CORRELATION OF CD4+T LYMPHOCYTES ACTIVATION WITH INTERLEUKIN IL-9, IL-17, IL-22 PROFILES IN THE PERIPHERAL BLOOD OF PATIENTS WITH PLAQUE PSORIASIS

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Psoriasis is an immune-mediated chronic inflammatory skin disease, with T-cell auto reactivity to a still unknown antigen leading to the inflammation. Disease is quite common, about 2% of the general population worldwide is affected [6,7]. Psoriasis most commonly primary manifests on the skin, although inflammatory processes can occur in other organs [6,7]. Disease greatly diminishes the quality of life of patients that is roughly equivalent to the data for patients with diabetes, myocardial infarction, and rheumatoid arthritis. Psoriasis is often genetically determined and both endogenous and exogenous factors are involved in disease pathogenesis. Many factors, such as infectious agents, mental or mechanical injuries, various drugs, and alcohol can trigger the start or relapse of this chronic condition [5].

The IL-23/TH17 immune axis is now thought to be central to the pathogenesis of psoriasis. The main cytokines involved in psoriasis pathogenesis, IL-23, TNF and IL-17, can be subdivided into regulatory and effector cytokines based on their mode of action [4]. IL-23 exerts regulatory effects on the maintenance of Th17 cells, whereas IL-17 and TNF mediate effector functions of innate (TNF) and adaptive (TNF, IL-17) immune cells [4].

Different subtypes of the regulatory T cells are involved in the conduct of psoriasis immunopathogenesis: among them are Th17, Th22 and Th9 cells. Their significance increases because they are involved in regulating the IL-23/Th17 axis that controls the pro-inflammatory rings of psoriasis cells. IL-23/Th17 axis controlling mainly the proinflammatory loop in psoriatic plaques which involves keratinocytes, dendritic cells, and T cells; especially γδ T cells also play a major role in the production of IL-17 and in the maintenance of inflammation in psoriatic plaques [1].

Recent advances in psoriasis research have provided new defined targets for therapeutic intervention, offering hope for safe and effective treatment [1].

The main goal of our study was to evaluate the ratio of T cell profile and IL-23/Th17 axis by evaluating IL17A, IL22, IL9 in peripheral blood of persons with moderate to severe plaque psoriasis. Based on the complex nature of the disease we aimed to identify the cells and cytokines which are leading the process in blood samples of patients and healthy groups. Also, we evaluated the expression of CD69 activation markers on $CD4^+$ T cells.

Material and methods. Before the start of experimental studies, the project was submitted to the Ethics Commission of the National Center for Disease Control and Public Health (NCDC). An ethical permit was taken to conduct the research in Georgia. During the research, the anonymity of the participants was preserved, voluntary engagement by each participant was certified by the signature of informed consent.

Peripheral blood was obtained from 18 patients with moderate-to-severe forms of plaque psoriasis aged between 18 and 65 years (median age 42) and 15 healthy age-matched volunteers. Only those patients who did not receive systemic therapy a month before the start of the study were included.

The severity of the disease was determined by standardized Psoriasis Area and Severity Index (PASI). The PASI of the patients included in the study ranged from 10 to 40, no other chronic inflammatory diseases were detected in persons of the main group.

Blood samples of patients were provided by partner dermatological clinics within the scope of the research. Obtaining, storage and delivery of the material was provided by the staff involved in the project. Ten ml of blood were collected in heparinized tubes (Sigma), stored at a room temperature and processed on the same day.

The experimental part of the project was implemented in the scientific laboratory of the Division of Immunology and Microbiology of Tbilisi State University.

Immunophenotyping - Phenotype of cells of peripheral blood. Expression of cell surface receptors was carried out following the standard immunophenotyping technique [8,9]. For staining there were used the following mAbs: PE-cyc5 conjugated anti-human CD3, FITC -cyc5 conjugated anti-human CD4, and PE conjugated anti-human CD69 (all – eBioscience). PE-cyc5-conjugated IgG1, FITC-conjugated IgG1 and PE-conjugated IgG1 were used as isotype controls (all – eBioscience). All samples were analyzed using FACScan flow cytometer (Becton&Dickinson) by gating on the Lymphocyte population in FSC/SSC dot plot.

