DOI 10.26724/2079-8334-2022-3-81-35-39
UDC 616.981.214.2:577.254

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FEATURES OF THE CYTOKINE TYPE OF REGULATION OF THE IMMUNE RESPONSE IN PATIENTS WITH ERYSIPELAS

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The study of the problems of changes in the cytokine status in erysipelas shows the absence in the studies of other authors of a precise distribution into the forms and types of the course of the disease. This makes it impossible to determine the cytokine type of immune response for the appointment of an individual pathogenetically substantiated treatment. We analyzed the state of the granulocytic link of the phagocytic system, cytokine status, types of immune response, taking into account the form (erythematous, bullous, phlegmonous-necrotic) and the type of course of erysipelas (primary and recurrent, which are complicated by sepsis). Incomplete phagocytosis against the background of a deficit in the functional and metabolic reserve of neutrophils is observed in groups with sepsis and in the recurrent course of the phlegmonous-necrotic form. Changes in immune response regulation from Th2-type (erythematous form) to Th1-type (sepsis) were revealed in primary erysipelas and all recurrent erysipelas. Bullous and phlegmonous-necrotic forms of primary erysipelas are characterized by an immune response of the Th1/Th2 type (with the predominance of the Th1 type). The revealed changes indicate a sharply increasing bacterial load and the severity of the disease. Determining the types of the regulation characteristic of patients with various forms and courses of the disease indicates the need for an individual approach to treatment and the mandatory prescription of drugs with an immunomodulatory effect.

Key words: erysipelas, microbial landscape, cytokines, phagocytosis, functional and metabolic status of neutrophils.

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ОСОБЛИВОСТІ ЦИТОКІНОВОГО ТИПУ РЕГУЛЯЦІЇ ІМУННОЇ ВІДПОВІДІ У ПАЦІЄНТІВ З РОЖИСТИМ ЗАПАЛЕННЯМ

Вивчення проблематики змін цитокінового статусу при бешиховому запаленні показує відсутність в дослідженнях інших авторів чіткого розподілу на форми і типи перебігу захворювання, що унеможливлює визначення цитокінового типу імунної відповіді для призначення індивідуального патогенетично обгрунтованного лікування. Нами проаналізовано стан гранулоцитарної ланки фагоцитарної системи, цитокіновий статус, типи імунної відповіді з урахуванням форми (еритематозна, бульозна, флегманозно-некротична) та типу перебігу бешихового запалення (первинним та рецидивуючим, що ускладнені сепсисом). Незавершений фагоцитоз на тлі дефіциту функціонально-метаболічного резерву нейтрофілів спостерігається в групах з сепсисом та при рецидивному перебігу флегманозно-некротичної форми. Виявлена зміна типів регуляції імунної відповіді від Th2-типу (еритематозна форма) до Th1-типу (сепсис) при первинній бешихі та всіх формах рецидивуючої бешихи. Бульозній та флегмонозно-некротичній формам первинної бешихи характерна імунна відповідь Th1/Th2-типу (з переважанням Th1-типу). Виявлені зміни вказують на різко наростаюче бактеріальне навантаження та тяжкість перебігу захворювання. Визначення типів регуляції, які властиві пацієнтам з різною формою та перебігом захворювання, вказує на необхідність індивідуального підходу до лікування та обов'язкового призначення препаратів з імуномодулюючою дією.

Ключові слова: бешиха, мікробний пейзаж, цитокіни, фагоцитоз, функціонально-метаболічний статус нейтрофілів.

The study is a fragment of the research project "Sepsis in patients with surgical forms of erysipelas", state registration No. 0121U110228.

The relevance of studying erysipelas is due to a high level of morbidity without a tendency to decrease. It occupies an important place in the structure of both temporary disability and disability [5, 9]. Despite many studies, there is still a need for further study of the pathogenesis of the disease. The pathogenetic processes in the skin are controlled by a complex of immune reactions [1], and the reparative generation of the skin directly depends on the functional state of the immune system [10].

Features of the immune response in erysipelas are caused by microbial associations, which are formed as a result of skin dysbiosis, which occurs when its barrier function is disrupted and local immunity is suppressed by pathogenic microorganisms [13]. So, along with gram-negative ones, fungi and aerobes, the leading role belongs to gram-positive flora (streptococci), the result of which is aimed at excessive production of cytokines by T-lymphocytes and neutrophils, the induction of which is a protective or pathological mechanism, i.e., a direct response to the presence of microorganisms [3, 9].

