Research

Annals of Clinical and Medical Case Reports

Synthetic Peptide LKEKK as Potential Antipsoriatic Drag

Elena V. Navolotskaya*, Vladimir B. Sadovnikov, Dmitry V. Zinchenko and Arkady N. Murashev

Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Science Avenue, 6, Pushchino, Moscow Region, 142290 Russian

***Correspondence author:**
Elena V. Navolotskaya,
Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Science Avenue, 6, Pushchino, Moscow Region, 142290 Russian Federation,
E-mail: navolotskaya@bibch.ru

Received Date: 01 Apr 2023
Accepted date: 25 Apr 2023
Published Date: 02 May 2023

1. Abstract

It has been established that the synthetic peptide LKEKK affects the ability of IL-12 to regulate the secretory activity of keratinocytes obtained from human psoriatic skin: in the concentration range of 100-1000 nM the peptide increases in a dose-dependent manner the production of IL-10 and IFN-γ as well as suppression of the production of IL- 17 induced by IL-12 in vitro. The peptide with the inverted sequence KKEKL was inactive, which indicates the high specificity of the peptide LKEKK action. An in vivo study of the peptide LKEKK activity in a model of imiquimod (IMQ)-induced psoriasis in mice showed that its daily application to the ear (150-500 μg) together with Aldara cream containing 5% IMQ for 6 days significantly suppresses the development of the inflammatory process.

2. Keywords:
Protein: Peptide, Receptor, Cytokine, Keratinocyte, Inflammation, Psoriasis, Skin

3. Introduction

The skin is the largest organ of the mammals with three main functions: protection, regulation and sensation. Mammalian skin is composed of the epidermis, dermis and adipose tissue. The outer layer - epidermis consists mainly of keratinocytes with an integral population of dendritic antigen-presenting cells. The dermis is formed by connective tissue containing various immune cells and several associated appendages (hair follicles, sweat and sebaceous glands, as well as muscle fibers that give the skin strength and elasticity). Adipose tissue forms the inner layer of the skin (Breitkreutz etal., 2009).

Keratinocytes are the main cells of the epidermis; they respond to various environmental factors and are directly involved in the regulation of the inflammatory response of the skin, producing pro- and anti-inflammatory cytokines (Guilloteau et al., 2010; Robeony et al., 2014).

Several years ago we synthesized the peptide LKEKK and found that it was able to suppress the inflammation in the human intestinal epithelium in vitro and in vivo: the peptide bound to human Caco-2 intestinal epithelial cells with high affinity and reduced the TNF-α-induced expression of pro-inflammatory cytokines IL- 6, IL-8, IL-1β and increased the expression of the anti-inflammatory cytokine IL-10 (Navolotskaya et al., 2018). In addition, the ability of the peptide to suppress the development of the inflammatory process induced by sodium dextran sulfate was demonstrated in a mouse model of colitis (Navolotskaya et al., 2019).

Recently we obtained similar results in the study of the effect of the peptide LKEKK on human keratinocytes: the peptide bound to these cells with high affinity, in the concentration range of 50-1000 nM it reduced IL-17A-induced production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1α) and increased the production of anti-inflammatory cytokine IL-10 in vitro The action of the peptide was mediated by the activation of soluble guanylate cyclase (Navolotskaya et al., 2020; Navolotskaya et al.2021a). We evaluated the anti-inflammatory activity of the peptide in mouse models of acute and chronic contact dermatitis induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). Experiments have shown that topical application of the peptide significantly reduced edema and associated pathological changes in the epidermis (Navolotsksys et al., 2021b). Based on the results obtained, it was concluded that the LKEKK peptide has significant therapeutic potential as an anti-inflammatory agent. It is known that the protective functions of the skin provide a variety of epidermal and immune elements that are part of the associated lymphoid tissue (SALT). The response of these cells to damage allows homeostasis to be restored. .Vulgar psoriasis is a widespread and most studied inflammatory skin disease, in the development of which most elements of SALT are involved to varying degrees (Lowes et al., 2014). In particular, keratinocytes are actively involved in the regulation of immune responses in SALT of psoriatic skin (Lowes et al., 2013). It has been shown that IL-17, IL-23, and TNF-α expressed by these cells play a key role in the development of psoriatic inflammation (Bouchaud et al., 2013).

