Allergy and Immunology

AIMS Allergy and Immunology, 8(4): 265–282.
DOI: 10.3934/Allergy.2024016
Received: 11 October 2024
Revised: 08 November 2024
Accepted: 20 November 2024
Published: 25 November 2024

https://www.aimspress.com/journal/Allergy

Research article

Serum cytokine concentrations correlate with the severity of imiquimod and IL-23-induced psoriatic inflammation in mice

Izabela Krzemień, Angelika Szatan, Paulina Skalska, Martyna Cieślik, Angelika Domagała, Magdalena Gębicka, Krzysztof Bryniarski and Katarzyna Nazimek*

Department of Immunology, Jagiellonian University Medical College, 18 Czysta St., 31-121 Krakow, Poland

* **Correspondence:** Email: katarzyna.nazimek@uj.edu.pl; Tel: +48126325865; Fax: +48126339431.

Abstract: Plaque psoriasis is dominated by a T helper cell-mediated autoimmune response, while interleukin (IL)-36 and neutrophil-induced autoinflammation underlies generalized pustular psoriasis; these pathomechanisms are characterized by similar dysregulation of cytokine circuits. Thus, monitoring serum cytokine concentrations seems to have a great diagnostic value. However, the exact relationship between serum cytokine levels and disease severity in psoriatic patients is not established, and no data are available on this relationship in mouse models of psoriasis. Therefore, we aimed to analyze the correlation between serum cytokine concentrations and severity of psoriatic inflammation induced in female C57BL/6 mice with either imiquimod (IMQ) or IL-23. To better reflect the heterogeneity of the systemic course of psoriasis, we diversified the study mouse population by jointly analyzing both models at two different time points and by calculating the cumulative score that simultaneously described the modified psoriasis area severity index (mPASI), ear swelling response, and splenomegaly index for each mouse separately. Compared to intradermally injected IL-23, topically administered IMQ causes significantly more severe psoriatic inflammation characterized by higher serum cytokine levels, mPASI scores, and ear swelling response together with spleen enlargement. Accordingly, serum concentrations of tumor necrosis factor alpha (TNFα) and p40 subunit of IL-12 and IL-23 positively correlated with disease severity, while IL-6 showed a negative correlation. Moreover, the mPASI value was not the only predictor of serum levels of these cytokines, which highlights the importance of assessing other clinical and laboratory parameters in psoriasis monitoring. This approach would enable a comprehensive analysis of systemic autoinflammatory and autoimmune reactions in psoriasis, determine the risk of complications and comorbidities, and select targeted therapy.

Keywords: autoimmunity; autoinflammation; cytokines; disease severity; imiquimod; IL-23; mouse models of psoriasis; PASI; pro-inflammatory cytokines; rodent models of psoriasis

1. Introduction

Psoriasis is a very complex systemic disease with an extremely heterogenous course. As reviewed elsewhere [1,2], helper T (Th) cell-dependent autoimmune response underlies plaque psoriasis, while interleukin (IL)-36 and neutrophil-driven autoinflammation is a main cause of generalized pustular psoriasis, and both extreme phenotypes are linked by cytokine network dysregulation. However, intermediate phenotypes of the disease predominate in the human population, disease course is heterogeneous in a given patient, and the immune-mediated pathogenesis has some important gaps [3]. This causes difficulties in diagnosis and assessment of the effectiveness of therapy, justifying the search for easily accessible markers with significant laboratory and diagnostic value. Monitoring of serum concentration of cytokines involved in psoriasis pathogenesis seems to have a great diagnostic value [4]. However, there is no consensus on the relationship between serum cytokine concentrations and disease severity due to the limited data availability [5].

Psoriasis-underlying immune-mediated pathomechanisms can be readily studied in various animal models [6], including imiquimod (IMQ) and IL-23-induced mouse models. Because there are significant knowledge gaps in the ability to monitor disease severity in mouse psoriatic inflammation using readily available laboratory methods, we aim to analyze the correlation between serum cytokine concentrations and disease severity in two commonly used mouse models of psoriasis.

The great advantage of animal models is the ability to study the pathomechanisms of a given disease without the influence of confounding factors commonly found in the human population, such as medical history and comorbidities as well as demographic, life-style-related and environmental factors. Moreover, inbred mice with identical genotype, microbiota composition, and breeding conditions show significantly reduced inter-individual variability in response to the model reaction trigger. On the other hand, these undoubted advantages limit the transfer of observations made in animal models directly to the clinical conditions. Therefore, in this study, we diversified the studied mouse population by inducing psoriasis with IMQ or IL-23 (differentiation by disease severity) at different numbers of administered doses (differentiation by disease duration).

2. Materials and methods

2.1. Laboratory animals

Ten to twelve-week-old female mice of the inbred C57BL/6 strain were obtained from the 2nd Animal Facility of the Faculty of Medicine, Jagiellonian University Medical College (Krakow, Poland), and kept under standard conditions with unrestricted access to autoclaved chow and water. Mice were randomly assigned to two treatment groups of 7–8 individuals.

2.1.1. Ethics approval of research

We followed the ARRIVE guidelines 2.0 and the study was approved by the 1st Local Ethics Committee in Krakow (approval number 659/2022).

2.2. IMQ-induced psoriatic inflammation

The dorsal skin of the mice was shaved under isoflurane anesthesia, and from the next day, the mice were topically treated with 62.5 mg of commercially available 5% Aldara cream (MEDA Pharmaceuticals, Solna, Sweden) on both sides of both ears and on the shaved dorsal skin using a glass spatula. The dose of cream used contains 3.125 mg of pure IMQ per mouse. The treatment lasted for 4 days (the peak) or 7 days (the endpoint).

2.3. IL-23-induced psoriatic inflammation

The mice were intradermally injected in both ears with 10 μL of 25 μg/mL DPBS-suspension of mouse recombinant IL-23 (BioLegend, San Diego, CA, USA, 250 ng of IL-23 per ear) using 30-gauge needle, under isoflurane anesthesia. The mice were treated every other day with a total of seven (the peak) or ten (the endpoint) doses of IL-23.

2.4. Assessment of psoriatic inflammation, ear swelling measurement, and splenomegaly evaluation

At the peak or endpoint of the experiment, the severity of psoriatic lesions on the ear skin was assessed macroscopically using the modified psoriasis area severity index (mPASI) scoring [7], taking into account the individual assessment of the following parameters: Erythema (redness), induration (thickness), and desquamation (scaling). Each parameter was assessed on a 0–4 scale (0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked), and points were summed to determine the total score for each mouse.

At the peak or endpoint, the ear thickness of each mouse was measured in a blinded manner using an engineer's micrometer (Mitutoyo, Tokyo, Japan), and the resulting ear swelling response was calculated by subtracting the background ear thickness measured before beginning of the treatment from the measure.

Spleens were individually collected from each sacrificed mouse and weighed on an electronic analytical balance. To calculate the splenomegaly index [8], spleen weight (in grams) was divided by the body weight of the mouse donor (in grams). A result equal to or greater than 0.005 was considered to indicate splenomegaly.

Since psoriasis is a systemic disease, we decided to calculate the cumulative score that simultaneously describes the mPASI value, ear swelling response, and splenomegaly index for each mouse separately, according to the selected criteria, as described below. Moreover, in order to diversify the studied population in terms of the duration and severity of psoriatic lesions and, consequently, to more accurately reflect the clinical situation, the experiment was completed at two time points, and the analysis was performed for all subjects as a whole and after division, according to the research model.