A distinctive feature of the pathogens of this pathological condition is their high level of tolerance to phagocytes, which has an important effect on the course of the disease [5].

Cytokines are a system that forms and regulates the whole complex of pathophysiological changes, determines the effectiveness of the immune response and the course of the inflammatory response in the body [14], while the choice of an immune system of a particular method of protection (Th_1, Th_2 type) depends on the type of antigen and its anti-effect of phagocytosis [7].

It should be noted that when assessing the immune status, conventional criteria are traditionally used, without taking into account the state of the phagocytosis system in the formation of nonspecific resistance of the organism.

Many studies in the literature [2, 8, 11, 14] are devoted to determining the cytokine status in patients with erysipelas. However, studies were conducted on a small number of patients; almost all the groups studied were mixed, i.e. without dividing as forms of erysipelas, both inflammation and the course of the disease (primary and recurrent). This complicates understanding the features of cytokine regulation inherent in a particular group. In our opinion, insufficient attention was paid to studying the state of the phagocytic system and its influence on the course of the disease.

Significant variability of the results presented in the literature sources available for study [2, 8, 11, 12] does not allow revealing significant differences between the norm and pathology, as well as determine the type of immune response (Th_1, Th_2- type), the significance of which in the pathogenesis of the disease study is insufficiently analyzed.

The purpose of the study was to establish features of the cytokine type of regulation of the immune response in patients with erysipelas.

Materials and methods. We examined 46 donors and 109 patients with erysipelas who were hospitalized in the purulent-septic center of the city hospital No. 3 in Zaporizhia. The age of the patients was 45–62 years.

The inclusion criterion in the study was erysipelas: erythematous, bullous, phlegmonous-necrotic forms; primary and recurrent course; with localization in the face, upper and lower extremities. Exclusion criteria from this study: recurrent and postpartum forms of erysipelas, type 1 and 2 diabetes mellitus, acute and chronic viral infections, intestinal infections, pneumonia, acute cardiovascular diseases, pregnancy. The diagnosis was verified based on clinical and anamnestic data according to the classification of V.L. Cherkasov (1986).

The control group comprised 46 practically healthy donors without acute and chronic infectious and autoimmune diseases and allergic reactions. The groups were comparable in terms of gender and age. All examined were born and live in the Zaporizhzhia region and gave an informative written agreement to participate in the study, which was approved by the bioethics committee of the state institution "Zaporizhzhia Medical Academy of postgraduate education of the Ministry of Health of Ukraine". The study was carried out according to the ethical, moral, and legal requirements of the Ministry of Health of Ukraine No. 281, dated November 1, 2000.

The studied patients were divided into groups.

– Group 1 – donors (conditionally healthy contingent) in the amount of 46 people.

– Group 2 – comparison group (patients with an erythematous form of erysipelas) in the amount of 24 people.

– Group 3 – 68 patients with primary erysipelas, which were divided into subgroups, depending on the form of the disease:

– Subgroup 3a – patients with a boolean form of erysipelas in the number of 20 people.

– Subgroup 3b – patients with a phlegmonous-necrotic form of erysipelas in the amount of 40 people.

– Subgroup 3c – patients with erysipelas complicated by sepsis in the amount of 8 people.

– Group 4 – 17 patients with recurrent erysipelas, who were divided into groups:

– Subgroup 4a – patients with a phlegmonous-necrotic form of erysipelas in the amount of 12 people.

– Subgroup 4b – patients with erysipelas complicated by sepsis in the amount of 5 people.

Patients with the bullous form of recurrent erysipelas were not studied because such patients are extra sporadic.

A bacteriological examination of the wound discharge of purulent-necrotic foci was carried out on an automated apparatus, "Vitek-2" (France).

To study the cellular factors of innate resistance, the phagocytic activity of neutrophils in the blood was determined. According to N.T. Frimel, the study is based on a method for determining their absorption and digestion capacity with a microbial test culture after joint preincubation.