The purpose of this study is to study the effect of the peptide LKEKK in psoriasis in a mouse model of the disease.

4. Materials and Methods

4.1. Chemicals

Human Keratinocyte Medium EpiGro was obtained from Cell Applications, Inc. (USA), IL-17A, TNF-a, IL-10, IL-12 and other

Annals of Clinical and Medical Case Reports

chemicals were obtained from Sigma (St. Louis, MO).

4.2. Peptides

Human thymosin-α_1 and human interferon-α_2 were obtained from Immundiagnostik AG (Germany). Peptides LKEKK (Np5) and KKEKL (iNp5) were synthesized on an Applied Biosystems Model 430A automatic synthesizer (USA) using the Boc/Bzl tactics of peptide chain elongation as described previously (Schnolzer et al, 1992). The peptides were purified to homogeneous state by preparative reverse-phase HPLC (Gilson chromatograph, France) on a Delta Pack C18 column, 100A (39×150 mm, mesh size 5 µm; flow rate 10 mL/min, elution with 0.1% TFA, gradient of acetonitrile 10–40% in 30 min). The molecular masses of peptides were determined by fast atom bombardment mass spectrometric analysis (Finnigan mass spectrometer, San Jose, CA). The data of amino acid analysis (hydrolysis by 6 M HCl, 22h, 110°C; LKB 4151 Alpha Plus amino acid analyzer, Sweden) and mass spectrum analysis are presented in Table 1.

Table 1: Main characteristics of the peptides

Peptide	Purity, %	Amino acid analysis data	Molecular mass, D
Np5	>98	Glu 1.08, Leu 1.00, Lys 3.32	645.4 (calculated value - 644.87)
iNp5	>97	Glu 1.14, Leu 1.05, Lys 3.30	648.5 (644.87)

4.3. Keratinocyte cultures

Normal human epidermal keratinocytes (Cryopreserved HEK, Adult: Frozen HEK (5x10^5), CAT.# 102-05a) were obtained from Cell Applications, Inc. (USA) and were cultured for 24 h in Keratinocyte serum-free medium EpiGro containing EpiLife undefined growth supplement (Thermo Fisher Scientific, USA) in a 5% CO_2 incubator at 37°C and were used at the second or third passage. Keratinocytes from the skin of psoriasis patients were kindly provided by Dr. Shcherbatova Yu.A. ("State Scientific Center for Dermatovenereology and Cosmetology" of the Ministry of Health of the Russian Federation, Moscow). Cells were cultured for 24 hours in a serum-free EpiGro medium containing 10% fetal calf as described above. Cells were preincubated with LKEKK or KKEKL peptide (10–1000 nM) for 1 hour before stimulation with IL-12 (250 nM).

4.4. Cytokine ELISAs

To measure the concentrations of cytokines in keratinocytes, cells were homogenized in three volumes of ice-cold PBS containing 1 mM PMSF, 10 µg/mL aprotinin, 10 µg/mL leupeptin, and 10 µg/mL pepstatin A (Sigma-Aldrich) using a POLYTRON® PT 1200 E (Kinematica AG., Switzerland) and centrifuged at 12,000 ×g for 10 min at 4°C. The protein concentration was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard. was measured by ELISA. Results are expressed as cytokine amount per total protein concentration. ELISAs were carried out using according to the manufacturer's instructions (BD Biosciences, San Jose, CA). Data are presented as mean ± SEM.

4.5. In vivo studies

In vivo experiments we used C57BL/6 mice at the age of 7–11 weeks (18–20 g, Nursery of Branch of Institute of Bioorganic Chemistry, Russian Academy of Sciences, Pushchino, Moscow Region). All procedures described below met the requirements for working with laboratory animals. To create a model of psoriasis used the method of van der Fits et al. (2009). Inflammation was induced by daily topical application of 60 mg Aldara cream containing 5% imiquimod (3M Health Care Ltd/UK) to the upper and lower surface of the right ear of the animals for 6 days. Skin thickness was measured daily with a digital caliper. The degree of inflammation was assessed by determining the change in the thickness of the treated skin compared to the control (thickness of the untreated skin on day 0). Peptide treatment (phosphate buffered solution 50-500 µg/ear) was administered concurrently with Addara's cream daily for 6 days.