2.5. Collection of blood sera and cytokine measurements

At the peak or endpoint of the experiment, blood was individually collected from each sacrificed mouse, and standardly separated blood sera were individually stored at −80 °C before cytokine measurements. In addition, sera from four naive mouse littermates were collected for measurements of background cytokine concentrations.

The concentration of cytokines in the serum of each mouse was determined separately, according to the manufacturer's procedures, using ELISA kits designed to measure: IL-6 (catalog number 555240, BD Biosciences, San Jose, CA, USA), as well as TNFα (catalog number 88-7324-88), IL-12/IL-23p40 (catalog number 88-7120-22), IFNγ (catalog number 88-7314-22), and IL-17A (catalog number BMS 6001) from Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA.

2.6. Statistical analysis

All statistical analyses were performed with the use of GraphPad Prism 8 (San Diego, CA, USA). The Shapiro-Wilk test was used to test the normality of the distribution of variables. Differences in mean cytokine concentrations as well as in mean values of mPASI score, ear swelling response, splenomegaly index, and cumulative score between both treatment groups were estimated using Student's t-test. The Spearman rank correlation coefficient (r) was calculated to evaluate the relationships between the dependent variable (cytokine concentration) and the independent variable (cumulative score). A multiple linear regression model (least squares) was used to find predictors (covariate variables) of cytokine concentration (outcome variable). In each case, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Only the serum concentration of TNFα differs significantly between the studied models

Initial comparison of both psoriasis models in terms of serum cytokine concentration showed that IL-6 level was undetectable in IL-23-treated animals at both time points, i.e., peak and endpoint of the reaction and was decreased at the endpoint of the IMQ-induced reaction compared to the peak (Figure 1A). Of note, the mean serum TNFα concentration was significantly higher in IMQ-exposed mice than in their IL-23-treated littermates (Figure 1B), while the levels of IL-12/IL-23p40, IFNγ and IL-17A were similar in both mouse populations (Figure 1C–E). Moreover, serum concentrations of IL-6 and TNFα were significantly higher in IMQ-treated mice than in naive littermates, and, similarly, both psoriatic models were characterized by increased serum levels of IL-12/IL-23p40, IFNγ, and IL-17A compared to untreated animals (Figure 1F).

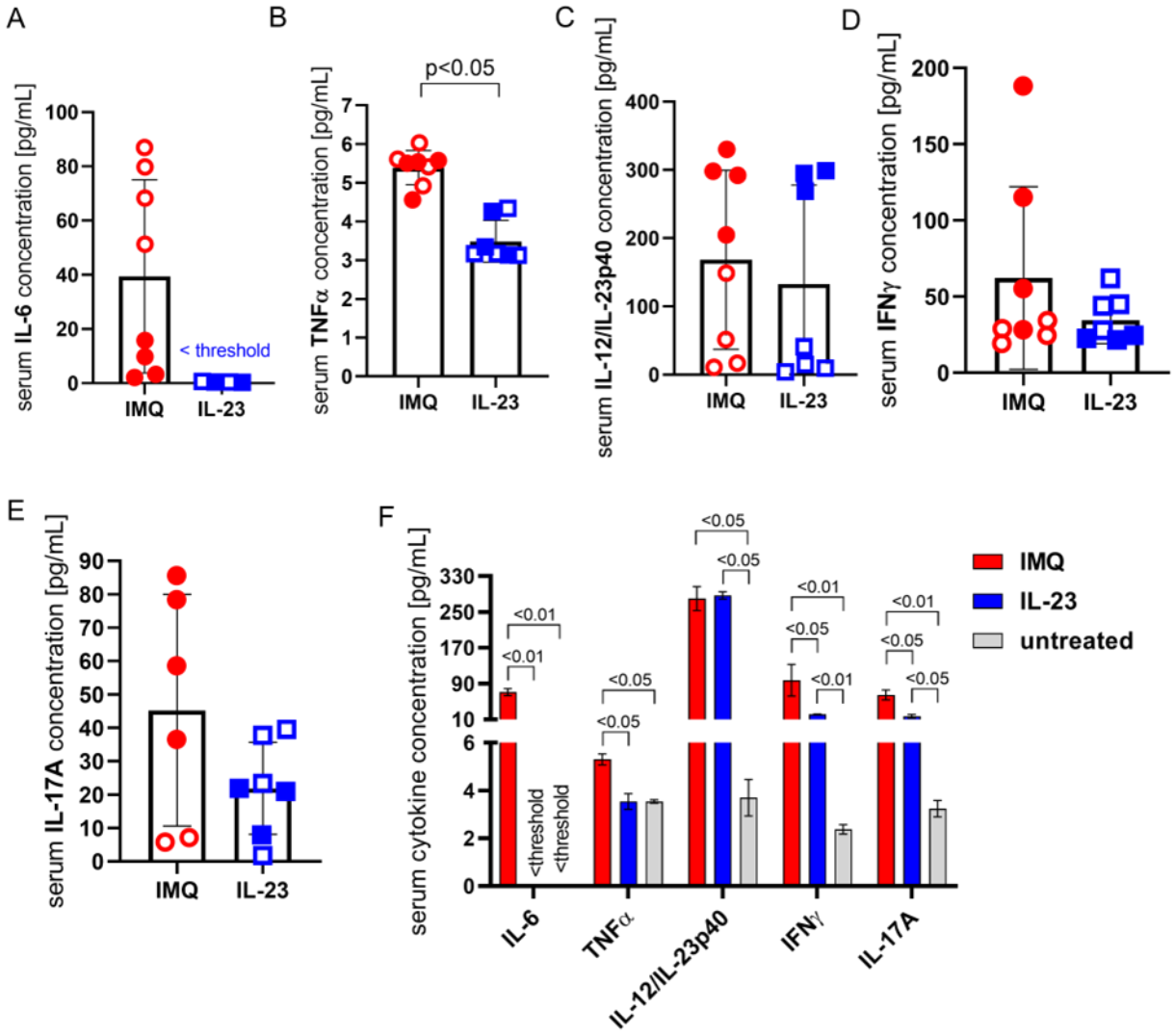
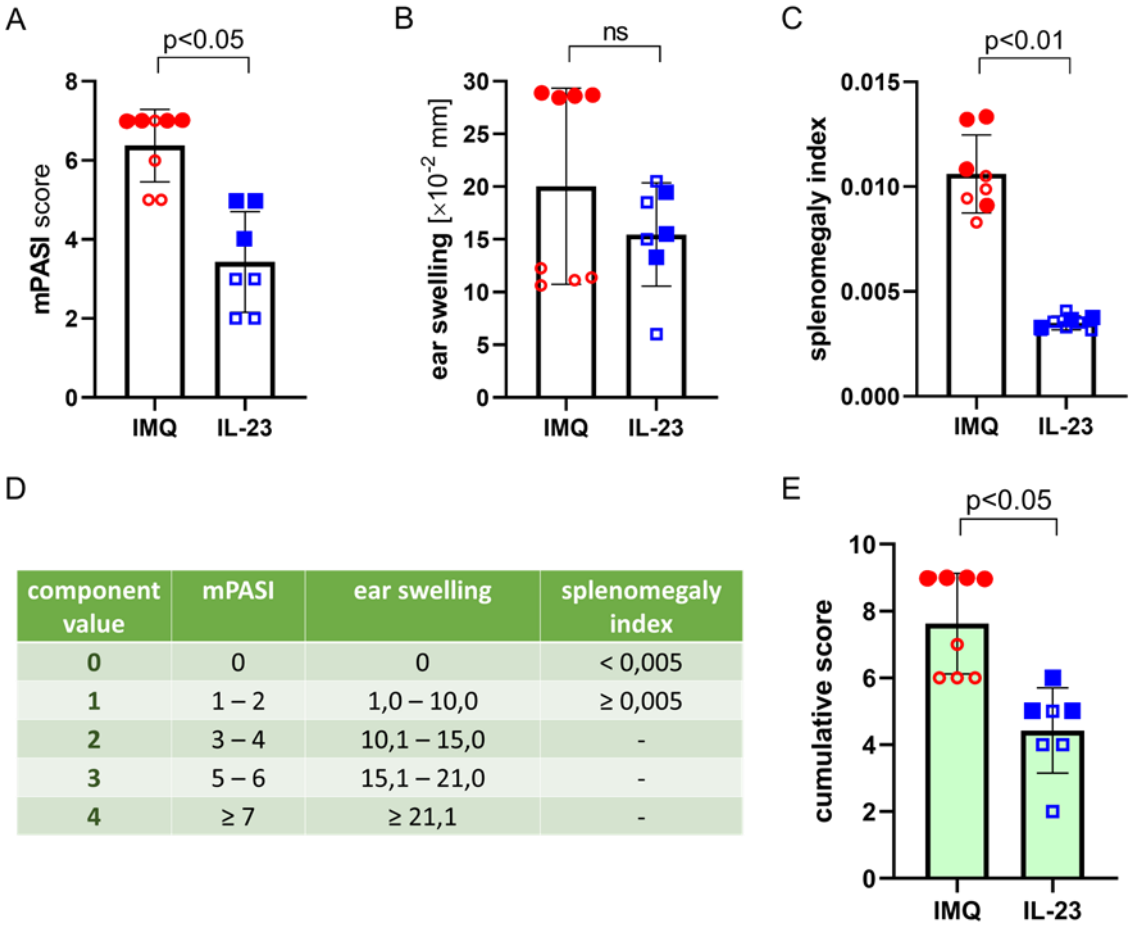


