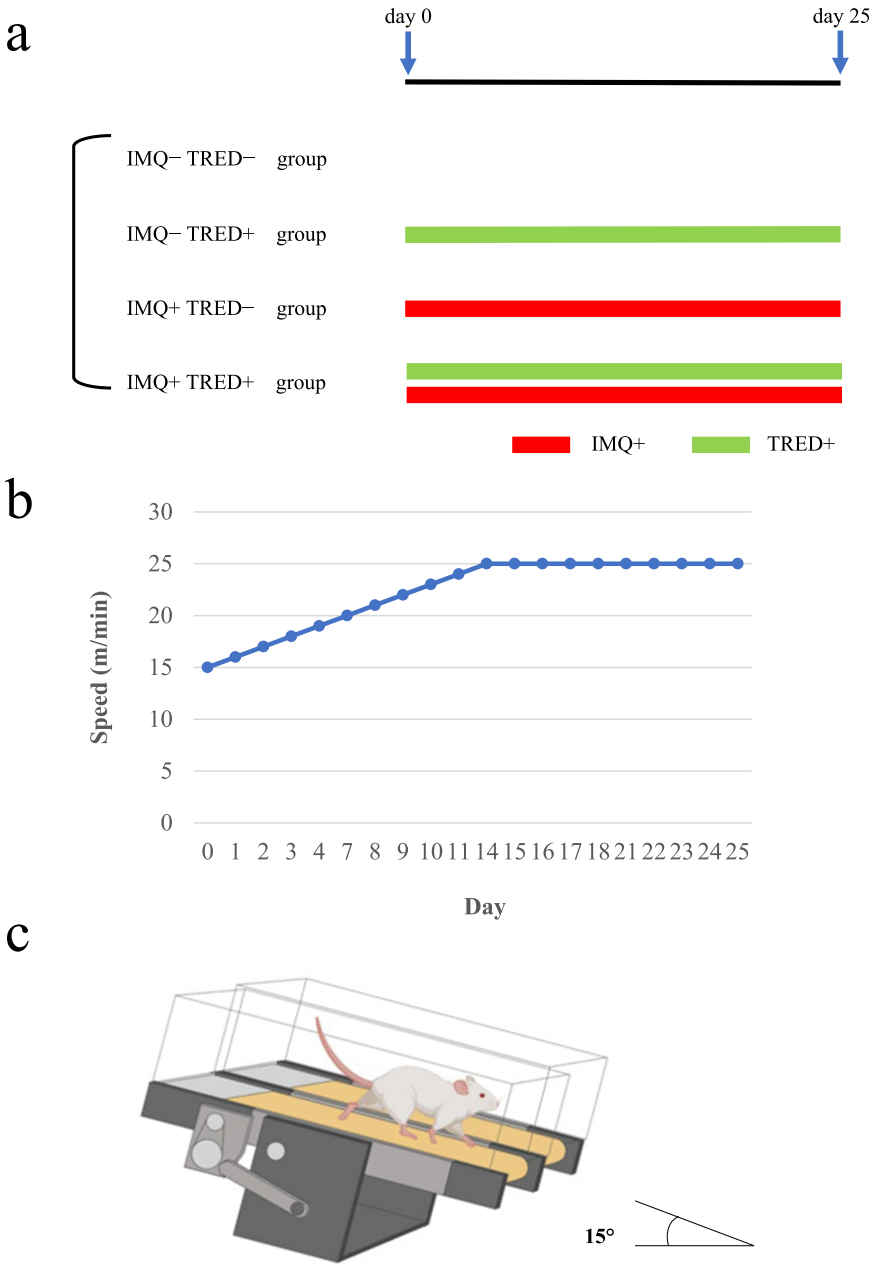

FIGURE 1 | (a) Experimental design of the psoriatic arthritis–induced mouse model. (b) Speed chart for the exercise intervention. (c) Treadmill set at 15° downhill. Created with http://BioRender.com. Abbreviations: IMQ, imiquimod; TRED, treadmill.

mounted. Under polarized light microscopy, larger collagen fibers appeared bright yellow or orange, and thinner fibers, including reticular fibers, appeared green. Uncalcified fibrocartilage (UFC) and calcified fibrocartilage (CFC) were observed under a light microscope (Olympus System Microscope Model BX53 LED; Olympus Co., Tokyo, Japan). The area of UFC was identified as orange-yellow over the tidemark. The CFC area was identified as the layer under the tidemark over the blue-green layer, subchondral bone. The areas of UFC and CFC were calculated using FIJI, ImageJ software (US National Institutes of Health, Bethesda, MD, USA). The area of fibrocartilage (FC) was defined as the total of the UFC and CFC areas. The ratio of UFC area to total FC area was then calculated.