4.6. Statistical Analysis

Data are expressed as means ± SEM. Student's t-test was used when comparisons were made only between the two groups. Differences were considered significant when $p < 0.05$.

5. Results and Discussion

The main characteristics of the synthesized peptides (purity, amino acid content, and molecular mass) are shown in Table 1.

The discovery of IL-6 and its receptor subunits laid the foundation for understanding the biology of a family of related cytokines: IL-12, IL-23, and IL-27. These cytokines have a similar spatial organization, use common receptors, and affect the outcome of infectious and inflammatory diseases (Wojno et al., 2019).

Interleukin-12 (IL-12) - a protein consisting of two 35 and 40 kDa subunits was isolated and characterized by Kobayashi et al. in 1989 (Kobayashi et al., 1989). The IL-12 receptor is a heterodimeric complex that includes unique α-chain providing specificity and gp130 chain common for the IL-6-related cytokine subfamily that mediates signaling through the JAK-STAT signaling cascade (Hirano et al., 1994; Taga et al., 1995; Presky et al., 1996).

In the mid-1990s, mice deficient of the IL-12 subunits were obtained, and, as expected, they were more susceptible to pathogens than wild-type

Annals of Clinical and Medical Case Reports

animals (Magram et al., 1996; Mattner et al., 1996; Decken et al., 1998; Cooper et al., 2002; Elkins et al., 2002; Lieberman et al., 2004). These results could not be explained by differences in IFN-γ levels, although the effects of IL-12 were initially believed to be due solely to its ability to stimulate IFN-γ production in T and NK cells (Trinchieri, 2003). Compared to wild-type mice both, p40(-) and p35(-)mice showed IFN-γ deficiency; at the same time, p35(-) mice showed increased expression of IL-4 and IL-10, while levels of lymphotoxin-α and TNF-α were reduced. These results suggested that there is a molecule other than the pro-inflammatory protein IL-12p70, using both subunits, capable of mediating signaling from IL-12 to SALT cells (Brombacher et al. 1999; Becher et al., 2002; Cua et al., 2003; Gran et al., 2002; Lynde et al., 2014).

The trigger of chronic skin inflammation in psoriasis is epidermal damage, followed by activation of mononuclear phagocytes and an increase in their secretion levels of IL-12, TNF, and IL-23. Subsequent immune responses, including the production of IL-17, IL-22, and IL-21 by T-helpers, provoke neutrophil infiltration and hyperactivation of keratinocytes which further fuel the pro-inflammatory cascade of reactions. (Perera et al., 2012). The first psoriasis drugs targeted TNF followed by a second generation that inhibited IL-12/IL-23 and IL-23 (Becher et al., 2012). IL-12 and IL-23 have a common p40 subunit associated with p35 and p19 subunits, respectively (Croxford et al., 2014). Mice that lacked the p40 subunit showed resistance to various patterns of chronic inflammation (Brombacher et al., 1999; Cua et al., 2003; Pantelyushin et al., 2012). Ustekinumab, the first monoclonal antibody (mAb) directed against p40, proved to be a more effective and safer treatment for psoriasis than TNF inhibitors (Leonardi et al., 2008).