Figure 1. Serum cytokine concentrations in IMQ and IL-23-treated female C57BL/6 mice. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Serum concentrations of IL-6 (A), TNFα (B), p40, a common subunit of IL-12 and IL-23 (C), IFNγ (D), and IL-17A (E), were measured in the serum of each animal separately and statistically analyzed using the Student's t-test. (F) Serum concentrations of tested cytokines compared in animals treated with seven doses of IMQ, ten doses of IL-23 or in untreated control mice and statistically analyzed using ANOVA with post hoc Tukey's test. Results are expressed as mean ± standard deviation (SD).

3.2. IMQ-induced psoriatic inflammation is more severe than the IL-23-induced reaction

Further comparison of both models studied demonstrated that IMQ-exposed mice develop more severe ear skin lesions than their IL-23-treated littermates, as reflected in significantly higher mean mPASI scores (Figure 2A), and enhanced ear swelling at the endpoint of psoriatic inflammation (Figure 2B). In

addition, IMQ treatment resulted in spleen enlargement, expressed by a significant increase in splenomegaly index (Figure 2C). To more accurately reflect the systemic nature of psoriasis, a cumulative score, simultaneously describing the mPASI value, ear swelling response, and splenomegaly index, was calculated for each mouse separately, according to the criteria presented in Figure 2D, and its mean value differed greatly between IMQ and IL-23-induced psoriasis models (Figure 2E).



component value	mPASI	ear swelling	splenomegaly index
0	0	0	< 0,005
1	1 – 2	1,0 – 10,0	≥ 0,005
2	3 – 4	10,1 – 15,0	-
3	5 – 6	15,1 – 21,0	-
4	≥ 7	≥ 21,1	-

Figure 2. Intensity of psoriatic inflammation induced in female C57BL/6 mice by IMQ or IL-23 administration. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Then, modified PASI score (A), ear swelling response (B), and splenomegaly index (C) were assessed for each animal separately, which allowed us to establish criteria for calculating the cumulative score (D), the average value of which was compared in both models (E). Results are expressed as mean ± standard deviation (SD), and were statistically analyzed using the Student's t-test.

3.3. Serum concentrations of IL-6, TNFα and IL-12/IL-23p40, but not IFNγ and IL-17A, correlate with psoriasis severity

To the best of our knowledge, IMQ- and IL-23-induced psoriatic inflammation models have not

been analyzed for correlations between serum cytokine concentrations and disease severity. In most studies, the latter is assessed using only the mPASI scoring system, while the calculated cumulative score combines mPASI values, ear swelling, and splenomegaly index, which reflects the intensity of psoriatic inflammation in animals more broadly. Furthermore, as mentioned above, the chosen study design allowed us to differentiate the mouse population in terms of disease severity and duration to mirror clinical conditions.

Under current experimental conditions, we observed a statistically significant negative correlation between serum IL-6 concentration and cumulative score in the total mouse population as well as in IMQ-exposed animals, whereas IL-23-treated littermates had undetectable serum IL-6 levels (Figure 3). Conversely, serum concentration of TNFα positively correlated with the cumulative score in the total mouse population, whereas in a separate analysis the opposite trend was observed (Figure 4).

Moreover, a strong positive correlation was found between serum concentration of p40, a common subunit of IL-12 and IL-23, and cumulative score in the total mouse population as well as in both models of psoriatic inflammation (Figure 5). Similarly, in the case of IFNγ (Figure 6) and IL-17A (Figure 7), we observed the positive correlation between serum cytokine concentration in the total mouse population (Figures 6A and 7A). However, a separate analysis revealed a positive correlation between cytokine concentrations and cumulative score in IMQ-exposed animals, whereas these variables correlated negatively in IL-23-treated littermates (Figures 6B and 7B).

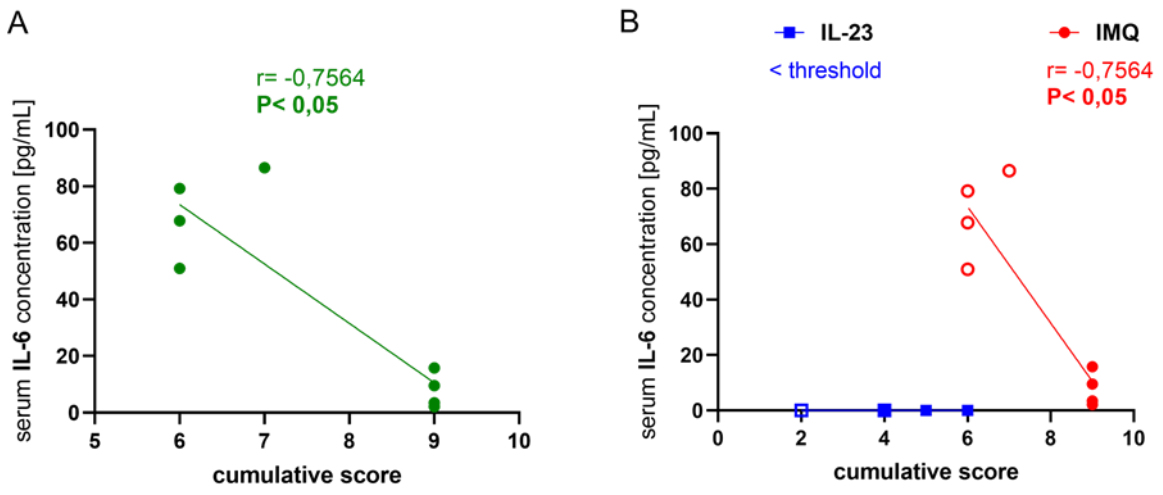


Figure 3. Correlation of serum IL-6 concentration and disease severity in female C57BL/6 mice treated with IMQ or IL-23. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Then, mPASI score, ear swelling response, and splenomegaly index were assessed to calculate the cumulative score for each animal separately, and blood sera were collected to measure cytokine concentration. The Spearman rank correlation was used to evaluate the relationships between serum IL-6 concentration and cumulative score in the total mouse population (A), and in both psoriasis models separately (B).