For immunohistochemical staining, tissues in paraffin blocks were sectioned at a thickness of 5 μm and mounted onto slides. The sections were then placed in an oven at 60°C for 30 min to allow for deparaffinization and dehydration and placed in 10 mM citrate buffer (pH 6.0) at 121°C for 10 min for antigen retrieval. The slides were incubated with 3% hydrogen peroxide (Fujifilm Wako Pure Chemical Co., Osaka, Japan) in phosphate-buffered saline (PBS) for 10 min and then immunostained with primary antibodies against IL-17A (5 μg/mL, ab79056; Abcam, Cambridge, UK), IL-23 (2 μg/mL, ab189300; Abcam), IHC-plus monoclonal hamster-Armenian anti-mouse TCR gamma + TCR delta (clone GL-3, FITC, IHC) (γδ T) (2 μg/mL, LS-B5684; LSBio, Seattle, WA), TNF-α (5 μg/mL, BS-2081R; Bioss Inc. Woburn, MN), or MMP-13 (2 μg/mL, ab39012; Abcam). Following overnight incubation at 4°C, the slides were washed twice with PBS for 5 min each. For secondary antibodies, Histofine simple stain mouse MAX PO(R) (414341; Nichirei Biosciences Inc., Tokyo,

Japan) was used for IL-17A, IL-23, TNF-α, and MMP-13 primary antibodies, and peroxidase-conjugated AffiniPure goat anti-Armenian hamster IgG (H + L) was used for the γδ T primary antibody (1.6 μg/mL, 127-035-099; Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA). Samples were stained with secondary antibodies at room temperature for 30 min. The slides were also washed twice with PBS for 5 min each and treated with diaminobenzidine (Nichirei Biosciences Inc.), controlling the degree of staining by monitoring with regular microscopy. The slides were stained with hematoxylin for 30 s to identify nuclei and then washed in distilled water again. Finally, the slides were dehydrated, cleared, and mounted. IL-17A, IL-23, and γδ T cells were analyzed in the back skin, spleen, and thymus tissues. TNF-α and MMP-13 were analyzed in shoulder tissue.

Areas positive for staining with these antibodies in one field of view were quantified using FIJI, ImageJ software. For five sections in each group, the positive area in representative high-power fields was measured. Two observers blinded concerning the experimental group counted the cells in the enthesis. For five immunostained sections in each group, the average number of positive cells in the enthesis was determined.

2.5 | Biochemical Analyses

Levels of serum cytokines (IL-17A, TNF-α, IL-23, IL-1α, IL-1β, IL-6, IL-10, IL-12p70, IL-27, MCP-1, interferon [IFN]-β, IFN-γ, and granulocyte macrophage–colony-stimulating factor [GM-CSF]) in mice were measured using LEGENDplex kits (BioLegend, San Diego, CA, USA) according to the manufacturer's protocols and assessed via a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA). Flow cytometry data files were analyzed using Qognit software (BioLegend).

2.6 | Statistical Analysis

All data were analyzed using GraphPad Prism 10 software and presented as the mean with 95% confidence interval (CI). Statistical analysis was performed using ordinary one-way analysis of variance (ANOVA), and Tukey's test was used for multiple comparisons. Significance was set at $p < 0.05$.

3 | Results

3.1 | Changes in Skin Lesions by Exercise

We initially evaluated the changes in skin lesions of each group in the first, second, third, and fourth weeks. Both the IMQ + TRED− and IMQ + TRED+ groups showed significant erythematous and scaly skin lesions in the first week, with erythema persisting after the second week (Figure 2a). Modified PASI scores were elevated in the IMQ + TRED− and IMQ + TRED+ groups in the first week but gradually declined thereafter (Figure 2a).

Histologically, the area positive for IL-23 in the skin stroma was significantly increased in the IMQ + TRED+ group compared with the other groups ($p = 0.011$ vs. IMQ − TRED− group, $p = 0.015$ vs. IMQ − TRED+ group, and $p = 0.016$ vs. IMQ + TRED− group). Although the IMQ − TRED+ and IMQ + TRED− groups exhibited greater positive areas than the IMQ − TRED− group, the differences were not statistically significant. The area positive for γδ T cells in the skin stroma was significantly increased in the IMQ + TRED− and IMQ + TRED+ groups compared with the other groups ($p = 0.017$ IMQ + TRED− group vs. IMQ − TRED− group, $p = 0.038$ IMQ + TRED− group vs. IMQ − TRED+ group, $p < 0.001$ IMQ + TRED+ group vs. IMQ − TRED− group, and $p < 0.001$ IMQ + TRED+ group vs. IMQ + TRED− group). In the IMQ+ groups, the positive area increased further with the addition of treadmill strenuous exercise. Similarly, the area positive for IL-17 followed the same pattern as that of γδ T cells ($p < 0.001$ IMQ + TRED− group vs. IMQ − TRED− group, $p < 0.001$ IMQ + TRED− group vs. IMQ − TRED+ group, $p < 0.001$ IMQ + TRED+ group vs. IMQ − TRED− group, and $p = 0.020$ IMQ + TRED+ group vs. IMQ + TRED− group) (Figure 2b). These results suggest that strenuous exercise exacerbates skin symptoms at the histological level.