Later, after preclinical trials of Ustekinumab, the p19 protein and, as a result, IL-23 were discovered (.Oppmann et al., 2000). Subsequent preclinical studies have shown that although mice lacking the IL-23-specific p19 subunit were resistant to the induction of many inflammatory diseases, including psoriasis, deficiency of IL-12p35 or the IL12rβ2 subunit of the IL-12 receptor resulted in an exacerbation of inflammation. On this basis, it was concluded that IL-23 mediates the development of psoriatic inflammation, while IL-12 plays a regulatory rather than pathological role in the pro-inflammatory cascade (Gordon et al., 2018). Recently, IL-23p19-specific mAbs, Guselkumab and Risankizumab have been obtained that are more effective than p40-targeted mAb Ustekinumab in the treatment of psoriasis (Langley et al., 2018). Taken together, these data indicated the dominant role of IL-23 in stimulating inflammation, while IL-12 performs regulatory and protective functions. However, it should be noted that although the protective role of IL-12 has been proven in preclinical models of psoriasis (Kulig et al., 2016), the cellular targets and mechanisms underlying it have not been established until recently.

A strain of Il12rb2 (KO)/reporter knockout mice was recently obtained (Zwicky et al., 2021). The creation of a conditional knockout (KO) and an Il12rb2 reporter allele in mice made it possible to identify IL-12 sensitive cells and their systematic deletion in the skin stroma and hematopoietic compartment. It was found that the deletion of Il12rb2 in the hematopoietic compartment does not affect the development of Aldara-induced psoriasis-like inflammation. At the same time, depletion of IL12rb2 in keratinocytes exacerbated the development of the disease. Thus, protective IL-12 signaling blocks keratinocyte hyperproliferation, maintains skin barrier integrity, and counteracts inflammation. The fact that IL-12 has a strong effect on the stroma of affected tissue supports the suggestion that psoriasis is not primarily the result of immune dysfunction, but may be a consequence of altered tissue homeostasis. Thus, it was found that the protective effect of IL-12 in psoriasis is directed to keratinocytes and consists in blocking the hyperproliferation of these cells, maintaining the integrity of the skin barrier and, as a result, counteracting the development of inflammation.

The results presented in this work show that the peptide LKEKK in the concentration range of 10-1000 nM does not affect the secretion of IL-10, IL-17, IFN-γ and TNF-α by keratinocytes obtained from the skin of patients with psoriasis; at the same time, IL-12 at concentrations of 50–1000 nM significantly increases the production of IL-10 and IFN-γ by these cells, suppresses their secretion of IL-17, and does not affect the level of TNF-α secretion (Table 2).

Table 2: Effect of peptide LKEKK (Np5) on IL-12-induced production of IL-10, IL-17, TNF-α by keratinocytes from human psoriatic skin

Np5/IL-12 (nM)	IL-10	IL-17	IFN-γ	TNF-α
	(pg/mg protein ± SEM)			
- (Control)	10 ± 2	336 ± 42	95 ± 14	272 ± 23
10	11 ± 2/ 12 ± 2	330 ± 45/ 324 ± 45	90 ± 12/ 99 ± 12	274 ± 27/ 278 ± 29
20	12 ± 2/ 15 ± 3	339 ± 40/ 308 ± 44	90 ± 15/ 120 ± 13*	270 ± 22/ 275 ± 25
50	10 ± 2/ 26 ± 4*	335 ± 39/ 265 ± 32*	98 ± 14/ 163 ± 19*	272 ± 20/ 277 ± 28
100	12 ± 2/ 44 ± 4*	333 ± 46/ 237 ± 28*	96 ± 15/ 276 ± 29*	269 ± 24/ 273 ± 23
500	10 ± 2/ 43 ± 6*	330 ± 38/ 228 ± 32*	95 ± 13/ 282 ± 34*	279 ± 28/ 271 ± 27

Annals of Clinical and Medical Case Reports

1000	11 ± 2/ 46 ± 5*	338 ± 45/ 230 ± 36*	97 ± 16/ 289 ± 37*	273 ± 24/ 275 ± 26

* Significant difference from the control (P < 0.05).

We studied the effect of the LKEKK peptide on the ability of IL-12 to regulate the secretory activity of psoriatic keratinocytes in vitro. For this, the cells were treated with peptide (10-1000 nM) and IL-12 (250 nmol/mL) was added to induce cytokine secretion. In parallel, the peptide with an inverted KKEKL sequence was tested as a negative control. Experiments showed that treatment of keratinocytes with the peptide LKEKK in the concentration range of 50-1000 nM increased the production of IL-10 and IFN-γ in a dose-dependent manner, decreased the secretion of IL-17, and did not affect the production of TNF-α (Table 3). The peptide with the inverted sequence was inactive, indicating a high specificity of the LKEKK peptide action.