Oxygen-dependent metabolism of neutrophils (nitroblue tetrazolium test, spontaneous, NBTsp) and functional reserve of cells (nitroblue tetrazolium test stimulated, NBTst) were determined by M. Ye. Viksman, A. N. Mayansky.

The content of pro-inflammatory and anti-inflammatory interleukins (IL): IL-1β, IL-2, IL-6, IL-10, and TNF-α was determined by enzyme immunoassay. The cytokine status was studied using appropriate monoclonal antibody sets of Vector-Best Test systems (Russia) on the Tecan-Sunrise ISA analyzer (Austria). The studies were carried out in the laboratories of the Department of CLD and ED of the "ZMAPO MOH of Ukraine" (certificate of technical competence No. 045/19 dated 10/25/2019, 067/20 dated 07/03/2020)

Statistical processing of the obtained data was performed using the STATISTICA package computer programs (StatSoft Statistic v.6.0, STA 862D175437Q).

Statistical significance of the compared indicators with a non-normal distribution, determined by the Kolmogorov-Smirnov and Shapiro-Wilk goodness-of-fit test, was determined using the Wald-Wolfowitz run test at a critical level of statistical significance p=0.05. The considered data are presented as "median and interquartile range": Me (RQ=UQ-LQ).

Results of the study and their discussion. We carried out a microbiological study of the discharge from the places of violation of the skin. A total of 31 bacterial species have been identified. Among the gram-positive flora, the prominent representatives of the micrococcus genus were Staphylococcus aureus and Staphylococcus epidermidis. Small amounts of Enterococcus faecalis, Enterococcus faecium, and Enterococcus adalacties were isolated.

Gram-negative flora had a broad spectrum: Escherichia coli, Pseudomonas aeruginosa, and a small amount of Klebsiella pneumoniae, Proteus mirabilis, Klebsiella oxytoca, Pseudomonas putida.

Candida nonalbicans and Candida famata represented the fungi.

Factors of pathogenicity of pathogens, including opportunistic pathogens, affect the evolutionarily well-established mechanisms of regulation of the immune defence of the macroorganism in various ways. This play a key role in ensuring its homeostasis and minimizing the consequences of almost any process, mainly depending on the adequate functioning of different parts of the immune system. As a result of the study of the phagocytosis system in patients of the second group, a decrease in the absorption capacity of neutrophils (phagocytic index of neutrophils, FIN) by 30' and 120' by 25 % and 28 % was revealed. The digesting capacity of neutrophils (phagocytic number of neutrophils, FNN) was increased by 35 % and 15 % compared to the control group.

The indices of the spontaneous NBT test were increased by 16 % and stimulated by 11 % about the indicators of the control group (Table 1).

Table 1

State of the functional and metabolic status of neutrophils in patients with various forms of primary and recurrent erysipelas Me (RQ=UQ-LQ)

Donors (n=46)		Group 2 (n=24)	Erysipelas primary			Erysipelas recurrent	
			3a	3b	3c	4a	4b
			(n=20)	(n=40)	(n=8)	(n=12)	(n=5)
FIN for 30 minutes, %	67.5 68–66=2	50.6* 55.1–44.3 =10.8	54.3* 56.8–53.2 =3.4	55.3* 59.1–53.2 =5.9	32.4*,** 34.5–30.2 =4.3	42.3* 47.9–38.5 =9.4	28.0*,** 31.2–25.3 =5.9
FNN for 30 minutes, RU	2.8 2.9–2.7 =0.2	3.8 4.2–3.6 =0.6	4.0 4.2–3.7 =0.5	4.2* 4.7–3.8 =0.9	3.3 3.7–3.2 =0.5	3.1 4.0–2.6 =1.4	2.0** 2.4–1.4 =1.0
FIN for 120 minutes, %	60.1 64–58=6	43.4* 47.1–40.8 =6.3	50.2 55.7–49.2 =6.5	51.7 56.6–49.4 =7.2	28.1*,** 30.9–26.3 =4.6	38.0* 39–36.6 =2.4	25*,** 30.1–22.7 =7.4
FNN for 120 minutes, RU	3.4 4.3–3.0 =1.3	3.9 4.4–3.6 =0.8	4.2 4.4–4.1 =0.3	4.4 5.0–4.1 =0.9	3.9 4.4–3.3 =1.1	3.1 4.0–2.9 =1.1	2.5** 2.9–2.1 =0.8
NBTsp, RU	1.2 1.3–1.1 =0.2	1.4 1.9–1.1 =0.8	1.7 2.1–1.3 =0.8	1.5 1.6–1.4 =0.2	1.4 1.8–1.0=0.8	1.1 1.7–0.5 =1.2	0.8 1.2–0.5 =0.7
NBTst, RU	1.8 2.0–01.6 =0.4	2.0 2.0–1.8 =0.2	2.2 2.6–2.1 =0.5	1.4 1.7–1.1 =0.6	1.0 1.1–0.8 =0.3	0.9 1.1–0.6 =0.5	0.6** 0.7–0.4 =0.3