3.2 | Effects of Cutaneous Inflammation and Exercise on the Spleen and Thymus

To examine whether strenuous exercise was associated with alterations in systemic immune organs, we next evaluated changes in the spleen and thymus. The mice were weighed before being sacrificed, and then the spleen of each mouse was weighed. The weight of the spleen in the IMQ + TRED− and IMQ + TRED+ groups was greater than that of the spleen in the IMQ− groups (Figure 2c). The spleen index (spleen weight/body weight) was significantly higher in the IMQ + TRED− group compared with the other groups ($p < 0.001$) (Figure 2c). These data indicate that IMQ application causes splenomegaly, whereas treadmill running leads to a decrease in the spleen index. There was no significant difference in the area positive for γδ T cells and IL-17 expression in the spleen between these groups (Figure 2d). In the thymus, the area positive for γδ T cells and IL-17 expression was significantly greater in the IMQ + TRED+ group than in the other groups ($p = 0.003$ and $p = 0.006$ versus IMQ − TRED− group, $p = 0.003$ and $p = 0.007$ versus IMQ − TRED+ group, and $p = 0.004$ and $p = 0.02$ versus IMQ + TRED− group, respectively) (Figure 2e). Collectively, these results suggest that strenuous exercise reduces splenomegaly and promotes γδ T cell generation and IL-17 expression in the thymus of IMQ-induced psoriasis model mice.

3.3 | Serum Cytokine Change by Exercise

Given the observed immunological changes in the skin and lymphoid organs, we next assessed whether strenuous exercise influenced systemic inflammatory cytokine levels in the serum. Most cytokines (e.g., IL-17, TNF-α, IL-23, IL-1α, IL-1β, IL-6, IL-10, IL-12p70, IL-27, MCP-1, IFN-β, IFN-γ, and GM-CSF) are produced by innate immune cells, thus linking innate and adaptive immunity or bystander cells. Serum levels of IL-17, TNF-α, IL-23, IL-6, and IL-12p70 tended to be higher in the IMQ + TRED+ group compared with the other groups (Figure 3a). In particular, serum levels of IL-17 and TNF-α were significantly higher in the IMQ + TRED+ group than in the other groups ($p = 0.006$ and $p < 0.001$ versus IMQ − TRED−

