NK CELLS IN PSORIASIFORM DERMATITIS. FROM PSORIATIC SITE TO PERIPHERAL BLOOD, THROUGH SECONDARY IMMUNE ORGANS

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Summary

Among skin pathologies, psoriasis is one of the most challenging disorders in terms of therapy and clinical monitoring of the patient.Psoriasis is a chronic inflammatory skin condition that has an autoimmune mechanism mediated by T cells, with involvement of innate immune cells.An important role is played by different subsets of T-helper lymphocytes, as well as a number of pro-inflammatory cytokines that maintain the chronic inflammatory state. Although some evidence suggests that NK cells play animportant role in psoriasis, their contribution to disease's development and progression remains incompletely understood. Experimental models are an indispensable tool in psoriasis research for both pathogenesis evaluation and for possible therapeutic targets identification. Due to its advantages, experimental models such as the Imiquimod-based mice model is one of the most popular animalmodel to study psoriasiform dermatitis in mice. In order to highlight the role of NK cells in the development of psoriasis, we performed the Imiquimod-based mice model and analysed NK cells distribution and phenotype by flow cytometry. Skin inflammation induced by Imiquimodwas assessed based on in vivo parameters (erythema, desquamation, thickening), PASI score, evaluation of splenomegaly and pathological examination. NK cells distribution was evaluated on three immune levels: peripheral blood, spleen and skin. There were quantified NK cells subsets based on the expression of CD27 and CD11b, and NK1.1$^+$CD11c$^+$CD45R$^+$subset. The expression of CD43, KLRG1 and CD69, markers with an important role in NK cells maturation and activation, was analysed.Our data show that, although a somewhat neglected cell in psoriasis, NKs correlate with the gravity of the auto-immune disease.

Keywords: *imiquimod, experimental model, NK cells psoriasiform dermatitis.*

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Introduction

Psoriasis (Ps) is a chronic inflammatory disease mediated by T cells [1], with cutaneous and articular manifestations [2]. The global prevalence is 2-3%, with a similar distribution among men and women [3], it depends on age, ethnicity, geographical area and environmental factors [4]. The pathology has a significant impact on the quality of life of patients and has a strong psychosocial imprint [4]. Ps is also associated with other pathologies such as obesity [5], dyslipidemia [6], diabetes [7], cardiovascular diseases [3], kidney chronic diseases [8], autoimmune diseases [9], cancer [10]. These associated pathologies partially contribute to the of patients increased mortality and have important implications in clinical management.

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Histologically, Ps is characterized by abnormal and rapid proliferation of keratinocytes and infiltration of psoriatic lesions with immune cells, especially T and dendritic cells[11].Classical histological manifestations include acanthosis, hyperkeratosis, parakeratosis, elongation of rete ridges, increase in the number and size of the dermal vessel and an abundant inflammatory cell infiltrate, consisting mostly of dendritic cells, macrophages and T lymphocytes in the dermis, respectively of neutrophils and T cells in the epidermis (Munro microabscesses and Kogoj pustules) [12].

Although Ps has an autoimmune mechanism mediated by T cells, both innate and specific immune cellsare highly involved; an important role is played by different subsets of T-helper lymphocytes, as well as a number of pro-inflammatory cytokines that maintain the chronic inflammatory state[13].Two phases have been described in the pathogenesis of psoriasis: the initiation of psoriatic events and the maintaining of inflammation. The onset of psoriatic events occurs when, under the action of triggering factors (e.g. genetic, environmental, skin lesions, infections), keratinocytes and nonspecific immunity cells (NK cells, macrophages, dendritic cells) secrete TNF-α, IFN-γ, IL-1β, IL-6 that will activate myeloid dendritic cells. These activated dendritic cells secrete IL-12 and IL-23, and induce the differentiationof naïve T lymphocytes into Th1, Th17 and Th22 cells. These effectors will release TNF-α, IFN-γ, IL-17A/F and IL-22, thus activating keratinocytes,further producing mainly pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), chemokines (CXCL9-11), antimicrobial peptides (LL-37, β-defensin), S100 protein. Activated ker-atinocytes, through the secretion of chemokines, will promote the recruitment and activation of neutrophils and macrophages, thus propagating and maintaining the inflammation [14,15].