Determining the intracellular interleukins in the study material. For the evaluation of IL-17, IL-22 and IL-9 level and potential activity, the study cells was stained with T helper cell antibodies, followed by the permeabilization of plasma membrane of the cells and staining intracellular interleukins with PE conjugated anti-human IL17, PE conjugated anti-human IL-22 and PE conjugated anti-human IL-9 mAb (all – eBioscience). FITC-conjugated IgG1 and PE-conjugated IgG1 were used as isotype controls (both – eBioscience). The results were measured in percentages from T CD4 + cellular population and data was evaluated statistically. The samples were analyzed in a FACScan flow cytometer (Becton&Dickinson).

The data was statistically analyzed using Mann-Whitney non-parametrical U-test. The values represent averages (M) with standard deviation (SD).

Results and discussion. Our data demonstrated that there was no significant deviation of the immune cell balance compared to normal age-matched healthy volunteers with patients with psoriasis (Fig. 1).

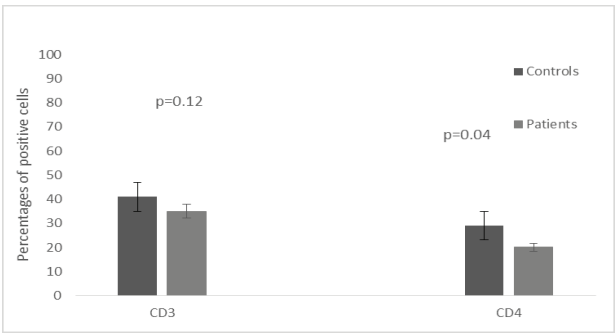

Fig. 1. CD3 and CD4 cell expression in patients with Psoriasis (patients (n=18) and normal controls (n=15)

The percentages of CD3+ T lymphocyte were no significant differences in individuals with psoriasis compared to normal healthy volunteers, which is not surprising since our patients had localized form of disease, therefore no change in the total number of T lymphocytes in peripheral blood was observed. It is noteworthy that, in contrast to control group, the expression level of CD4+ T cells was decreased in the patients, which may be due to mobilization of T helper lymphocytes into the inflammatory areas, which may be result in Th cell number partial decrease in the peripheral blood of patients with psoriasis.

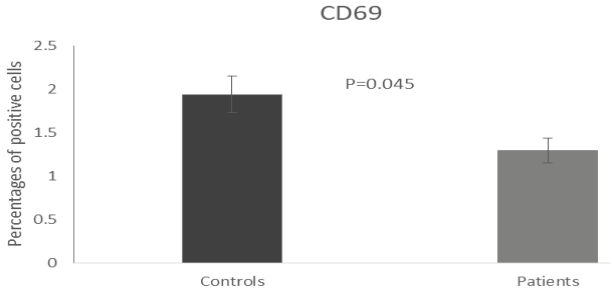

Fig. 2 Evaluation of CD 69 activation marker expression in T helper cells (patients (n=18) and healthy controls (n=15)

Our results indicate that the level of CD69 expression on T helper cells in blood samples of patients with psoriasis were found slightly lower than in healthy groups (Fig.2). CD69 is known to be a marker of Th cell activation, although recent studies have suggested that downregulation of the CD69 marker in psoriatic inflammation areas is considered to be a positive prognostic marker, and its activation results in increased expression of pro-inflammatory interleukins on Th17 cells, such as IL-22 [2]. Therefore, the role of CD69 as a pro-inflammatory marker in peripheral blood appears to be unclear.