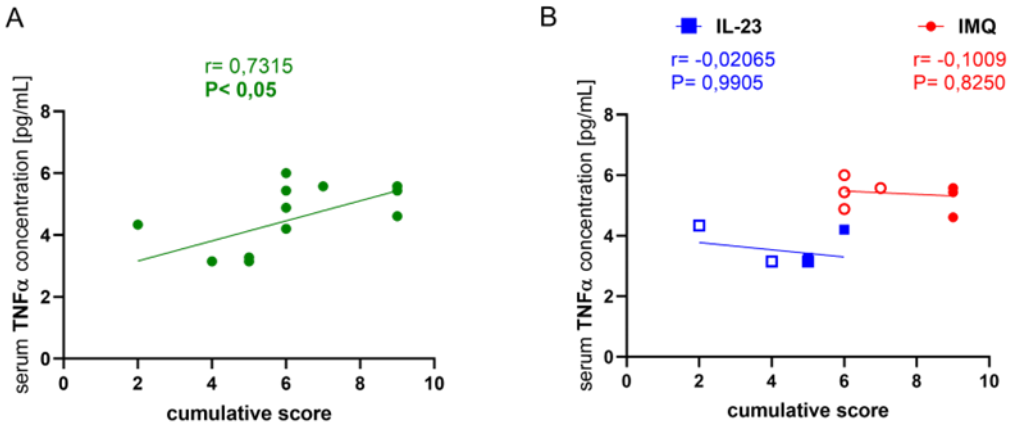


Figure 4. Correlation of serum TNFα concentration and disease severity in female C57BL/6 mice treated with IMQ or IL-23. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Then, mPASI score, ear swelling response, and splenomegaly index were assessed to calculate the cumulative score for each animal separately, and blood sera were collected to measure cytokine concentration. The Spearman rank correlation was used to evaluate the relationships between serum TNFα concentration and cumulative score in the total mouse population (A), and in both psoriasis models separately (B).

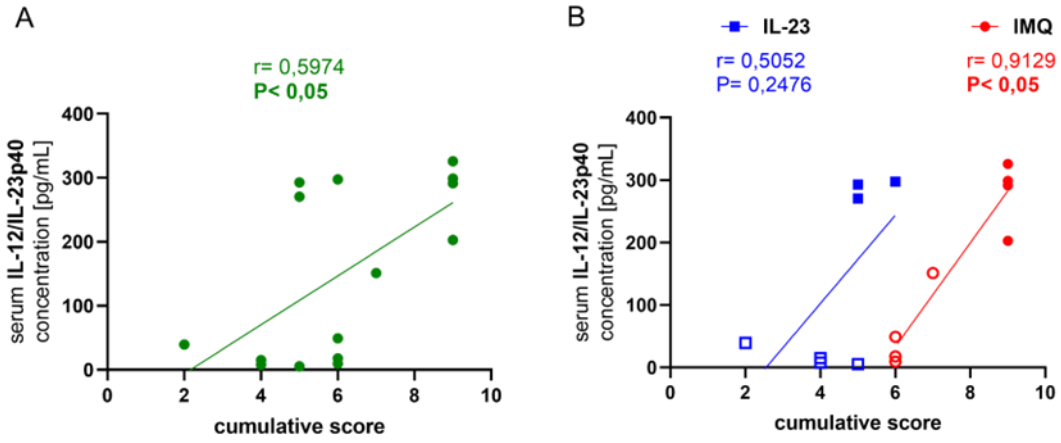


Figure 5. Correlation of serum concentration of IL-12/IL-23p40 and disease severity in female C57BL/6 mice treated with IMQ or IL-23. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Then, mPASI score, ear swelling response, and splenomegaly index were assessed to calculate the cumulative score for each animal separately, and blood sera were collected to measure cytokine concentration. The Spearman rank correlation was used to evaluate the relationships between serum concentration of IL-12/IL-23p40 and cumulative score in the total mouse population (A), and in both psoriasis models separately (B).

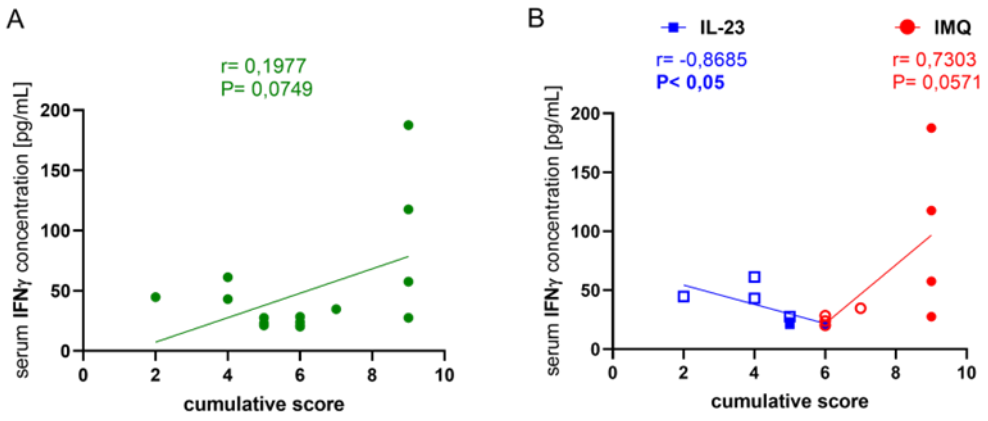


Figure 6. Correlation of serum IFNγ concentration and disease severity in female C57BL/6 mice treated with IMQ or IL-23. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Then, mPASI score, ear swelling response, and splenomegaly index were assessed to calculate the cumulative score for each animal separately, and blood sera were collected to measure cytokine concentration. The Spearman rank correlation was used to evaluate the relationships between serum IFNγ concentration and cumulative score in the total mouse population (A), and in both psoriasis models separately (B).