Although some evidence suggests that NK cells play animportant role in Ps, their contribution to diseases development and progression is relatively under studied and remains incompletely understood.NK cells are important cellular components of the innate immune system with an active role in anti-tumour and anti-viral defence [16]. Phenotypically, human NK cells are defined as $CD3^{-}CD56^{+}CD16^{+}$ cells; other markers specific only for NK cells are NKp46 and NKp30. Depending on CD56 level of expression, NK cells are divided into two subsets with distinct functional properties: cytotoxic subset (90% of circulating NK cells) with low density of CD56 expression, characterized by abundant granules (perforin, granzime) and regulatory subset, with high density of CD56 expression, characterized by reduced cytolytic activity and higher ability to produce cytokines (IFNγ, TNF, IL-10, IL-13, GM-CSF)[17]. NK cells appear to play an important role in Ps by releasing cytokines (IFN-γ, TNF and IL-22), but their pathogenic importance is not yet fully understood. NK cells are able to produce large amounts of IFN-γ thus inducing keratinocyte's activation. These activated keratinocytes, through the production of chemokines (CXCL10, CCL5, CCL20), attract NK-$CD56^{bright}$ cells, thus exacer-bating inflammation [18].

In order to elucidate as many aspects of the pathogenesis of Ps, several experimental models have been developed in recent years and these include transgenic mice, knockout mice, and reconstituted Ps models [19]. Since 2009[20], the Imiquimod (IMQ)-induced mice model has befall one of the most popular experimental models to study psoriasiform dermatitis in mice.IMQ (1-isobutyl-1H-imidazo[4,5-c]quinolin-4-amine) is able to activate proinflammatory signalling pathways through the activation of toll-like receptor (TLR) 7/8.This compound is used for its anti-viral and anti-neoplastic properties in the treatment of genital warts, squamous cell carcinoma and actinic keratosis.The IMQ-based mice model has several advantages: the relatively quick and reproducible inflammatory skin response, the induced dermatitis mirrorpsoriatic pathogenesis in human, it is easy to use, provides quick resultsand it is relatively inexpensive[21].

To elucidate some aspects of NK involvement in the development of experimental Ps we have done a murine model of psoriatic dermatitis by local application of IMQ for 5 consecutive days. Skin inflammation induced by IMQwas assessed based on *in vivo*parameters (erythema, desquamation, thickening), PASI score, evaluation of splenomegaly and pathological examination.

The immunological changes induced by IMQ treatment in NK cells distribution and phenotype were evaluated by flow cytometry. NK cells distribution was evaluated on three levelsthat sustain the immune functions: peripheral blood (PB), spleen and skin. There were quantified NK cells subsets based on the expression of CD27 and CD11b, and NK1.1$^+$CD11c$^+$CD45R$^+$subset.The expression of CD43, KLRG1 and CD69, markers with an important role in NK cells maturation and activation, was analysed.

Material and Methods

IMQ – based murine model of psoriasiform dermatitis

C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME), males and females (1:1), aged 8-10 weeks, were provided by the Animal Husbandry from Victor Babeș National Institute. The animals were accommodated in individual cages (open cage system) and kept in optimal conditions (temperature 22 ± 2°, humidity 55 ± 10%, artificial ventilation, 12/12 - light / dark cycle). The animals were fed and watered *ad libidum* with standard granulated feed and water (filtered and sterilized). All cages were maintained under a rigorous cleaning and sanitation program, and the animals were monitored daily. The experiments were performed in accordance with recognized principles of laboratory animal care in the framework of EU Directive 2010/63/EU for animal experiments [22]. The protocols of this study were approved by the Ethics Committee of Victor Babeș National Instituteand by the National Sanitary Veterinary and Food Safety Authority through the project authorization 388 / 22.03.2018.

The IMQ - based experimental model was performed according to the protocols described in literature [20]. In order to perform the experimental model there were used two groups of C57BL/6 mice (average weight 22.2 ± 3 g): **IMQ group** (10 mice) –received a daily topical dose of 62.5 mg IMQ cream (5% Aldara Cream, MEDA AB Sweden) on the shaved back for 5 consecutive days, and **Control group** (10 mice)-healthy mice.

The mice were monitored daily for assessment of the IMQ-induced inflammation severity by scoring of erythema, desquamation and thickeningon a scale from 0 to 4 (0 - none; 1 - slight; 2 - moderate; 3 - marked; 4 - very marked). On each day of treatment, a modified PASI score was calculated cumulatively, by summing the daily scores of erythema, desquamation and induration (0-12 scale).