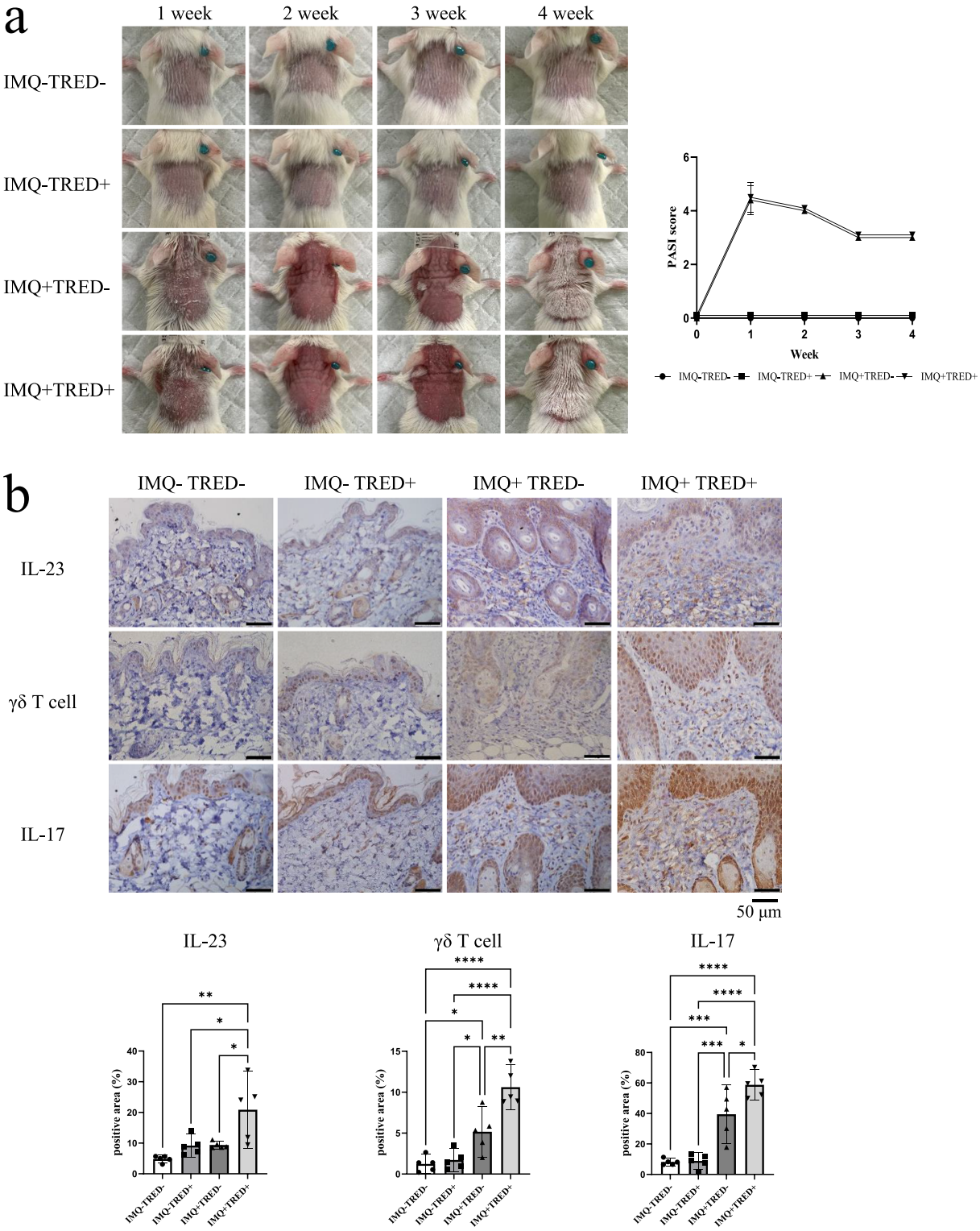

FIGURE 2 | (a) Representative images of mouse back skin in the first, second, third, and fourth weeks. Weekly mean disease severity of skin lesions of each group assessed using the modified PASI score. (b) Representative immunohistochemistry images showing IL-23, γδ T cells, and IL-17 in the skin (scale bars = 50 μm). Comparison of, IL-23–, γδ T cell–, and IL-17–positive areas in the skin. (c) Photographs of spleens (scale bars = 1 cm). Spleen index (spleen weight/body weight). (d) Representative immunohistochemistry images showing γδ T cell infiltration and IL-17 expression in the spleen (scale bars = 50 μm). Comparison of γδ T cell– and IL-17–positive areas in the spleen. (e) Representative immunohistochemistry images showing γδ T cell infiltration and IL-17 expression in the thymus (scale bars = 50 μm). Comparison of γδ T cell– and IL-17–positive areas in the thymus. Data are presented as the mean with 95% confidence interval (CI). Each experimental group consisted of $n = 5$ mice. Statistical comparisons were performed among all four groups (IMQ − TRED−, IMQ − TRED+, IMQ + TRED−, and IMQ + TRED+) using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