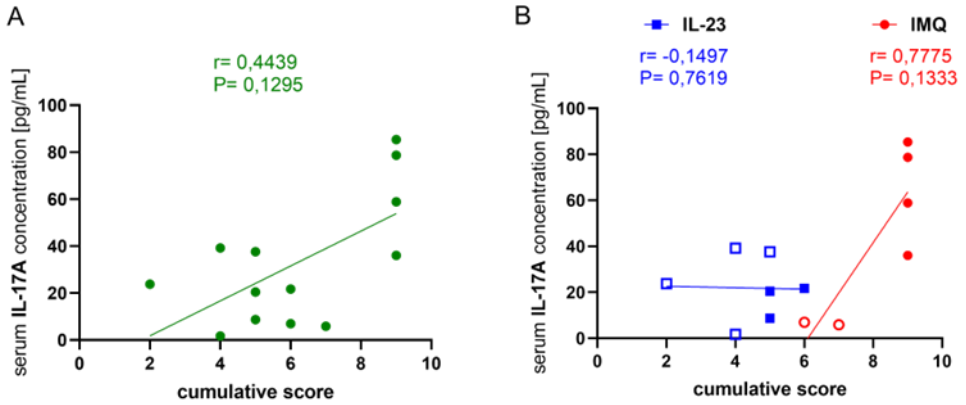


Figure 7. Correlation of serum IL-17A concentration and disease severity in female C57BL/6 mice treated with IMQ or IL-23. Mice were either topically administered with four (the peak, open red circles) or seven (the endpoint, solid red circles) doses of IMQ, or intradermally injected with seven (the peak, open blue squares) or ten (the endpoint, solid blue squares) doses of IL-23. Then, mPASI score, ear swelling response, and splenomegaly index were assessed to calculate the cumulative score for each animal separately, and blood sera were collected to measure cytokine concentration. The Spearman rank correlation was used to evaluate the relationships between serum IL-17A concentration and cumulative score in the total mouse population (A), and in both psoriasis models separately (B).

3.4. Serum concentrations of IL-6, TNFα and IL-12/IL-23p40 are predicted by different variables

In the last stage of analysis, we used a multiple linear regression model to find predictors of serum concentration of each assessed cytokine. Namely, serum IL-6 concentration was determined by the mPASI score and ear swelling, serum TNFα concentration was determined by ear swelling and the value of the splenomegaly index, while ear swelling response determined the serum concentration of the p40 subunit shared by IL-12 and IL-23 (Table 1).

Table 1. Estimation of the impact of individual components of the cumulative score on the serum concentrations of selected cytokines carried out using a multiple linear regression model.

dependent variable	independent variable	t-test	P value
serum IL-6 concentration	mPASI	4.186	0.0138
	ear swelling	9.463	0.0007
	splenomegaly index	1.114	0.3277
serum TNFα concentration	mPASI	1.050	0.3165
	ear swelling	2.209	0.0493
	splenomegaly index	3.756	0.0032
serum IL-12/IL-23p40 concentration	mPASI	1.899	0.0841
	ear swelling	2.807	0.0171
	splenomegaly index	2.054	0.0645
serum IFNγ concentration	mPASI	0.2539	0.8042
	ear swelling	0.6719	0.5155
	splenomegaly index	1.828	0.0948
serum IL-17A concentration	mPASI	0.3191	0.7569
	ear swelling	1.026	0.3319
	splenomegaly index	1.899	0.0901

4. Discussion

Despite obvious differences between species that do not allow a full reflection of the pathogenesis of psoriasis in humans, mouse models are commonly used to study the complex pathomechanism of the disease [9]. Among various mouse strains, genomic studies revealed that IMQ-induced reaction in C57BL/6 mice best reflects the histopathology of human skin psoriatic lesions [10]. Similar plaque changes, but accompanied by a milder inflammatory reaction, can also be induced by intradermal injection of IL-23 [11,12], and, in both cases, the activated cytokine circuits are analogous to those that cause skin pathology in psoriasis patients [13]. Therefore, intradermal injection of IL-23 and topical administration of IMQ have become the most commonly used methods to induce acute psoriatic inflammation in laboratory mice [6].

In mice, IMQ-induced psoriatic inflammation is triggered by TLR7/NF-κB and/or

inflammasome-dependent signaling, which stimulates the secretion of proinflammatory cytokines by keratinocytes and peritoneal macrophages [14–16]. This causes the proinflammatory activation of skin-resident macrophages and dendritic cells (DCs) as well as infiltration of the epidermis by neutrophils and monocytes, which amplifies local acute inflammatory reaction and leads to epidermal hyperplasia and keratinocyte death accompanied by the release of antimicrobial peptides (AMPs) [14,17]. As a result, keratinocytes become an important source of autoantigens that trigger the autoimmune component of psoriatic inflammation, involving activation of Th17, Th22, and Th1 lymphocytes [18], by macrophage and DC-derived IL-23 and IL-12, respectively. Since IL-12 plays a dual role in psoriatic inflammation [19], Th1 lymphocytes can also be activated by IFNα derived by plasmacytoid DCs [18]. Interestingly, researchers uncovered that immune cell-released granzyme K stimulates macrophages to secrete IL-23 [20], which begins early after initial IMQ application and peaks approximately 72 hours later [14]. At that time, Th17 lymphocytes together with γδ T cells produce large amounts of IL-17A and other IL-17 family members [14,21], while Th22 lymphocytes deliver IL-22 to synergistically amplify the effector immune response and resulting epidermal pathology [14,22]. Furthermore, Th1 lymphocyte-derived IFNγ drives Th17 cell activity [23]. Apart from local cutaneous inflammation, systemic response is activated by cytokine-secreting peritoneal macrophages [14]. Interestingly, researchers found a unique phenotype induced by IL-23 treatment in peritoneal macrophages of C57BL/6 mice. These IL-23-treated macrophages were shown to release large amounts of IFNγ, IL-17A, and IL-22 [24], which seems to systemically amplify psoriatic immune response. Although it is unclear how peritoneal macrophages contribute to systemic complications of psoriatic inflammation [25,26], they seem to migrate to other tissues and organs in a CX3CR1 and CX3CL1-dependent manner [27]. In turn, macrophages can activate T cells in draining lymph nodes, and the presence of germinal centers in lymph nodes recently observed in mice with psoriatic inflammation has been associated with the systemic nature and severity of the disease [13]. Additionally, the number of macrophages increases in the enlarged spleens of animals treated with IMQ. However, the relationship between IMQ, psoriatic inflammation and systemic complications, including splenomegaly, dehydration, and transient weight loss, remains unclear [28]. Especially since the severity of systemic complications in IMQ-treated mice are significantly enhanced by IMQ ingestion [29]. In addition, robust systemic inflammation along with the tachyphylaxis effect of IMQ prevent long-term observations, therefore development of comorbidities cannot be observed in the IMQ-induced model [29]. It is also worth noting that IMQ-induced reaction in mice is strain-dependent, and detail comparative studies confirmed that immune mechanisms induced in C57BL/6 strain were most consistent with those observed in psoriatic patients and best reflected the histopathology of skin plaque lesions [10], including epidermal hyperplasia, neo-angiogenesis, erythema, scaling, as well as inflammatory infiltrate and skin barrier dysfunction [13]. In addition, C57BL/6 mice develop less severe epidermal hyperplasia than BALB/c mice, which seems to result from reduced activity of Th22 lymphocytes [14]. Therefore, the selection of the C57BL/6 strain appears to be best suited for the study of IMQ-induced psoriatic inflammation and the application of the "3 Rs" principle to ensure animal welfare.