Note: * – p-level=0.05 in relation to the control group; ** – p-level=0.05 in relation to group 2.

In subgroup 3a, the absorption capacity of neutrophils decreased by 30' and 120' by 22 % and 16 %, while the digestion capacity increased by 42 % and 23 % relative to the comparison group.

Indices of NBTsp and NBTst tests were increased by 21 % and 10 % in the comparison group.

In patients of subgroup 3b at 30' and 120', the absorption capacity of neutrophils was reduced by 18 % and 14 %, and the digesting capacity was increased by 50 % and 29 % in the indicators of the comparison group.

NBTsp and NBTst indicators were increased by 25 % and 15 % among the indicators of the comparison group.

In subgroup 3b, there was a decrease in the absorption capacity of neutrophils by 30' and 120' by 48 % and 47 % and an increase in the digestive function by 18 % and 15 % in relation to the indicators of the comparison group.

NBTsp indices were increased by 16 %, and NBTst were reduced by 45 % about the indicators of the comparison group.

Patients in subgroup 4a showed a decrease in the absorption capacity of neutrophils by 30' and 120' by 63 % and 63 %. The digestion capacity was slightly increased by 30' – by 11 % and decreased by 120' – 9 % about the indicators of the comparison group.

NBTsp indices were reduced by 8 %, and NBTst by 50 % about the indicators of the comparison group.

Patients in subgroup 4b showed a decrease in the absorption capacity of neutrophils by 30' and 120' by 59 % and 53 %, while the digesting capacity decreased by 71 % and 73 % in relation to the indicators of the comparison group.

When studying the state of the cytokine status in patients of group 2, an increase in the level of cytokines IL-1β was observed by 242 %, and a decrease in the level of IL-2 by 19 %. In contrast, TNF-α, IL-6, and IL-10 remained unchanged relative to the control group (Table 2).

Table 2

State of the cytokine status in patients with various forms of primary and recurrent erysipelas Me (RQ=UQ-LQ)

Donors (n=46)		Group 2 (n=24)	Erysipelas primary			Erysipelas recurrent	
			3a	3b	3c	4a	4b
			(n=20)	(n=40)	(n=8)	(n=12)	(n=5)
IL-1β pkg/mL	2.1 2.8–1.7=1.1	5.1 7.3–4.5=2.8	5.8 6.2–4.1=2.1	17.3*,** 23.3–15.1 =8.2	41.7*,** 49.4–36.8 =12.6	23.5*,** 29.7–18.9 =10.8	58.3*,** 649–49.1 =15.8
IL-2 pkg/mL	4.2 5.3–3.4=1.9	3.4 4.8–2.7=2.1	3.1 3.9–2.0 =1.9	2.8 3.0–2.7 =0.3	2.0*,** 2.4–1.9 =0.5	2.5* 3.0–1.8=1.2	1.9*,** 2.2–1.1=1.1
TNF α pkg/mL	3.3 5.0–2.4=2.6	3.4 4.3–2.9 =1.4	5.5*,** 12.4–6.7 =5.7	6.8*,** 4.8–2.4 =2.4	7.2*,** 3.6–1.5 =2.1	8.3*,** 2.3–1.0=1.3	9.5*,** 2.7–0.9=1.8
IL-6 pkg/mL	9.8 13.0–6.9 =6.1	10.2 12.7–8.1 =4.6	3.2*,** 4.6–2.0 =2.6	12.4 16.8–9.7 =7.1	8.7 10.9–6.8 =4.1	8.2 10.3–6.7=3.6	7.4*,** 9.0–5.9=3.1
IL-10 pkg/mL	3.8 4.2–3.3=0.9	3.8 4.8–2.9=1.9	3.5 4.0–2.9 =1.1	3.0 4.1–2.5 =1.6	2.4 4.8–1.7 =3.1	2.8 3.3–1.9=1.4	2.0* 3.7–0.8=2.9

Note: * – p-level=0.05 in relation to the control group; ** – p-level=0.05 in relation to group 2.