On the sixth day of the experiment, the animals were weighed and anesthetized with ketamine / acepromazine(100/5 mg/kg, Ketaset; Wyeth/Fort Dodge Animal Health, Overland Park, KS, USA; Vedco, St. Joseph, MO, USA) for blood sampling. PB was collected by retro-orbital puncture in K2-EDTA coated tubes (Microvette, Sarstedt AG & Co, Numbrecht, Germany). All animals were sacrificed, spleen and skin samples were collected.

The collected spleens were weighed (Balance AEP-1500 A, Adam Equipment Co Ltd, Milton Keynes, UK) for the evaluation of splenomegaly, by calculating the ratio between spleen weight (SW) and body weight (BW).The harvested skin sampleswere fixed in 10% buffered formalin and embedded into paraffin; the paraffin blocks were sectioned (5 μm thick sections), stained with hematoxylin-eosin (H&E) and examined by pathologist.

The spleens and skin samples were harvested in RPMI 1640 media with 5% FBS (Biochrom GmbH, Germany) and passed through a 70 μm cell strainer (BD Falcon - BD Biosciences, San Jose, CA, USA) after mechanical disruption. The suspensions were centrifuged (5 min, 350 xg, 20°), resuspended in RBC Lysis Buffer (BioLegend, San Diego, CA, USA), and incubated 5 min on ice. The lysis was stopped by adding 10 mL Cell Staining Buffer (BioLegend, San Diego, CA, USA). SCS were centrifuged (5 min, 350 xg, 20°)and the cell pellet was resuspended in Cell Staining Buffer at 1 x 10^6 cells/mL.

Quantification and phenotypic characterization of NK cells by flow cytometry

NK cells analysis was performed by flow cytometry, based on the expression of surface markers.We have used a BD FACSCanto II

cytometer with BD FACSDiva v.6.1 software (BD Biosciences, San Jose, CA, USA) and FlowJo v.10 software (TreeStar, BD Biosciences).

PB and cell suspensions obtained from the spleens and skin samples were labeled with monoclonal antibodies for analysis by flow cytometer. After an incubation (20 min) with TruStainfcX (anti-mouse CD16/32, isotype Rat IgG2a, λ) Antibody (BioLegend, San Diego, CA, USA) in order to block non-specific antibody binding, all samples were incubated for 20 min (room temperature and dark) with the following monoclonal anti-mouse antibodies conjugated with fluorochromes: CD3ε FITC (clone 145-2C11, isotype Armenian Hamster IgG) (BioLegend), NK1.1 BV 510 (PK136, Mouse IgG2a, κ) (BD Biosciences), CD27 PerCP/Cy5.5 (LG.3A10, Armenian Hamster IgG)(BioLegend),CD11b APC (M1/70, Rat IgG2b, κ) (BioLegend), CD43 APC/Cy7 (RA3-6B2, Rat IgG2a, κ) (BioLegend), KLRG1PE (2F1, Syrian Hamster IgG) (BioLegend), CD69 PE/Cy7 (H1.2F3, Armenian Hamster IgG) (BioLegend),CD11c PerCP/Cy5.5 (N418, Armenian Hamster IgG) (BioLegend), CD45R APC/Cy7 (RA3-6B2, Rat IgG2a, κ) (BioLegend). Surface staining was followed by red blood cells lysis (BD Pharm Lyse, BD Biosciences) for 3 min at 37°C and dark and centrifugation (5 min, 350 xg). After two washing steps with Cell Staining Buffer, the suspensions were analysed by flow cytometry. Data were expressed as percentages of $NK1.1^+$ cells, gated from $CD3\varepsilon^-$ lymphocytes. As controls were used unlabeled cells and in order to avoid non-specific fluorescence signals obtained due to spectral overlap,automatically compensation was performed (UltraCompeBeads, Invitrogen by Thermo Fischer Scientific, San Diego, CA, USA). CST beads (BD Cytometer Setup & Tracking Beads Kit, BD Biosciences) were used to setup the cytometer performances.

Data were analysed with Microsoft Excel (Microsoft, Redmond, CA) and theresults were expressed as mean ± SD values. In the statistical analysis, to assess the differences between the experimental groups, Student's t-test (two-tailed, assuming equal variance) was used. A p value less than 0.05 was considered statistically significant.

Results

Experimental model of psoriasiform dermatitis induced by IMQ

The experimental model was performed using C57BL/6 mice, by topically applying of IMQ-based cream for 5 consecutive days. Mice were monitored daily and the progress and severity of inflammation were assessed based on *in vivo* measurements, PASI score, splenomegaly and histopathological evaluation.