Along these lines, IL-23-induced psoriatic inflammation is much milder than IMQ-induced inflammation. This is very likely due to the fact that intradermally injected IL-23 directly activates the immune response of effector T cells, bypassing the activation of TLR and inflammasome-dependent pathways. Moreover, this allows to induce chronic inflammatory reaction in mice that results in the development of comorbidities, including psoriatic arthritis, in IL-23-treated mice [6,30]. IL-23 is

mostly delivered by macrophages and DCs and polarizes $CD4^+$ T lymphocytes towards Th17 phenotype [22]. In addition, IL-23 was shown to activate Th17-like regulatory T lymphocytes that seem to drive psoriatic inflammation [31] together with IL-17A-releasing γδ T cells [32] as well as IL-22-producing Th22 lymphocytes [33]. Consequently, IL-17A and IL-22 are the major downstream mediators of psoriasis-related epidermal pathology [34], and their effector action was suggested to be tightly controlled by IL-6 [35]. On the other hand, some studies implied that IL-23-induced epidermal hyperplasia is mediated by TNFα [36], which suggests the existence of an IL-17-independent pathway involved in the development of psoriatic skin lesions and confirms the downstream effector activity of TNFα in psoriatic inflammation. These observations provide evidence for the involvement of Th1 lymphocytes in the pathogenesis of psoriasis by promoting epidermal injury through the production of TNFα and IFNγ [23]. Moreover, recent studies revealed the possibility of inducing Th1-like lymphocytes by IL-23R-dependent signaling in autoimmune conditions [37]. Altogether, these findings confirm that IL-23 potently induces an autoimmune response that involves Th17, Th22, Th1 lymphocytes and γδ T cells together with corresponding cytokine circuits in psoriatic mice. However, to the best of our knowledge, both models have not been examined for the correlation between serum cytokine concentrations and disease severity.

Cytokines involved in mouse psoriatic inflammation can be classified as inductors of local and systemic inflammation (innate immune cell and keratinocyte-derived IL-1β, IL-6 and TNFα), T cell activators (antigen presenting cell-derived IL-12 and, especially, IL-23), as well as effector mediators (T cell-derived members of IL-17 family, IL-22, IFNγ and, to some extent, TNFα) [23]. We have currently focused on the most representative cytokines from these groups, and observed that serum IL-6 concentration was undetectable and TNFα level was significantly lower in animals treated with IL-23, while serum concentrations of IL-12/IL-23p40, IFNγ and IL-17A were comparable between both models, but higher than in untreated littermates (Figure 1). Moreover, our current research findings proved that IL-23 induces milder psoriatic inflammation than IMQ, as expressed by lower mPASI scores, reduced ear swelling and the lack of spleen enlargement that is typical for IMQ-exposed animals (Figure 2). Collectively, this resulted in the significantly lower values of the calculated cumulative score (Figure 2), which has been correlated with serum cytokine concentrations.

Although some Authors reported the positive correlation of serum IL-6 concentration with PASI score in psoriatic patients [38,39], under current experimental conditions, serum IL-6 concentration correlated negatively with disease severity and was higher at the peak of IMQ-induced reaction (Figure 3). Interestingly, similar negative correlation was observed between serum IL-6 levels and Nail Psoriasis Severity Index scores in Polish cohort of psoriatic patients. In addition, a median concentration of this cytokine was higher in patients without psoriatic arthritis [40], collectively suggesting that elevated IL-6 levels characterize earlier stages of the disease and likely its severe relapses [41]. Thus, one can assume that the rapidly released and endocrine-acting IL-6 triggers psoriatic inflammation and is then gradually consumed during the effector response [35].

Conversely, we have shown that serum TNFα concentration is positively correlated with disease severity in the total mouse population. However, separate analysis revealed weak negative correlation in both, IMQ and IL-23-treated mice (Figure 4). Analogous weak negative correlation between serum TNFα level and PASI score was found in Turkish patients [42], while Japanese and Chinese studies demonstrated a positive correlation with PASI score and disease course [39,43,44]. These discrepancies seem to result from the fact that TNFα concentration depends on the type of psoriasis. Specifically, it was found to be higher in patients with pustular psoriasis than in patients with plaque

psoriasis [45]. On the other hand, during the course of psoriatic inflammation, this cytokine is released not only by macrophages, DCs and keratinocytes, but also by Th1 lymphocytes, thus playing an inducing and effector roles [23]. In this regard, our results indicate the usefulness of TNFα concentration measurement as a marker of disease severity in a heterogeneous population of psoriatic patients.

Polish research demonstrated that serum IL-12p70 and IL-23 levels negatively correlate with disease duration, but IL-12p70 concentration positively correlates with PASI [40]. Furthermore, previous studies showed significant positive correlation between serum concentration of IL-12/IL-23p40 and PASI in Turkish and Japanese patients [42,43]. The latter findings are in line with currently observed strong positive correlation of serum levels of p40 subunit of IL-12 and IL-23 with disease severity in the total population of female C57BL/6 mice as well as in both models separately (Figure 5). However, the interpretation of this observation is not unambiguous. On the one hand, IL-12 plays an IFNγ-independent role in immune regulation and epithelial homeostasis [19,46]; on the other hand, it promotes the psoriasis-related immune response of IFNγ-secreting Th1 lymphocytes. The latter likely explains the observed better clinical efficacy of Tofacitinib treatment in patients with higher baseline concentrations of IFNγ [47]. Moreover, these findings show that the activity of the IL-12/IFNγ cytokine axis varies across the patient population and may be therapeutically significant in some cases.

Along these lines, serum IFNγ level was positively correlated with PASI score in Turkish and Chinese patients [39,42]. A similar trend was observed in the total mouse population as well as in IMQ-exposed animals, while a strong negative correlation of serum IFNγ level with disease severity was shown in IL-23-treated littermates (Figure 6). This discrepancy suggests that systemic Th1 cell activity persists longer and is more pronounced in the IMQ-induced model. The close interplay between Th1 and Th17 lymphocytes contributes to the pathology of psoriasis through the secretion of effector cytokines, mostly IFNγ and IL-17, respectively [48]. It is worth noting that IFNγ stimulates antigen presenting cells to activate Th17 lymphocytes [49]. Thus, one can assume that increased serum IFNγ levels drive the systemic activity of Th17 lymphocytes in IMQ-exposed mice.

Accordingly, under the experimental conditions, the correlation of serum IL-17A concentration with disease severity (Figure 7) showed the same pattern as for IFNγ (Figure 6), i.e., positive correlation in the total population and in IMQ-exposed mice and negative correlation in IL-23-administered animals. Therefore, one can speculate that IL-23-induced psoriasis is mostly associated with local inflammatory reaction, while IMQ induces systemic inflammation characterized by higher serum levels of effector cytokines.

Furthermore, correlation between serum IL-17 and PASI score was strongly negative for Hindu patients without nail involvement [50], but was shown positive in Turkish and Chinese populations [39,42,44]. Altogether, this inconclusive data confirms that, although dysregulated cytokine networks are responsible for the self-perpetuating nature of psoriatic inflammation [2], peripheral blood testing is not sufficient to determine the exact pattern of cytokines characteristic of individual courses of psoriasis. On the other hand, however, serum TNFα levels appear to most precisely reflect the severity of psoriatic inflammation and skin pathology [45].