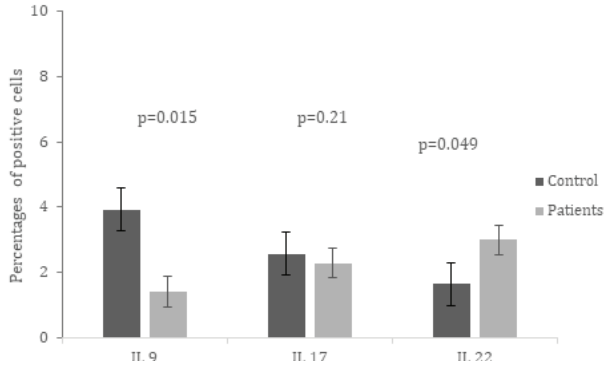

Fig. 3. Evaluation of intracellular IL-9 IL-17 and IL-22 expression in CD4+ T cells in peripheral blood samples in patients with psoriasis (n=*18) and healthy control group (n=15)*

As is well known in the development of psoriasis, CD4+ T cells activated by IL-23, release various cytokines in the inflammatory site and therefore increase the number of interleukins.

In the immunopathogenesis of psoriasis, Th17 cells are actively involved in the initiation and amplification phase of the skin inflammatory process, and Th22 resident cells play a major role as the memory cells, and the role of Th9 cells in the development of recurrent psoriasis is also particularly important. Fig.3 shows that in the peripheral blood samples of patients with psoriasis, elevated levels of IL-22 can be found in T helper cells, as the function of IL-22 is reorganization of the non-immune tissue involved in the inflammatory processes of psoriasis [10], these results therefore are highly logical. Whereas, IL-9 and IL-17 expression levels are decreased in peripheral blood Th cells, which may be explained by mobilization of the corresponding Th9 and Th17 cells into the inflammatory site.

Conclusion. The T cell profile and the IL-23/Th17 axis functional activity levels were significantly different from the literature data obtained about the inflammatory region (psoriatic lesions on the skin). IL-9 and IL-17 expression levels are decreased in peripheral blood CD4 $^{+}$ T cells, which may be explained by mobilization of the corresponding Th9 and Th17 cells into the inflammatory site.

The investigation continues to identify the level of activation of Th17, Th22, Th9 cells in correlation with psoriasis severity.

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SUMMARY

CORRELATION OF CD4+T LYMPHOCYTES ACTIVATION WITH INTERLEUKIN IL-9, IL-17, IL- 22 PROFILES IN THE PERIPHERAL BLOOD OF PATIENTS WITH PLAQUE PSORIASIS

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Psoriasis is a T cell mediated chronic inflammatory skin disease affecting about 2% of the population worldwide. Recently has established the central role of IL-23/Th17 immune axis in the pathogenesis of psoriasis and different subclasses of T cells including Th1 and Th17 cells are involved in initiation and amplification of the skin inflammation process, in addition, in cases of recurrent psoriasis, Th22 cells play the role of memory cells with the help of Th9 cells, which are also important in this process.

The main goal was to evaluate the ratio of T cell profile and IL23/Th17 axis by evaluating IL17A, IL22, IL9 in peripheral blood of persons with moderate to severe plaque psoriasis.

We have estimated the activation of IL-23/Th17 axis by evaluating the level of IL-17A, IL-22 and IL-9 in peripheral blood of patients with plaque psoriasis (n=18) with different severity of the disease (PASI from 10 to 40) comparing the results with data obtained from healthy persons (n=15). The expression of CD69 activation marker on T helper cells has been evaluated as well. The results were analyzed using FACScan flow cytometer (Becton Dickinson).

The percentage of CD3 + T lymphocytes in the peripheral blood of patients with psoriasis was not significantly different compared to normal healthy volunteers, however, the level of expression of CD4 + T cells was reduced. We observed a dramatic increase in IL22 along with a decrease in the level of expression of IL-9 and IL-17, the expression of Th activation marker (CD69) was also decreased in comparison with the control group.

The T cell profile and the IL-23/Th17 axis functional activity levels were significantly different from the literature data obtained about the inflammatory region (psoriatic lesions on the skin). IL-9 and IL-17 expression levels are decreased in peripheral blood Th cells, which may be explained by mobilization of the corresponding Th9 and Th17 cells into the inflammatory site.