In vivo parameters - erythema, desquamation and thickening- were scored daily on a 0-4 scale. Started with day 2 the signs of inflammation were visible and it increased in intensity by the end of the experimentTwo days after the start of IMQ treatment, the back skin of the mice started to display signs of erythema. Desquamation and thickening became visible from day 3. Evolution of erythema, desquamation and induration scores during the IMQ treatment is presented in Figure 1 A.

As a measure of the severity of inflammation, on each day of IMQ-application, a modified PASI score was calculated cumulatively, on a 0-12 scale, by summing the daily scores of erythema, desquamation and thickening (Fig. 1 B).The PASI score increased progressively, the value reached at the end of the experiment being 10.2.

At the end of the experiment, the animals were weighed and anesthetized for blood sampling, and then sacrificed for spleens and skin samples collecting. In order to evaluatethe splenomegaly, the collected spleens were weighed separately and the ratio between spleen weight (SW) and body weight (BW) was calculated. We found a significant spleen enlargement (with a 2x increase in weight) in IMQ group. Both spleens weight and SW/BW ratio were significantly increased in IMQ-mice as compared to control group (SW: 0.225±0.03 versus 0.11±0.02; $p = 2.5x10^{-4}$; SW/BW ratio: 0.011±0.002 versus 0.005±0.0006; $p = 3.1x10^{-4}$).

Histopathological evaluationof H&E sections from IMQ-mice revealed acanthosis, hyperkeratosis and elongation of rete ridges, typical for human psoriasis (Figure 2 A). None of these features were found in normal skin samples from control group (Figure 2 B).

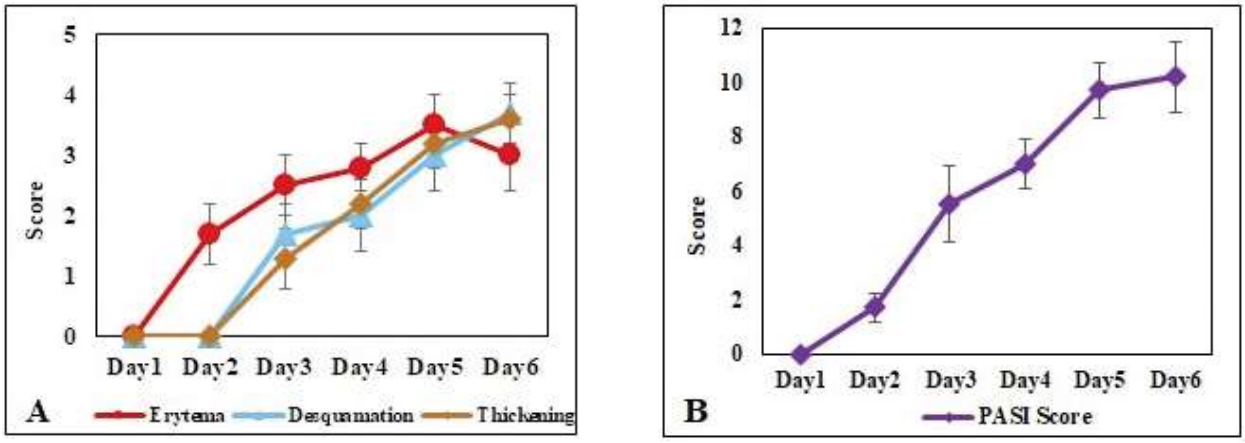

Figure 1. Evaluation of skin inflammatory parameters:

A.Evolution of i*n vivo* parameters scores. Erythema, desquamation and thickening were scored daily on a 0-4 scale: Day1: 0; 0; 0; Day2: 1.7±0.5; 0; 0; Day3: 2.5±0.5; 1.7±0.5; 1.3±0.5; Day4: 2.8±0.4; 2±0.6; 2.2±0.4; Day5: 3.5±0.5; 3±0.6; 3.2±0.4; Day6: 3±0.6; 3.7±0.5; 3.6±0.4.

B.PASI score evolution during IMQ treatment. PASI cumulative score was calculated daily by summing the daily scores of erythema, desquamation and thickening: 0; 1.7±0.5; 5.5±1.4; 7±0.9; 9.7±1; 10.2±1.3. Data are presented as mean score ± SD.