It is also worth noting that different independent variables determined serum levels of assayed cytokines (Table 1). Exactly, serum concentration of IL-6 was found to be determined by mPASI score and ear swelling intensity. The latter alone determined serum levels of IL-12/IL-23p40, while together with splenomegaly index value was a predictor of serum TNFα concentration. Interestingly, the PASI score determines the serum IL-6 levels in Polish cohort of psoriatic patients [51]. However, we showed

that the concentration of a given cytokine in the blood serum of laboratory animals is determined by more variables than the mPASI score. It can, therefore, be assumed that the concentrations of individual cytokines in blood serum will reflect the severity and clinical course of psoriasis in a given person when related to a larger number of laboratory and clinical parameters. Such an approach could allow for a more accurate assessment of the risk of developing complications and comorbidities of psoriasis, as well as the selection of more adequate therapy thanks to a more comprehensive analysis of the systemic autoinflammatory-autoimmune reaction in psoriasis. Our observations may therefore encourage physicians to use cytokine measurements to monitor the course of the disease in patients and contribute to a deeper understanding of the pathomechanism of the immune reaction induced by IMQ or IL-23 in laboratory animals.

5. Conclusions

Our study provides new insights into the understanding of the complex interplay between serum cytokine levels and severity of psoriasis in mice that can be translated to clinical settings. We have shown that, in contrast to TNFα, IL-6 levels decrease rapidly in serum, suggesting a more significant role of TNFα in the chronic systemic psoriatic inflammation, which is additionally driven by IFNγ and IL-17A. Importantly, the fact that circulating cytokine concentrations are determined not only by mPASI score highlights the importance of evaluating other clinical and laboratory parameters, including patient's and physician's global assessments, affected body surface area index, as well as serum C-reactive protein level and lipid profile, in psoriasis assessments. Therefore, future detailed studies are needed to establish easily accessible criteria for monitoring psoriasis, predicting therapeutic outcomes, and assessing the risk of comorbidities.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Acknowledgments

We would like to thank Mr. Maciej Panek, MSc, and Mrs. Jolanta Wątor for housing and taking care of the laboratory animals. This work was supported by Polish Ministry of Science and Higher Education under awards number N41/DBS/001026 and N41/DBS/001285 to KN.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

IK performed the experiments, analyzed the data and drafted the manuscript. AS performed the experiments and analyzed the data. PS performed the experiments and ELISA tests. MC, AD and MG participated in the experiments. KB conceived and supervised the study and revised the manuscript.

KN conceived and designed the study, performed the experiments, analyzed the data, drafted the manuscript and received funding. All authors have read and approved the final manuscript.

References

1. Liang Y, Sarkar MK, Tsoi LC, et al. (2017) Psoriasis: A mixed autoimmune and autoinflammatory disease. *Curr Opin Immunol* 49: 1–8. https://doi.org/10.1016/j.coi.2017.07.007
2. Rendon A, Schäkel K (2019) Psoriasis pathogenesis and treatment. *Int J Mol Sci* 20: 1475. https://doi.org/10.3390/ijms20061475
3. Fry L, Baker BS, Powles AV, et al. (2015) Psoriasis is not an autoimmune disease? *Exp Dermatol* 24: 241–244. https://doi.org/10.1111/exd.12572
4. Solberg SM, Sandvik LF, Eidsheim M, et al. (2018) Serum cytokine measurements and biological therapy of psoriasis-Prospects for personalized treatment? *Scand J Immunol* 88: e12725. https://doi.org/10.1111/sji.12725
5. Alsabbagh MM (2024) Cytokines in psoriasis: From pathogenesis to targeted therapy. *Hum Immunol* 85: 110814. https://doi.org/10.1016/j.humimm.2024.110814
6. Gangwar RS, Gudjonsson JE, Ward NL (2022) Mouse models of psoriasis: A comprehensive review. *J Invest Dermatol* 142: 884–897. https://doi.org/10.1016/j.jid.2021.06.019
7. Neu SD, Strzepa A, Martin D, et al. (2021) Myeloperoxidase inhibition ameliorates plaque psoriasis in mice. *Antioxidants (Basel)* 10: 1338. https://doi.org/10.3390/antiox10091338
8. Surcel M, Munteanu A, Huică R, et al. (2019) Reinforcing involvement of NK cells in psoriasiform dermatitis animal model. *Exp Ther Med* 18: 4956–4966. https://doi.org/10.3892/etm.2019.7967
9. Asbóth D, Bánfi B, Kocsis D, et al. (2024) Rodent models of dermatological disorders. *Ital J Dermatol Venerol* 159: 303–317. https://doi.org/10.23736/S2784-8671.23.07700-9
10. Swindell WR, Michaels KA, Sutter AJ, et al. (2017) Imiquimod has strain-dependent effects in mice and does not uniquely model human psoriasis. *Genome Med* 9: 24. https://doi.org/10.1186/s13073-017-0415-3
11. Bocheńska K, Smolińska E, Moskot M, et al. (2017) Models in the research process of psoriasis. *Int J Mol Sci* 18: E2514. https://doi.org/10.3390/ijms18122514
12. Parab S, Doshi G (2023) The experimental animal models in psoriasis research: A comprehensive review. *Int Immunopharmacol* 117: 109897. https://doi.org/10.1016/j.intimp.2023.109897
13. Jabeen M, Boisgard AS, Danoy A, et al. (2020) Advanced characterization of imiquimod-induced psoriasis-like mouse model. *Pharmaceutics* 12: 789. https://doi.org/10.3390/pharmaceutics12090789
14. Flutter B, Nestle FO (2013) TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol* 43: 3138–3146. https://doi.org/10.1002/eji.201343801
15. Keppler M, Straß S, Geiger S, et al. (2023) Imidazoquinolines with improved pharmacokinetic properties induce a high IFNα to TNFα ratio *in vitro* and *in vivo*. *Front Immunol* 14: 1168252. https://doi.org/10.3389/fimmu.2023.1168252
16. Li ZJ, Sohn KC, Choi DK, et al. (2013) Roles of TLR7 in activation of NF-κB signaling of keratinocytes by imiquimod. *PLoS One* 8: e77159. https://doi.org/10.1371/journal.pone.0077159
17. Zhang S, Zhang J, Yu J, et al. (2021) Hyperforin ameliorates imiquimod-induced psoriasis-like murine skin inflammation by modulating IL-17A-producing γδ T cells. *Front Immunol* 12: 635076. https://doi.org/10.3389/fimmu.2021.635076