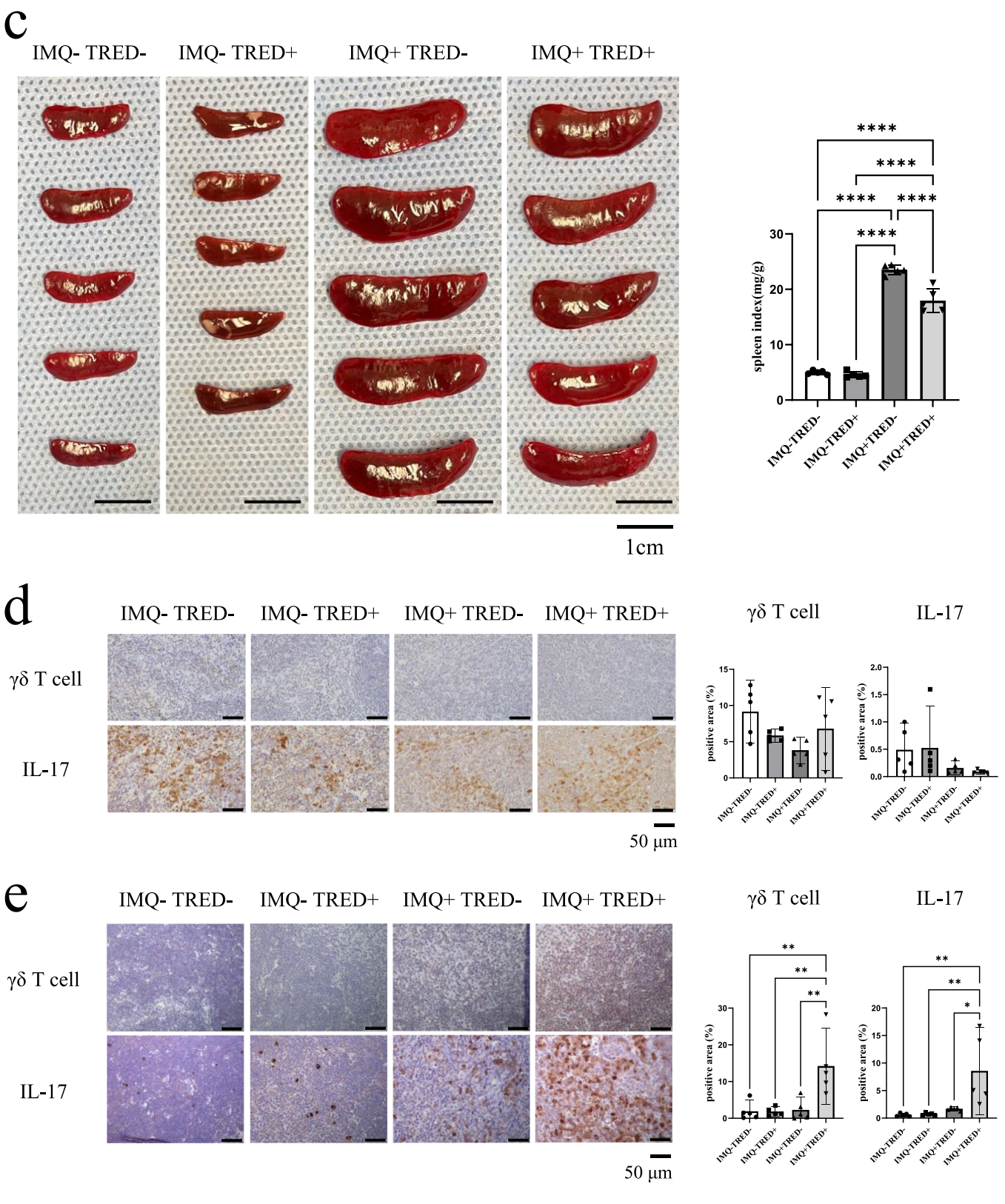

FIGURE 2 | (Continued)