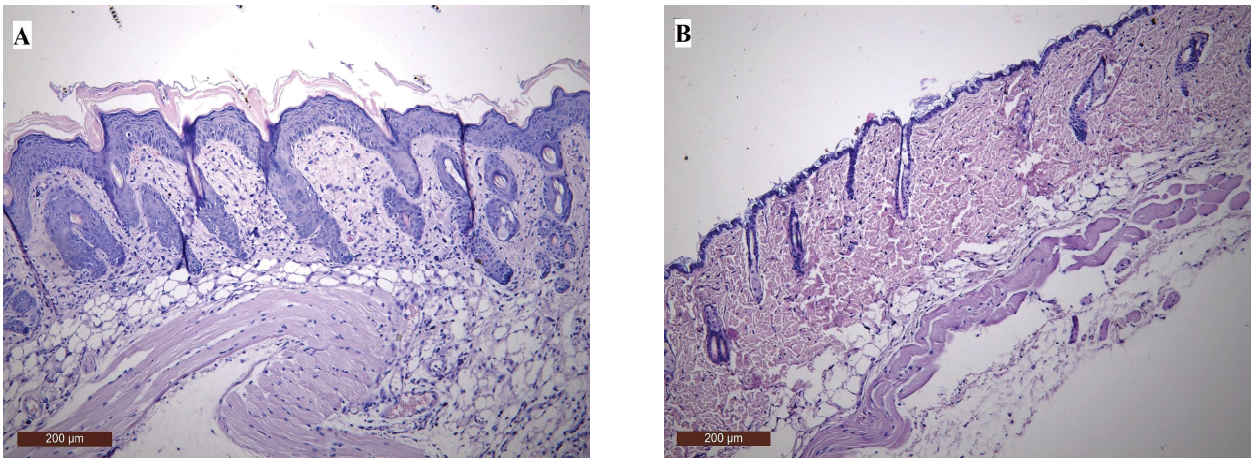

Figure 2. H&E sections from skin samples:

A.Section from a IMQ-mouse.
B. Section from a healthy mouse (control group).

NK cells distribution in peripheral blood, spleen cell suspension and skin samples

NK cell distribution was assessed by flow cytometry based on lineage markers NK1.1 and CD3ε. In both PB and spleen cell suspension (SCS)the level of NK cells washigher for IMQ-mice as compared to the control group (Figure 3.A).Statistically significant differences between the tested groups were obtained in PB ($p=6.5x10^{-5}$).

For skin samples, the expression of NK cells from was evaluated using Overton Subtraction. This method was chosen because the flow cytometry analysis revealed a continuous fluorescence that did not allow the delimitation of negative and positive populations. Elevated values for OvertonSubtraction were obtained for

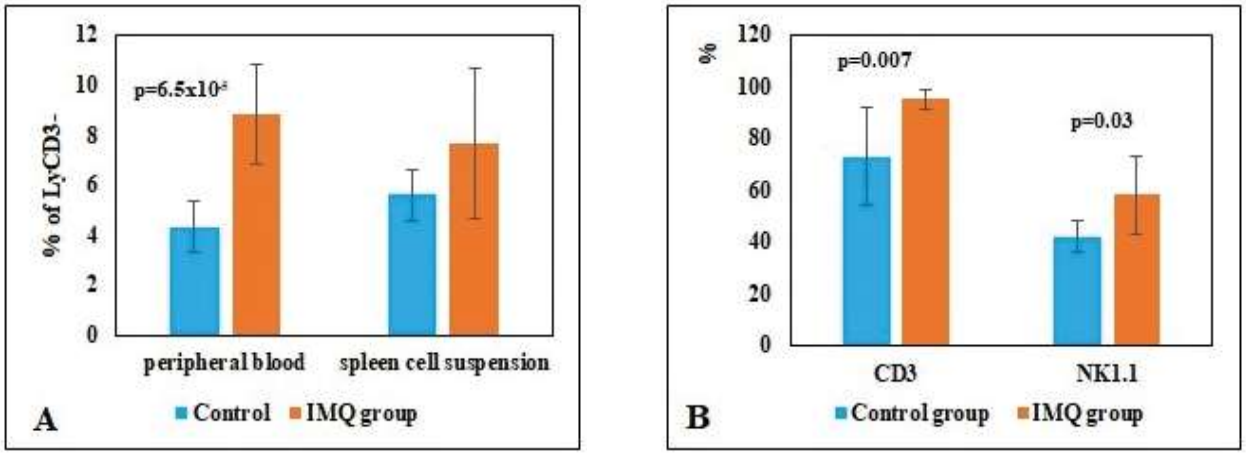

Figure 3. NK cells levels in PB, SCS and skin:

A. Distribution of NK cells in PB and SCS. Distribution of mean percentage values of NK cells for IMQ-treated mice compared to control group in PB (8.84±2 versus 4.33±1; $p=6.5x10^{-5}$) and in SCS (7.66±3 versus 5.6±1). Data are presented as percentage of $CD3\varepsilon^-$ lymphocytes (mean ± SD);

B.Levels of fluorescence in skin samples. Mean values of Overton Subtraction for $CD3\varepsilon^+$ and NK1.1+ cellsin IMQ-mice (95±4; 58±15) and control group (73±19; 42±6).

both $CD3\varepsilon^+$ and $NK1.1^+$ cells which indicate an increased level of fluorescence for these cells and the differences between the experimental groups were statistically significant (p=0.007; p=0.03) (Figure 3 B).

Phenotypic changes of NK cells in PB and SCS

Phenotypic characterization of NK cells was assessed by flow cytometryfrom PB and SCS, and the values obtained for IMQ-mice were compared tocontrol group.

For IMQ-mice we found significantly decreased values for the immature stages ($CD27^-CD11b^-$ and $CD27^+CD11b^-$) and higher percentages for mature subsets ($CD27^+CD11b^+$ and $CD27^-CD11b^+$) in PB. Statistically significant differences between the experimental groups were obtained for immature (p=0.001) and early mature NK cells (p=0.01) (Figure 4.A). In SCSthe immature NK cells were significantly decreased for IMQ-mice (p=0.0003), the values increase for early mature and mature NK cells (p=0.01), and then decrease for late mature NK cells (Figure 4 B).

In order to highlight the changes in the phenotype of NK cells in IMQ-mice, the expression of CD43, KLRG1 and CD69 on NK cells was analysed. In PB, statistically significant differences were obtained for all investigated markers, their levels on NK cells being significantly increased in IMQ-mice (p=0.01; $p=2.9x10^{-9}$; $2.4x10^{-6}$) (Figure 5 A). A similar distribution was obtained in the SCSand statistically significant differences between the experimental groups were obtained for KLRG1 (p=0.02) and CD69 ($p=1.5x10^{-8}$) (Figure 5.B).

Evaluation of $NK1.1^+CD11c^+CD45R^+$subset revealed increased values in both PB and SCS for IMQ-mice compared to controls. Statistically significant differences between the experimental groups were obtained in SCS ($p = 3.4x10^{-5}$) (Figure 6).

Discussions

Experimental models are an indispensable research tool and try to explain the pathogenesis of psoriasis and to identify possible therapeutic targets. Ps does not occur naturally in laboratory animals and for this reason, developing an experimental model who reflects all aspects of human Ps remains a challenge for researchers. Since 2009 [20], the IMQ-based mice model has become one of the most popular experimental

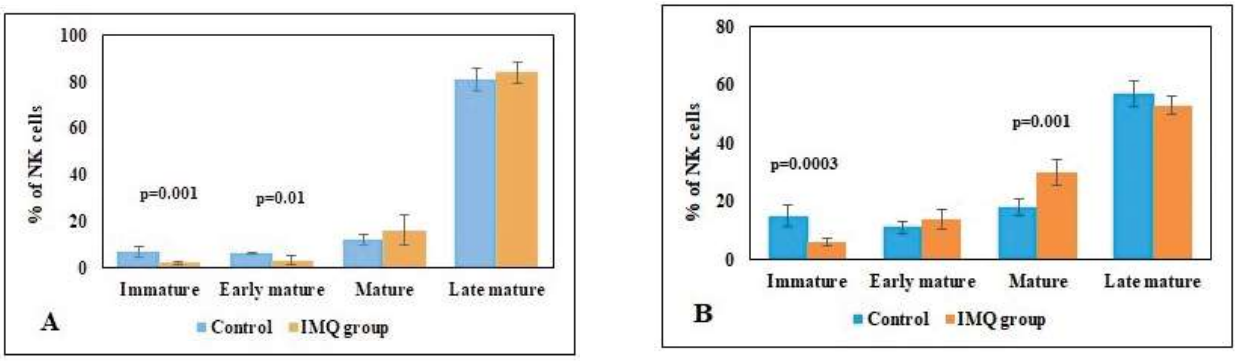

Figure 4. NK cells subsets distribution.