In subgroup 3a, an increase in the level of IL-1β and TNF-α – by 176 % and 61 % were observed, while the levels of IL-2, IL-6 and IL-10 were reduced by 13 %, 218 % and 8 %, respectively, relative to indicators of the comparison group. In patients of subgroup 3b, an increase in the level of IL-1β, TNF-α, and IL-6 was observed by 239 %, 100 % and 21 %, while the levels of IL-2 and IL-10 were reduced by 16 % and 21 %, respectively, relative to comparison groups.

In subgroup 3c, there was an increase in the level of IL-1β and TNF-α by 717 %, 111 % and a decrease in the level of IL-2, IL-6, IL-10 by 42 %, 25 % and 37 % relative to the comparison group.

In patients of subgroup 4a, an increase in IL-1 and TNF-α by 360 % and 144 % were observed, while IL-2, IL-6, and IL-10 were reduced by 27 %, 20 % and 26 % relative to the comparison group.

In patients of subgroup 4b, an increase in the level of IL-1β, and TNF-α, by 1143 % and 179 % was observed, while the levels of IL-2, IL-6, IL-10 were reduced by 56 %, 37 % and 47 % relative to indicators of the comparison group.

After analyzing the specificity of cytokine status, it was found that in patients with erysipelas. In group 2, the immune response was of the Th2 type. In subgroups 3a, and 3b, it was shifted towards the Th2/Th1 type, with a predominance of the Th1 type, while in 3c, 4a, and 4b, it corresponded to the Th1 type.

A number of authors [3, 5, 6], who study the problems associated with erysipelas, note that not only the presence of microbial associations contributes to the rapid course of the disease and the development of complications. but also, the full functioning of immune mechanisms, in particular, cytokine regulation, which is fully consistent with the results of our research.

Excessive bacterial burden, as one of the main components of erysipelas, depending on the form and course of the disease (which was not found in the available works), affects the main mechanisms of

immune defence. In particular, phagocytosis (in patients 3c, 4a, and 4b, incomplete phagocytosis was observed against the background of a severe deficiency of the functional and metabolic reserve), which leads to long-term persistence within the outer cover and contributes to the formation of a chronic relapsing course of the disease [7].

Analysis of literary data on the treatment of erysipelas indicates that the use of antibiotics, in addition to positive effects, has a depressive impact on phagocytosis [5, 6]. Consequently, therapy should be based on a combination of antibacterial and immunomodulatory agents (nevertheless, there is no data on which forms and types of erysipelas should be treated with immunomodulatory therapy) [2]. In our work, cytokine types of the immune response have been identified, which can be markers of complications in the course of the disease and can be used to prescribe immunomodulatory therapy.

Changing the typical immune response from Th_2 - type (erythematous form) to Th_1- type in patients with primary erysipelas (sepsis) and all conditions of recurrent erysipelas, with a change in types from Th_2-type to Th_1-type, with a predominance of Th_1-type (bullous, phlegmonous-necrotic forms) with primary erysipelas, indicates a sharply increasing bacterial load and the severity of the disease, which does not allow forming an adequate immune response [8].

The result of this study is the need to prescribe pathogenetically substantiated treatment with immunomodulators, which will increase the duration of remission in the case of primary erysipelas and increase the inter-relapse period in the case of recurrent erysipelas.

Conclusions

1. Violation of granulocytic protection in patients with erysipelas is directly related to the frequency of relapses and complications, making it possible to predict the outcome of the disease.

2. The results obtained during the research made it possible to identify the types of immune regulation inherent in patients with various forms and courses of the disease, which indicates the need for an individual approach to treatment.

3. Based on the study's results, patients with primary and recurrent erysipelas complicated by sepsis and with recurrent erysipelas, phlegmonous-necrotic form, need to be prescribed drugs with an immunomodulatory effect.