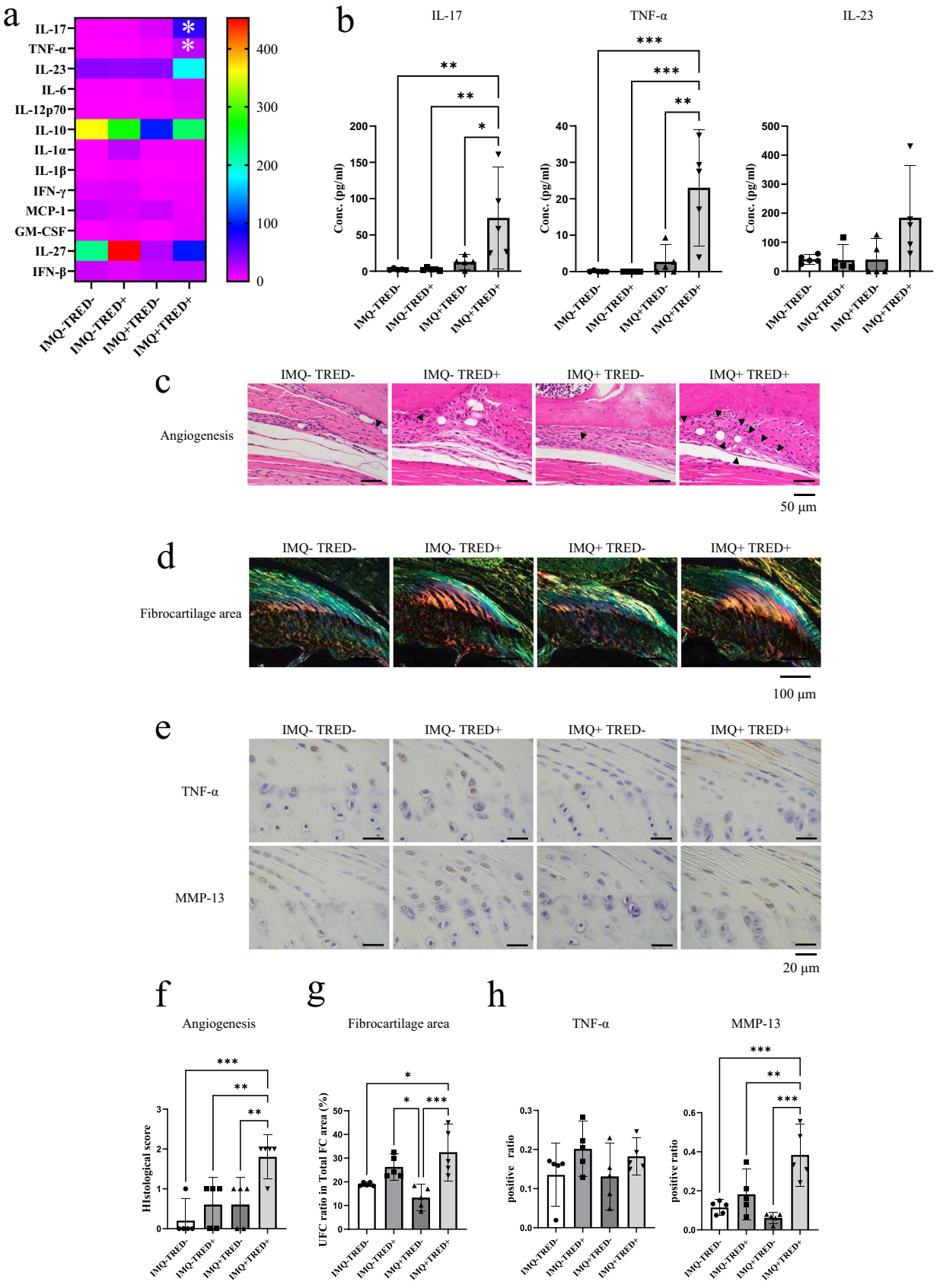

FIGURE 3 | Legend on next page.

group, $p = 0.006$ and $p < 0.001$ vs. IMQ − TRED+ group, and $p = 0.02$ and $p = 0.001$ vs. IMQ + TRED− group, respectively) (Figure 3b). These results suggest that strenuous exercise increases IL-17 and TNF-α levels in the serum of IMQ-induced psoriasis model mice.

3.4 | Effects of Exercise on Histological Changes and Inflammatory Factors in the Shoulder

Finally, to elucidate the effects of systemic immune responses, including elevations of IL-17, IL-23, and γδT cells in the skin, thymus, and serum, and strenuous exercise on the tendon enthesis, we examined histological and molecular changes in the shoulder enthesis. Subacromial bursa tissue was stained with HE to calculate the modified histological score of angiogenesis (Figure 3c). The score in the IMQ + TRED+ group was significantly higher compared with the other groups ($p < 0.001$ vs. IMQ − TRED− group, $p = 0.008$ vs. IMQ − TRED+ group, and $p = 0.008$ vs. IMQ + TRED − group) (Figure 3f). Excluding the IMQ + TRED+ group, the scores were very similar between the groups.

The ratio of UFC area to total FC area was significantly higher in the IMQ + TRED+ group compared with the IMQ − TRED− and IMQ + TRED− groups ($p = 0.01$ and $p < 0.001$, respectively), although there was no significant difference between the IMQ − TRED+ and IMQ + TRED+ groups ($p = 0.37$) (Figure 3g).

The ratio of TNF-α–positive cells in the IMQ + TRED+ did not differ significantly compared with the other groups ($p = 0.59$ vs. IMQ − TRED− group, $p = 0.96$ vs. IMQ − TRED+ group, and $p = 0.52$ vs. IMQ + TRED− group), although the ratios of the IMQ+ groups tended to be higher compared with the other groups (Figure 3h). The ratio of MMP-13–positive cells was significantly higher in the IMQ + TRED+ group compared with the other groups ($p < 0.001$ vs. IMQ − TRED− group, $p = 0.009$ vs. IMQ − TRED+ group, and $p < 0.001$ vs. IMQ + TRED− group). Collectively, these results suggest that the mechanical stress associated with strenuous exercise affects the entheses and the synovium in the shoulder in IMQ-induced psoriasis.

4 | Discussion

In this study, we demonstrated that strenuous exercise is associated with enhanced cutaneous, systemic, and enthesis-related inflammatory changes in an IMQ-induced psoriasis mouse model.

When IMQ cream is applied to the dorsal skin of mice, specific dendritic cells produce cytokines such as TNF-α and IL-23 [14, 15]. IL-23 induces the recruitment of IL-17–producing γδ T cells to the skin, which further contribute to IL-17 production [10, 18, 19]. Ultimately, IL-17 acts on the epidermis, causing skin cell infiltration and inflammation [14, 20]. Additionally, mechanical stress has been shown to stimulate cytokine production, suggesting that it also exacerbates the symptoms of psoriasis [21, 22]. Consistent with these previous observations, our study also demonstrated increased expression of IL-23, γδ T cells, and IL-17 in the skin, suggesting that similar inflammatory processes might be operative in our model.