A. PB. Distribution of immature, early mature, mature and late mature NK cells in IMQ-mice (2.2±0.7, p=0.001; 3±1.9, p=0.01; 16±6.5 and 84±4.6) and in control group (7±2.2, 6±0.3, 12±2.1 and 81±4.8).
B. SCS. Distribution of immature, early mature, mature and late mature NK cells in IMQ-mice (6±1.1, p=0.0003; 14±3.4;30±4.3, p=0.001 and 53±3) and in control group (15±3.5, 11±2, 18±2.8 and 57±4.5). Data are presented as a percentage from NK1.1+ cells (mean ± SD).

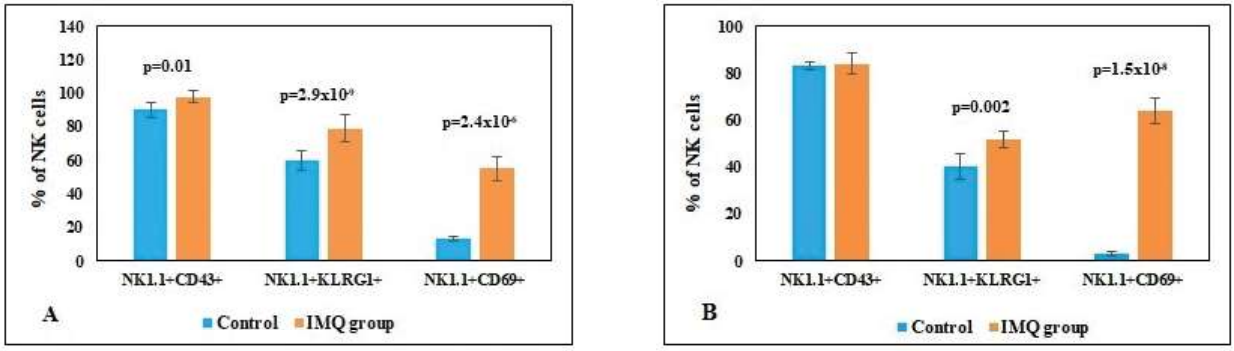

Figure 5. The expression levels of CD43, KLRG1 and CD69 on NK cells.

A. PB. Distribution ofNK cells positive forCD43, KLRG1 and CD69 in IMQ-mice (98±3.5, p=0.01; 79±8.3, p=2.9x10^{-9} and 55±7, p=2.4x10^{-6}) and in control group (90±4.6, 60±5.8 and 13±1.2).
B. SCS. Distribution ofNK cells positive forCD43, KLRG1 and CD69 in IMQ-mice (84±4.5; 52±3.5, p=0.002 and 64±5.3, p=1.5x10^{-8}) and in control group (83±1.6, 40±5.4 and 3±1). Data are presented as a percentage from NK1.1+ cells (mean ± SD).

models to study psoriasiform dermatitis in mice.This model has several advantages: quick and reproducible inflammatory skin response, the induced dermatitis mirrorspsoriatic pathogenesis in human,it is relatively easy to develop the model in standard Husbandries, provides quick resultsand it is relatively inexpensive[21].In this study, the murine model of psoriatic dermatitis was performed by local application of IMQ for 5 consecutive days. Daily *in vivo* measurements revealed progressive increases in erythema, desquamation and induration in the sites were IMQ was applied. Also, the PASI cumulative score was calculated daily and had a progressive evolution, reaching values close to the maximum at the end of the experiment. Evaluation of splenomegaly revealed that in IMQ-mice, the weight of the spleen and the SW:BW ratio are significantlyhigher compared to controls. Histopathological examination con-

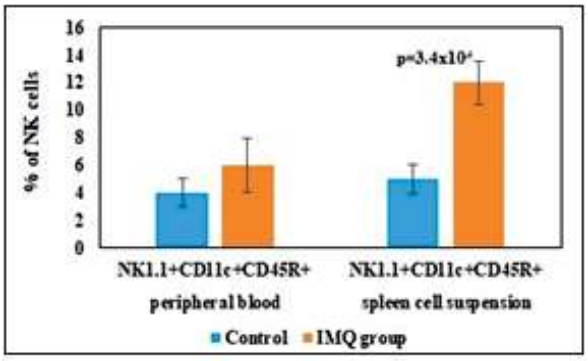

Figure 6. NK1.1$^+$CD11c$^+$CD45R$^+$ cells in PB and SCS.
Distribution of NK1.1$^+$CD11c$^+$CD45R$^+$ cells in IMQ-mice (6±2 in PB and 12±1.6, p=3.4x10-5 in SCS) and in control group (4±1 in PB and 5±1.1 in SCS).Data are presented as percentage from NK1.1+ cells (mean ± SD).

firmed that IMQ application induces hyperkeratosis, parakeratosis, acanthosis and elongation of the red ridges, histopathological features that are typical for human psoriatic lesions. The results showed that the experimental model of psoriasiform dermatitis induced by IMQ showed specific aspects of human Ps.