Keywords: psoriasis, T cells activation, cell marker, peripheral blood sample, Interleukin.

РЕЗЮМЕ

КОРРЕЛЯЦИЯ АКТИВАЦИИ CD4+T ЛИМФОЦИТОВ С ПРОФИЛЯМИ ИНТЕРЛЕЙКИНОВ IL-9, IL-17, IL-22 В ПЕРИФЕРИЧЕСКОЙ КРОВИ БОЛЬНЫХ БЛЯШЕЧНЫМ ПСОРИАЗОМ

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Псориаз - хроническое воспалительное заболевание кожи, опосредованное Т-клетками. Псориазом поражено около 2% населения всего мира. На сегодняшний день установлена центральная роль IL-23/TH17 иммунной оси в патогенезе псориаза. Немаловажную роль в развитии псориаза играют различные подклассы Т-клеток, включая Th1 и Th17, участвующих в инициации и усилении процесса воспаления в псоритической коже. Кроме того, при рецидиве псориаза Th22 клетки с помощью Th9 клеток играют роль клеток памяти, что весьма значимо в патогенезе псориаза.

Целью исследования явилась оценка соотношения профиля Т-клеток и иммунной оси IL-23/Th17 в периферической крови больных бляшечным псориазом средней и тяжелой степени тяжести.

Оценка активации оси IL-23/Th17 и экспрессии маркера активации CD69 на Т-хелперных клетках в периферической крови пациентов с бляшечным псориазом (n=18; PASI от 10 до 40) проведена с использованием проточного цитометра FACScan (Becton Dickinson). Результаты сравнены с соответствующими данными здоровых лиц (n=15).

Процент CD3+Т-лимфоцитов в периферической крови пациентов с псориазом существенно не отличался от данных здоровых добровольцев, однако, уровень экспрессии CD4+Т-клеток был понижен. Наблюдалось резкое увеличение IL-22 с одновременным снижением уровня экспрессии IL-9 и IL-17, а экспрессия маркера активации CD69 на Th клетках была понижена в сравнении с контролем.

Полученные в результате проведенного исследования данные о соотношении Т-клеток и уровня функциональной активности оси IL-23/Th17 в периферической крови больных бляшечным псориазом значительно отличаются от существующих литературных данных псориатического очага. Уровень экспрессии IL-9 и IL-17 в Th-клетках периферической крови был понижен, что, по всей вероятности, объясняется мобилизацией соответствующих Th9 и Th17 клеток в местах воспаления.

რეზიუმე

CD4+T ლიმფოციტების აქტივაციის კორელაცია ინტერლეიკინ IL-9, IL-17, IL-22 პროფილთან ბალთოვანი ფსორიაზით დაავადებულ პირთა პერიფერიულ სისხლში

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ფსორიაზი T უჯრედებით წარმოადგენს პირობადებულ ქრონიკულ დერმატოზს, რომლის ინციდენტობა მსოფლიოში დაახლოებით 2% შეადგენს. ბოლო პერიოდში დადასტურდა IL-23/Th17 იმუნური ღერძის ცენტრალური როლი ფსორიაზის პათოგენეზში. ანთების ინიციაციასა და შენარჩუნების პროცესში მნიშვნელოვან როლს ასრულებენ T უჯრედების სხვადასხვა ქვეკლასები, Th1 და Th17-ის ჩათვლით. ასევე, ფსორიაზის რეციდივის დროს Th22 უჯრედები Th9-ის დახმარებით ასრულებენ მეხსიერების უჯრედების როლს, რაც მნიშვნელოვანია ფსორიაზის პათოგენეზში.

კვლევის მიზანს წარმოადგენდა საშუალო და მძიმე ბალთოვანი ფსორიაზით დაავადებულ პაციენტთა პერიფერიულ სისხლში T უჯრედების პროფილისა და IL23/Th17 იმუნური ღერძის შეფასება.