Table 3: Effect of the peptide LKEKK on IL-12-induced production of cytokines by keratinocytes from the skin of patients with psoriasis

Peptide (nM)	Cytokine (pg/mg protein ± SEM)		
	IL-10	IFN-γ	IL-17
Control (250 nM IL-12)	43.3 ± 3.6	273 ± 24	332 ± 36
10	46.7 ± 4.3	282 ± 27	305 ± 39
50	60.9 ± 4.6*	303 ± 29	270 ± 21*
100	68.8 ± 6.0*	347 ± 35*	242 ± 25*
250	78.5 ± 6.2*	359 ± 30*	232 ± 23*
500	79.3 ± 6.7*	363 ± 32	224 ± 32*
1000	78.8 ± 7.3*	366 ± 38*	220 ± 36*

* Significant difference from the control (P < 0.05).

As noted above, we recently showed that the LKEKK peptide binds with high affinity and specificity to normal human keratinocytes and, in the concentration range of 50–1000 nM, reduces IL-17A-induced production of TNF-α, IL-6, IL-1α and increases production of IL-10 in vitro (Navolotskaya et al., 2019; Navolotskaya etal., 2020). The results presented in this work show that the peptide is not able to directly influence the secretory activity of keratinocytes in psoriasis-affected skin: its effect on the production of IL-10, IFN-γ, and IL-17 by these cells mediates IL-12.

Table 4: Effect of the peptide LKEKK (Np5) on changes in ear thickness in mice treated with Aldara cream

Action	Increase in ear thickness (mm)/experiment day					
	1	2	3	4	5	6
Aldara[1]	0.7 ± 0.1	1.5 ± 0.2	1.8 ± 0.2	2.7 ± 0.2	3.2 ± 0.3	3.8 ± 0.2
Aldara + Np5[2]	0.8 ± 0.1	1.4 ± 0.3	1.7 ± 0.3	2.5 ± 0.2	2.9 ± 0.4	3.2 ± 0.3
Aldara + Np5[3]	0.6 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.8 ± 0.3*	2.3 ± 0.3*	2.5 ± 0.2*
Aldara + Np5[4]	0.5 ± 0.2	0.8 ± 0.3	1.0 ± 0.2*	1.5 ± 0.2*	1.7 ± 0.3*	1.9 ± 0.3*
Aldara + Np5[5]	0.5 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	1.3 ± 0.2*	1.6 ± 0.2*	1.7 ± 0.3*

Note: 1 - Aldara cream 60 mg daily for 6 days; 2 - Addara 60 mg + Np5 50 mcg/ear daily for 6 days; 3 - Addara 60 mg + Np5 150 mcg/ear daily for 6 days; 4 - Addara 60 mg + Np5 300 mcg/ear daily for 6 days; 5 - Addara 60 mg + Np5 500 mcg/ear daily for 6 days.
* P<0.05 compared with Addara's cream treatment, mean values ± SEM are shown.