The immunological changes induced by the experimental psoriasis in NK cells distribution and phenotype were evaluated by flow cytometry. NK cells distribution was evaluated on three immune levels: peripheral blood, spleen and skin. In both PB and SCS the level of NK cells washigher in psoriatic mice as compared to the control group. Analysis of NK cells in skin samples revealed elevated values for Overton Subtraction which indicate an increased level of fluorescence for these cells and the differences between the experimental groups were statistically significant. These data may support the involvement of these cells in psoriatic lesions.

CD27 is a key marker of the NK cell lineage and splitthe mature NK cells into two functionally distinct subsets. The CD27 high NK cell subset displays a greater effector function and responsiveness to chemokines than CD27 low NK cell subset [23]. In mice, the surface density of CD27 and CD11b divides NK cells into four subsets and represents their level of maturation: immature NK cells (NK1.1$^+$CD27$^-$ CD11b$^-$), early mature NK cells (NK1.1$^+$CD27$^+$ CD11b$^-$), mature NK cells (NK1.1$^+$CD27$^+$CD11b$^+$) and late mature NK cells (NK1.1$^+$CD27$^-$CD11b$^+$) [24]. Our data showed significantly decreased values for the immature stagesand higher percentages for mature subsets for psoriatic mice in PB.Statistically significant differences between the experimental groups were obtained for immature (p=0.001) and early mature NK cells (p=0.01). In SCSthe immature NK cells were significantly decreased (p=0.0003), the values increase for early mature and mature NK cells (p=0.01), and then decrease for late mature NK cells in psoriatic mice.These results show the commitment to an activating profile of NK cells and may indicate the usefulness of evaluating the intensity of psoriasis in correlation with the profile of circulating NK cells.

In order to highlight the changes in the phenotype of NK cells in psoriatic mice, the expression of CD43, KLRG1 and CD69 on NK cells was analysed.

CD69 is a differentiation antigen expressed shortly after activation on T lymphocytes and other cells of haematopoietic origin, including NK cells.CD69 is rapidly induced in NK cells shortly after activation by different stimuli (such as phorbol 12-myristate 13-acetate, IL-2, IL-12, IFN-α) and its role in NK cytotoxicity has been demonstrated both in human and mice. A high level of CD43 on NK cells surface is associated with the capability of NK cell to produce large amounts of IFN-γ, while CD27$^-$CD11b$^+$KLRG1$^+$ NK cells are terminally differentiated NK cells, with reduced capacity to proliferate and effector function during viral infection [25]. In our study we found statistically significant differencesfor all investigated markers in PB, the expressions of these markers on NK cells being significantly increased in psoriatic mice (p=0.01; p=2.9x10^{-9}; 2.4x10^{-6}). A similar distribution was obtained in SCSand statistically significant differences between the experimental groups were obtained for KLRG1 (p=0.02) and CD69 (p=1.5x10^{-8}).

NK1.1$^+$CD11c$^+$CD45R$^+$cells, an activated NK cells subset, was described as analogous to human NKCD56^{bright} cells [26–28]. In our experimental model, the evaluation of thissubset revealed increased values in both PB and SCS for IMQ-treated mice compared to controls. Statistically significant differences between the

experimental groups were obtained in SCS ($p=3.4x10^{-5}$).These data highlight once again the activation of this subset of lymphocytes.

The increased expression of CD43 and KLRG1 on NK cells as well as the high percentages of NK cells in the final stages of maturation indicate a poor cytotoxic phenotype and focused mainly on the production of cytokines. These data obtained for IMQ-based murine model emphasize once again the importance of the study of NK phenotype in correlation with the degree of psoriatic lesions.

Conclusions

The IMQ-based experimental model of psoriasiform dermatitiswas performed and evaluated clinically and histopathologically; skin-induced inflammation and disease severity were assessed by *in vivo* measurements (independent scores for erythema, desquamation and induration), PASI score, splenomegaly assessment, and histopathological examination. Data obtained for all parameters showed that the IMQ-based murine model showed specific features of the human disease. Evaluation of NK cells distribution and phenotype revealed important immunological changes in psoriatic mice as compared to controls. The main finding was the activation phenotype of NK cells that correlate with the gravity of the autoimmune disease.

Acknowledgements

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Conflict of interest
NONE DECLARED

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