We next examined whether cutaneous inflammation associated with strenuous exercise was accompanied by alterations in systemic immune organs. Topical application of IMQ to the dorsal skin of mice induced splenomegaly, consistent with previous reports [16, 23, 24]. A previous study reported a significant increase in the infiltration of immune cells—including neutrophils, dendritic cells, macrophages, and B cells—in the spleen of IMQ-induced psoriasis model mice, which correlated with increased spleen weight [25]. However, no significant increases in γδ T cell infiltration or IL-17 expression were observed in the spleen. In contrast, treadmill strenuous exercise was associated with a reduction in spleen size, in agreement with previous findings [25–28].

In the thymus of IMQ-induced psoriasis model mice subjected to strenuous exercise, a marked increase in γδ T cells and IL-17 expression was observed. Strenuous exercise also elevated IL-23 expression in both psoriatic skin and serum in this model. Previous reports have shown that γδ T cells are activated by IL-23 and differentiate into a pro-inflammatory subset (γδT17) that produces IL-17 [29]. Therefore, the observed increase in IL-23 may contribute to the enhanced accumulation of γδ T cells and the upregulation of IL-17 production in the thymus.

Previous studies have reported increased serum levels of IL-23 and IL-17 in IMQ-induced psoriasis model mice and in IL-23–induced psoriasis-like mouse models [7]. High serum levels of IL-23 and IL-17 have also been observed in psoriasis patients [30]. These findings are consistent with the results of the present study.

In IMQ-induced psoriasis model mice, strenuous exercise was associated with angiogenesis in the subacromial bursa, expansion of the uncalcified fibrocartilage region, and increased expression of MMP-13 in the shoulder enthesis suggesting that strenuous exercise exacerbates enthesitis in psoriasis. These findings are consistent with previous studies demonstrating that excessive mechanical loading influences enthesis structure and inflammatory status. Although γδ T cell infiltration was not markedly increased in the enthesis (Figure S1), systemic elevations in IL-17, IL-23, and TNF-α may indirectly contribute to enthesis pathology.

FIGURE 3 | (a) Heatmaps of serum cytokine levels. (b) Comparison of the serum concentrations of IL-17, TNF-α, and IL-23. Abbreviation: conc, concentration. (c) Representative images of HE staining of shoulder tissue. Black arrowheads indicate areas of angiogenesis (scale bars = 50 μm). (d) Representative images of toluidine blue and picrosirius red staining of shoulder tissue (scale bars = 100 μm). (e) Representative immunohistochemistry images showing TNF-α and MMP-13 expression in shoulder tissue (scale bars = 20 μm). (f) Comparison of the modified histological scores for angiogenesis in the subacromial bursa. (g) Comparison of the ratio of UFC area to total FC area. Abbreviations: FC, fibrocartilage; UFC, uncalcified fibrocartilage. (h) Comparison of TNF-α– and MMP-13–positive cell ratios in the enthesis. Data are presented as the mean with 95% confidence interval (CI). Each experimental group consisted of $n = 5$ mice. Statistical comparisons were performed among all four groups (IMQ − TRED−, IMQ − TRED+, IMQ + TRED−, and IMQ + TRED+) using one-way ANOVA followed by Tukey's multiple comparisons test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