18. Albanesi C, Madonna S, Gisondi P, et al. (2018) The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. *Front Immunol* 9: 1549. https://doi.org/10.3389/fimmu.2018.01549
19. Kulig P, Musiol S, Freiberger SN, et al. (2016) IL-12 protects from psoriasiform skin inflammation. *Nat Commun* 7: 13466. https://doi.org/10.1038/ncomms13466
20. Richardson KC, Aubert A, Turner CT, et al. (2024) Granzyme K mediates IL-23-dependent inflammation and keratinocyte proliferation in psoriasis. *Front Immunol* 15: 1398120. https://doi.org/10.3389/fimmu.2024.1398120
21. Qi C, Wang Y, Li P, et al. (2021) Gamma delta T cells and their pathogenic role in psoriasis. *Front Immunol* 12: 627139. https://doi.org/10.3389/fimmu.2021.627139
22. Di Cesare A, Di Meglio P, Nestle FO (2009) The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol* 129: 1339–1350. https://doi.org/10.1038/jid.2009.59
23. Hu P, Wang M, Gao H, et al. (2021) The role of helper T cells in psoriasis. *Front Immunol* 12: 788940. https://doi.org/10.3389/fimmu.2021.788940
24. Hou Y, Zhu L, Tian H, et al. (2018) IL-23-induced macrophage polarization and its pathological roles in mice with imiquimod-induced psoriasis. *Protein Cell* 9: 1027–1038. https://doi.org/10.1007/s13238-018-0505-z
25. Kamata M, Tada Y (2022) Dendritic cells and macrophages in the pathogenesis of psoriasis. *Front Immunol* 13: 941071. https://doi.org/10.3389/fimmu.2022.941071
26. Nazimek K, Bryniarski K (2024) Macrophage functions in psoriasis: Lessons from mouse models. *Int J Mol Sci* 25: 5306. https://doi.org/10.3390/ijms25105306
27. Morimura S, Oka T, Sugaya M, et al. (2016) CX3CR1 deficiency attenuates imiquimod-induced psoriasis-like skin inflammation with decreased M1 macrophages. *J Dermatol Sci* 82: 175–188. https://doi.org/10.1016/j.jdermsci.2016.03.004
28. Shinno-Hashimoto H, Eguchi A, Sakamoto A, et al. (2022) Effects of splenectomy on skin inflammation and psoriasis-like phenotype of imiquimod-treated mice. *Sci Rep* 12: 14738. https://doi.org/10.1038/s41598-022-18900-7
29. Hawkes JE, Gudjonsson JE, Ward NL (2017) The snowballing literature on imiquimod-induced skin inflammation in mice: A critical appraisal. *J Invest Dermatol* 137: 546–549. https://doi.org/10.1016/j.jid.2016.10.024
30. Chen L, Deshpande M, Grisotto M, et al. (2020) Skin expression of IL-23 drives the development of psoriasis and psoriatic arthritis in mice. *Sci Rep* 10: 8259. https://doi.org/10.1038/s41598-020-65269-6
31. Kannan AK, Su Z, Gauvin DM, et al. (2019) IL-23 induces regulatory T cell plasticity with implications for inflammatory skin diseases. *Sci Rep* 9: 17675. https://doi.org/10.1038/s41598-019-53240-z
32. Cai Y, Shen X, Ding C, et al. (2011) Pivotal role of dermal IL-17-producing γδ T cells in skin inflammation. *Immunity* 35: 596–610. https://doi.org/10.1016/j.immuni.2011.08.001
33. Chan TC, Hawkes JE, Krueger JG (2018) Interleukin 23 in the skin: Role in psoriasis pathogenesis and selective interleukin 23 blockade as treatment. *Ther Adv Chronic Dis* 9: 111–119. https://doi.org/10.1177/2040622318759282
34. Rizzo HL, Kagami S, Phillips KG, et al. (2011) IL-23-mediated psoriasis-like epidermal hyperplasia is dependent on IL-17A. *J Immunol* 186: 1495–1502. https://doi.org/10.4049/jimmunol.1001001

35. Lindroos J, Svensson L, Norsgaard H, et al. (2011) IL-23-mediated epidermal hyperplasia is dependent on IL-6. *J Invest Dermatol* 131: 1110–1118. https://doi.org/10.1038/jid.2010.432
36. Chan JR, Blumenschein W, Murphy E, et al. (2006) IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med* 203: 2577–2587. https://doi.org/10.1084/jem.20060244
37. Pawlak M, DeTomaso D, Schnell A, et al. (2022) Induction of a colitogenic phenotype in Th1-like cells depends on interleukin-23 receptor signaling. *Immunity* 55: 1663–1679.e6. https://doi.org/10.1016/j.immuni.2022.08.007
38. Muramatsu S, Kubo R, Nishida E, et al. (2017) Serum interleukin-6 levels in response to biologic treatment in patients with psoriasis. *Mod Rheumatol* 27: 137–141. https://doi.org/10.3109/14397595.2016.1174328
39. Wang Q, Yan D, Zheng S, et al. (2022) Cytokine profiles and the relationship of disease severity in patients with psoriasis. *Indian J Dermatol* 67: 204. https://doi.org/10.4103/ijd.ijd_79_22
40. Michalak-Stoma A, Bartosińska J, Raczkiewicz D, et al. (2022) Multiple cytokine analysis of Th1/Th2/Th9/Th17/Th22/Treg cytokine pathway for individual immune profile assessment in patients with psoriasis. *Med Sci Monit* 28: e938277. https://doi.org/10.12659/MSM.938277
41. Alves NR de M, Kurizky PS, da Mota LMH, et al. (2024) Elevated serum IL-6 levels predict treatment interruption in patients with moderate to severe psoriasis: A 6-year real-world cohort study. *An Bras Dermatol* 99: 34–42. https://doi.org/10.1016/j.abd.2023.03.002
42. Arican O, Aral M, Sasmaz S, et al. (2005) Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm* 2005: 273–279. https://doi.org/10.1155/MI.2005.273
43. Takahashi H, Tsuji H, Hashimoto Y, et al. (2010) Serum cytokines and growth factor levels in Japanese patients with psoriasis. *Clin Exp Dermatol* 35: 645–649. https://doi.org/10.1111/j.1365-2230.2009.03704.x
44. Wu LTY, Qiao ZH, Tian JB, et al. (2023) The effect of secukinumab treatment for psoriasis on serum cytokines and correlation with disease severity. *Skin Res Technol* 29: e13405. https://doi.org/10.1111/srt.13405
45. Ovcina-Kurtovic N, Kasumagic-Halilovic E (2022) Serum levels of tumor necrosis factor-alpha in patients with psoriasis. *Mater Sociomed* 34: 40–43. https://doi.org/10.5455/msm.2022.33.40-43
46. Zwicky P, Ingelfinger F, Silva de Melo BM, et al. (2021) IL-12 regulates type 3 immunity through interfollicular keratinocytes in psoriasiform inflammation. *Sci Immunol* 6: eabg9012. https://doi.org/10.1126/sciimmunol.abg9012
47. Vincken NLA, Welsing PMJ, Silva-Cardoso SC, et al. (2022) Suppression of IL-12/IL-23 p40 subunit in the skin and blood of psoriasis patients by Tofacitinib is dependent on active interferon-γ signaling in dendritic cells: Implications for the treatment of psoriasis and interferon-driven diseases. *Exp Dermatol* 31: 962–969. https://doi.org/10.1111/exd.14566
48. Hsieh CL, Yu SJ, Lai KL, et al. (2024) IFN-γ, IL-17A, IL-4, and IL-13: Potential biomarkers for prediction of the effectiveness of biologics in psoriasis patients. *Biomedicines* 12: 1115. https://doi.org/10.3390/biomedicines12051115
49. Kryczek I, Bruce AT, Gudjonsson JE, et al. (2008) Induction of IL-17 + T cell trafficking and development by IFN-gamma: Mechanism and pathological relevance in psoriasis. *J Immunol* 181: 4733–4741. https://doi.org/10.4049/jimmunol.181.7.4733

50. Bhoi AK, Grover C, Singal A, et al. (2024) Serum levels of tumour necrosis factor (TNF-α) and interleukin-17 (IL-17) in patients with nail psoriasis: A cross-sectional study. *Indian J Dermatol Venereol Leprol* 1–5. https://doi.org/10.25259/IJDVL_367_2023
51. Pietrzak A, Chabros P, Grywalska E, et al. (2020) Serum concentration of interleukin 6 is related to inflammation and dyslipidemia in patients with psoriasis. *Postepy Dermatol Alergol* 37: 41–45. https://doi.org/10.5114/ada.2018.78028

AIMS Press