შეფასებულია ბალთოვანი ფსორიაზით პაციენტების (n=18; PASI 10-დან 40-მდე) პერიფერიულ სისხლში IL-23/Th17 ღერძის აქტივაცია, ასევე აქტივაციის მარკერის CD69-ის ექსპრესია T ჰელპერებზე. კვლევაში გამოყენებულია FACS გამდინარე ციტომეტრი (Becton Dickinson). შედეგები შედარებულია ჯანმრთელი მოხალისეების (n=15) ანალოგიურ მაჩვენებლებთან.

ფსორიაზით დაავადებულ პაციენტთა პერიფერიულ სისხლში CD3 + T ლიმფოციტების პროცენტული მაჩვენებელი მნიშვნელოვნად არ განსხვავდებოდა ჯანმრთელი მოხალისეების იგივე მაჩვენებლებისგან, თუმცა, CD4 + T უჯრედების ექსპრესიის დონე აღმოჩნდა შემცირებული. გამოვლენილია IL-22-ის დონის მნიშვნელოვანი ზრდა, IL-9 და IL-17-ის ექსპრესიის კლებით. Th-ის აქტივაციის მარკერის CD69 ექსპრესია T ჰელპერებზე ასევე იყო შემცირებული საკონტროლო ჯგუფის ანალოგიურ მაჩვენებელთან შედარებით.

ბალთოვანი ფსორიაზით დაავადებულ პაციენტთა პერიფერიული სისხლის კვლევის შედეგად მიღებული T უჯრედების პროფილის და IL-23/Th17 ღერძის ფუნქციური აქტიურობის მონაცემები მნიშვნელოვნად განსხვავდება ფსორიაზულ კერაში (კანში) ლიტერატურაში არსებული მონაცემებისგან. პერიფერიულ სისხლის Th უჯრედებში შემცირებულია IL-9 და IL-17 ექსპრესის დონე, რაც შეიძლება აიხსნას შესაბამისი Th9 და Th17 უჯრედების ანთებით კერებში მობილიზაციით.

COMORBIDITY OF TYPE 1 DIABETES MELLITUS WITH OTHER CHRONIC PATHOLOGY IN CHILDREN

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The most important features of modern human pathology are the predominance of chronic diseases, the genesis of which is multifactorial, the prevalence of diseases characterized by systemic damage, as well as comorbidity, or the coexistence of several in one person - two or more diseases [6].

Diabetes mellitus type 1 (DM1) is one of the most common chronic diseases developing in childhood, characterized by absolute insulin deficiency following autoimmune-mediated destruction of pancreatic beta cells. The incidence of the disease in children increases for unknown reasons at a rate from 3 to 5% every year worldwide. About 1 in every 400-600 children and adolescents has DM1 [9]. Complex interactions between environmental and genetic factors contribute to the development of DM1 in genetically predisposed patients [2]. The DM1-induced or is one manifestation of systemic autoimmune process can also affect other organs, resulting in development of additional autoimmune or other chronic diseases in the patient, thereby impeding diabetes control. The most common DM1 comorbidities include autoimmune thyroid diseases, celiac disease, and autoimmune gastritis; additionally, diabetes can be a component of Polyglandular Autoimmune Syndrome [1].

Diabetes causes musculoskeletal changes that lead to symptoms such as joint pain and stiffness; swelling; nodules under the skin, particularly in the fingers; tight, thickened skin; trigger finger; carpal tunnel syndrome; painful shoulders; and severely affected feet. Several studies focus on the diabetes as a risk factor and the risk of different types of arthritis [3, 11]. What starts off as a hormonal problem can evolve into joint problems, in addition to the widely known cardiovascular problems. Diabetes raises your risk of having arthritis, including rheumatoid arthritis (RA) and arthritis-related issues, by about 20% [12]. RA and type 1 diabetes mellitus (T1DM) are both autoimmune diseases. Chronic inflammation from diabetes may pave the way for arthritis or other chronic autoimmune disorders [11].