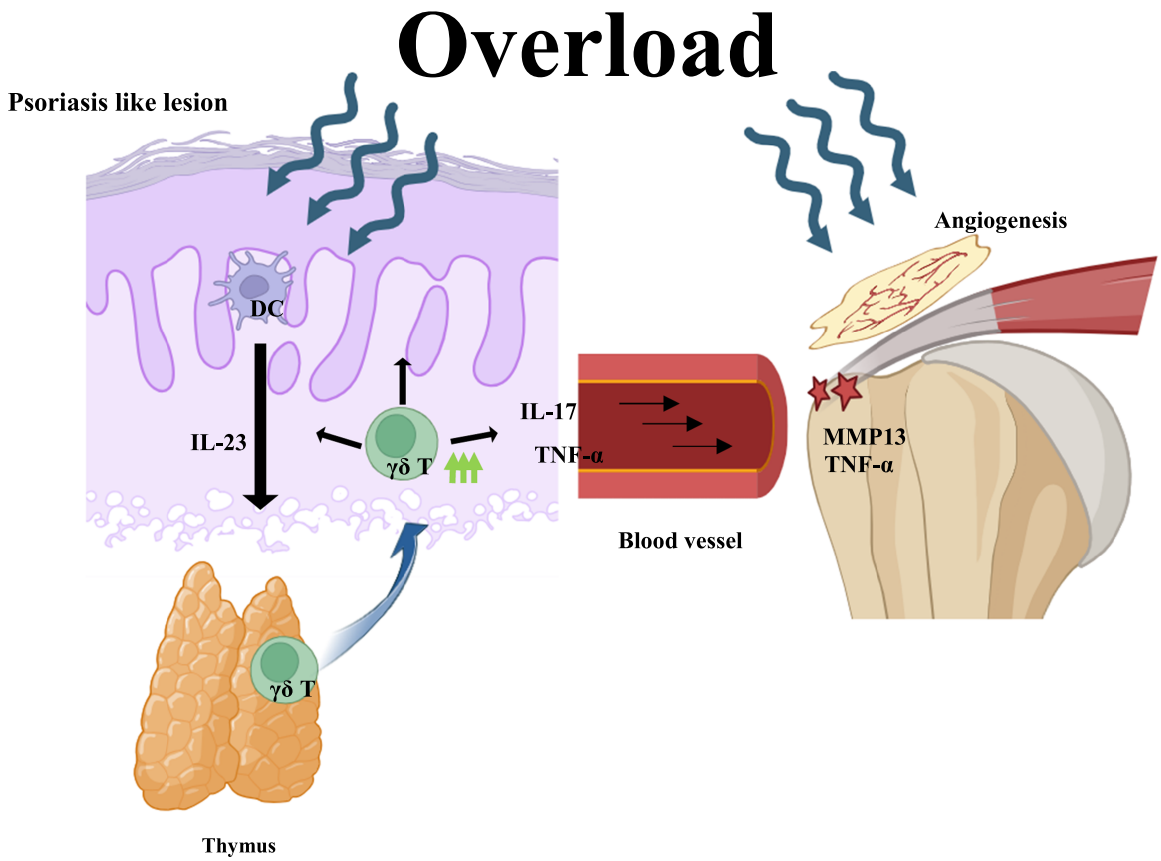

FIGURE 4 | Schematic representation of the mechanism underlying the effect of increased strenuous exercise on IMQ-induced psoriasis model mice. Created with http://BioRender.com. Abbreviations: γδ T, gamma delta T cell; DC, dendritic cell.

Based on findings of this study and previous research, we propose a schematic model illustrating a possible link between strenuous exercise–associated cutaneous inflammation, systemic immune modulation, and enthesis pathology (Figure 4).

This study has some limitations. First, our current IMQ-induced psoriasis model uses mice. Thus, the effects of hard exercise on γδ T cell populations in human psoriasis and the pathogenesis of strenuous exercise-induced γδ T cell accumulation in the skin have not been fully characterized. Moreover, the present study was not long term. Several animal studies have shown that long-term mild to moderate exercise exerts anti-inflammatory effects [31, 32]. Four months of voluntary wheel running in mice decreased intestinal lymphocyte expression of TNF-α and other inflammatory mediators [31]. In older mice, 10–14 weeks of voluntary aerobic exercise reversed age-associated arterial inflammation, normalizing NF-κB activation and macrophage infiltration [32]. These findings suggest that some favorable effects of physical exercise generally appear after longer periods of adaptation rather than in the short term. Because the present study evaluated only a 4-week protocol, the potential beneficial effects associated with longer-term exercise adaptation could not be assessed. However, most previous studies demonstrating anti-inflammatory effects utilized mild to moderate workloads, whereas the present protocol involved high-intensity downhill treadmill running, which imposes substantial mechanical loading stress. Strenuous exercise has been reported to exacerbate tissue inflammation and worsen enthesitis in murine models [33, 34]. Therefore, the exacerbation of psoriatic pathology and enthesis alterations observed in our study may be specifically attributable to the high-intensity nature of the workload.

In this study, biochemical and histological alterations induced by strenuous exercise were observed not only at the enthesis but also in the skin, thymus, spleen, and serum. So this research provides valuable insights regarding the mechanism underlying psoriasis that could lead to the development of new therapies. However, there are still many unknowns in the pathology of psoriasis and its relationship with mechanical stress. Thus, further studies are needed to explore how strenuous exercise and mechanical stress stimulate IL-23 production and affect γδ T cell development.

Author Contributions

Tomoki Furutani: formal analysis, writing – original draft. **Taichi Saito:** conceptualization, writing – original draft, writing – review and editing. **Asahi Ikeda:** data curation. **Kenta Mashima:** data curation. **Natsumi Yukihiro:** investigation. **Satoki Kusakabe:** investigation. **Ryo Nakamichi:** conceptualization. **Aki Yoshida:** formal analysis. **Keiichiro Nishida:** conceptualization. **Toshifumi Ozaki:** writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated in the present study are available from the authors upon reasonable request.

Transparency Statement

The lead author Taichi Saito affirms that this manuscript is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary figure: Representative immunohistochemistry images showing γδ T cell infiltration in shoulder tissue (scale bar = 50 μm).