Annals of Clinical and Medical Case Reports

References

1. Burkhard Becher, Brigit G Durell, Randolph J Noelle. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. J. Clin. Invest. 2002; 110: 493-497. DOI: 10.1172/JCI15751
2. Becher B, Pantelyushin S. Hiding under the skin: Interleukin-17-producing γδ T cells go under the skin? Nat. Med. 2012; 18: 1748-1750.
3. Bouchaud G, Gehrke S, Krieg C, Kolios A, Hafner J, Navarini AA, et al. Epidermal IL-15Rα acts as an endogenous antagonist of psoriasiform inflammation in mouse and man. J. Exp. Med. 2013; 210: 2105-2117.
4. Breitkreutz D, Mirancea N, Nischt R. Basement membranes in skin: Unique matrix structures with diverse functions? Histochem. Cell Biol. 2009; 132: 1-10.
5. Brombacher F, Dorfm€uller A, Magram J, Dai WJ, Köhler G, Wunderlin A, et al. IL-12 is dispensable for innate and adaptive immunity against low doses of Listeria monocytogenes. Int. Immunol. 1999; 11: 325-332.
6. Chorachoo J, Lambert S, Furnholm T, Roberts L, Reingold L, Auepemkiate S, et al. The small molecule rhodomyrtone suppresses TNF-α and IL-17A-induced keratinocyte inflammatory responses: A potential new therapeutic for psoriasis. PLoS One. 2018; 13: e0205340.
7. Cooper AM, Kipnis A, Turner J, Magram J, Ferrante J, Orme IM. Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. J. Immunol. 2002; 168: 1322-1327.
8. Croxford AL, Kulig P, Becher B. IL-12-and IL-23 in health and disease. Cytokine Growth Factor Rev. 2014; 4: 415 421.
9. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature. 2003; 421: 744-748.
10. Decken K, Köhler G, Palmer-Lehmann K, Wunderlin A, Mattner F, Magram J, et al. Interleukin-12 is essential for a protective Th1 response in mice infected with Cryptococcus neoformans. Infect. Immun. 1998; 66: 4994-5000.
11. Elkins KL, Cooper A, Colombini SM, Cowley SC, Kieffer TL. In vivo clearance of an intracellular bacterium, Francisella tularensis LVS, is dependent on the p40 subunit of interleukin-12 (IL-12) but not on IL-12 p70. Infect. Immun. 2002; 70: 1936-1948.
12. Gordon KB, Strober B, Lebwohl M, Augustin M, Blauvelt A, Poulin Y, et al. Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials. Lancet. 2018; 392: 650-661.
13. Gran B, Zhang G-X, Yu S, Li J, Chen X-H, Ventura ES, et al. IL-12p35-deficient mice are susceptible to experimental autoimmune encephalomyelitis: evidence for redundancy in the IL-12 system in the induction of central nervous system autoimmune demyelination. J. Immunol. 2002; 169: 7104-7110.
14. Guilloteau K, Paris I, Pedretti N, Boniface K, Juchaux F, Huguier V, et al. Skin Inflammation Induced by the Synergistic Action of IL-17A, IL-22, Oncostatin M, IL-1α, and TNF-α Recapitulates Some Features of Psoriasis. J. Immunol. 2010; 184: 5263-5270.
15. Hirano T, Matsuda T, Nakajima K. Signal transduction through gp130 that is shared among the receptors for the interleukin 6 related cytokine subfamily. Stem Cells. 1994; 12: 262 277.
16. Kobayashi M, Fitz L, Ryan M, Hewick RM, Clark SC, Chan S, et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. Exp. Med. 1989; 170: 827-845.
17. Kulig P, Musiol S, Freiberger SN, Schreiner B, Gyülveszi G, Russo G, et al. IL-12 protects from psoriasiform skin inflammation. Nat. Commun. 2016; 28: 7. 13466. DOI: 10.1038/ncomms13466
18. Langley RG, Tsai TF, Flavin S, Song M, Randazzo B, Wasfi Y, et al. Efficacy and safety of guselkumab in patients with psoriasis who have an inadequate response to ustekinumab: results of the randomized, double-blind, phase III NAVIGATE trial. Br. J. Dermatol. 2018; 178: 114-123. DOI: 10.1111/bjd.15750
19. Leonardi CL, Kimball AB, Papp KA, Yeilding N, Guzzo C, Wang Y, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). Lancet. 2018; 371: 1665-1674.
20. Lieberman LA, Cardillo F, Owyang AM, Rennick, DM, Cua DJ, Kastelein RA, IL-23 provides a limited mechanism of resistance to acute toxoplasmosis in the absence of IL-12. et al. Immunol. 2004; 173: 1887–1893. DOI: 10.4049/jimmunol.173.3.1887.
21. Lowes M.A, Suárez-Farinas M, Krueger JG. Immunology of Psoriasis. Ann. Rev. Immunol. 2014; 32: 227-255. DOI: 10.1146/annurev-immunol-032713-120225.
22. Lowes M.A, Russell CB, Martin DA, Towne JE, Kruege r JG. The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses. Trends Immunol. 2013; 34: 174-181. DOI: 10.1016/j.it.2012.11.005
23. Lowry OH, Rosebbrough NJ, Farr OL, Randal RJ. Protein measurement with the Folin phenol reagent. J.Biol. Chem. 1951; 193: 265-275.
24. Lynde CW, Poulin Y, Vender R, Bourcier M, Khalil S. Interleukin 17A: toward a new understanding of psoriasis pathogenesis. J.Am. Acad. Dermatol. 2014; 71: 141-150. DOI: 10.1016/j.jaad.2013.12.036.
25. Magram J, Connaughton SE, Warrier RR, Carvajal DM, Wu CY, Ferrante J, et al. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. Immunity. 1996; 4: 471-481. DOI: 10.1016/s1074-7613(00)80413-6.
26. Mattner F, Magram J, Ferrante J, Launois P, Di Padova K, Behin R, et al. Genetically resistant mice lacking interleukin-12 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response. Eur. J. Immunol. 1996; 26: 1553-1559. DOI: 10.1002/eji.1830260722.
27. Navolotskaya EV, Sadovnikov VB, Lipkin VM, Zav'yalov VP. Binding of cholera toxin B subunit to intestinal epithelial