Prospects for further research. Taking into account the above facts, it is planned to determine the presence of correlations between the composition of the microbial discharge from the wound surface, the level of cytokines and markers of inflammation, taking into account the severity and course of the disease. This would suggest that the microbiome affects the severity of the disease.

References

1. Bashkina OA, Samotrueva MA, Azhikova AK, Pakhnova LR. Neiroimmunologicheskaya reguliatsiya fiziologicheskikh i patologicheskikh protcessov v kozhe. Meditsinskaya immunologiya. 2019; 5: 807–20. DOI.org/10.15789/1563-0625-2019-5-807-820. [in Russian]
2. Bekenova NB, Gribovski AM, Mukovozova LA. Soderzhanye tsytokynov u bolnykh rozhei. Nauka i zdravookhranenye. 2015; 6: 55–66. Available from: https://cyberleninka.ru/article/n/soderzhanie-tsitokinov-u-bolnyh-rozhey. [in Russian]
3. Brazhnik EA, Ostroushko AP. Rozhistoe vospalenie v khirurgicheskoy praktike. Nauchnoe obozrenie. Meditsinskie nauki. 2016; 4: 14–7. Available from: https://science-medicine.ru/ru/article/view?id=903. [in Russian]
4. Vasylevska LA. Vplyv mikrobnoho peizazhu na rozvytok destruktyvnykh form beshykhy. Suchasni medychni tekhnolohiyi. 2019; 2: 17–9. DOI: https://doi.org/10.34287/MMT.2(41).2019.3 [in Ukrainian]
5. Yeroshenko DV, Korobov VP. Adgeziia stafilokokkov: pervyi shag k obrazovaniyu bioplenok. Uspekhi sovremennoy biologii. 2017; 1: 100–12. Available from: https://rucont.ru/efd/592010. [in Russian]
6. Gopatsa GV, Ermakova LA. Rozha: sovremennoe sostoianie problemy. Nauchnyi almanakh. 2016; 1-2(15): 364–66. DOI: 10.17117/na.2016.01.02.364. [in Russian]
7. Kazmirchuk VE, Kovalchuk LV, Maltsev DV. Klinicheskaya immunologiya i allergologiya s vozrastnymi osobennostiami. Uchebnik. Kiev: VSI Meditsina; 2012. 520 p. [in Russian]
8. Nikolov VV, Ospanbekova NK, Denefil EV. Immunnye i neiroreguliatornye narusheniya pri rozhistoy infektsii. Klinichna imunolohiya, alergolohiya, infektolohiya. 2015; 1: 43–8. [in Russian]
9. Shapoval SD. Hniyno-septychna khirurhiya. Navchalnyi posibnyk. Kyiv: Medytsyna; 2019. 192 p. [in Ukrainian]
10 Boyce ST, Lalley AL. Tissue engineering of skin and regenerative medicine for wound care. Burns Trauma. 2018; 6: 4. DOI: 10.1186/s41038-017-0103.
11. Brishkoska-Boshkovski V, Dimitrovska I, Kondova-Topuzovska I. Clinical Presentation and Laboratory Characteristics in Acute and Recurrent Erysipelas. Open Access Maced J. Med. Sci. 2019; 7(5): 771–774. DOI: 10.3889/oamjms.2019.213.
12. Brishkoska-Boshkovski V, Kondova-Topuzovska I, Damevska K, Petrov A. Comorbidities as Risk Factors for Acute and Recurrent Erysipelas. Open Access Maced J Med Sci. 2019; 7(6): 937–942. DOI: 10.3889/oamjms.2019.214.
13. Dreno B, Araviiskaia E, Berardesca E, Gontijo G, Sanchez Viera, Xiang L, Martin R, Bieber T. Microbiome in healthy skin, update for dermatologists. J. Eur. Acad. Dermatol. Venereol. 2016; 30(12): 2038–47. DOI: 10.1111/jdv.13965.
14. Hali F, Belanouane S, Zarouali Ouariti K, Sodqi M, Chiheb S. Erysipelas of the leg: A cross-sectional study of risk factors for recurrence. Ann. Dermatol. Venereol. 2022; 149(2): 119–122. DOI: 10.1016/j.annder.2021.08.006.

Стаття надійшла 26.08.2021 р.