cells. Toxicology in Vitro. 2018; 47: 269–273. DOI: 10.1016/j.tiv.2017.12.010.

28. Navolotskaya EV, Sadovnikov VB, Lipkin VM, Zav'yalov VP. Cholera Toxin B Subunit and Peptide LKEKK Inhibit TNF-α Signaling in Intestinal Epithelial Cells and Reduce Inflammation in a Mouse Model of Colitis. J. Clin. Exp. Immunol. 2019; 4: 1-6. DOI: doi.org/10.33140/JCEI.04.04.03
29. Navolotskaya EV, Sadovnikov VB, Zinchenko DV, Zolotarev YA, Lipkin VM, Murashev AN. Effect of the LKEKK Peptide on Human Keratinocytes. Rus J Bioorg Chem. 46, 1038-1043. DOI: 10.1134/S1068162020060229
30. Navolotskaya EV, Sadovniko VB, Zinchenko DV, Zav'yalov VP, Murashev AN. Anti-inflammatory Effect of the Peptide LKEKK. J. Clin. Exp. Immunol. 2021a; 6: 356-361. DOI: doi.org/10.33140/JCEI.06.05.02
31. Navolotskaya EV, Sadovnikov VB, Zav'yalov VP, Murashev A.N b. Anti-inflammatory Effect of Peptide LKEKK on Keratinocytes. J. Clin. Immunol. Immunother. 2021; 7: 064. DOI: 10.24966/CIIT-8844/1000064
32. Oppmann, B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000; 13: 715-725.
33. Pantelyushin S, Haak S, Ingold B, Kulig P, Heppner FL, Navarini AA, Becher B. Rorγt+ innate lymphocytes and γδ T cells initiate psoriasiform plaque formation in mice. J. Clin. Invest. 2012; 122: 2252-2256. DOI: 10.1172/JCI61862
34. Perera GK, Meglio PDi, Nestla FO, Psoriasis. Annu. Rev. Pathol. 2012; 7: 385-422. DOI: 10.1146/annurev-pathol-011811-132448
35. Presky DH, Yang H, Minetti LJ, Chua AO, Nabavi N, Wu CY, et al. A functional interleukin 12 receptor complex is composed of two beta-type cytokine receptor subunits. Proc Natl. Acad. Sci. USA. 1996; 93: 14002-14007. DOI: 10.1073/pnas.93.24.14002
36. Robeony H, Petit-Paris I, Garnier J, Barrault C, Pedretti N, Guilloteau K, et al. Inhibition of Keratinocyte Differentiation by the Synergistic Effect of IL-17A, IL-22, IL-1α, TNFα and Oncostatin M. PLoS One. 2014; 9: e101937. DOI: 10.1371/journal.pone.0101937.
37. Schnolzer M, Alewood P, Jones A, Alewood D, Kent SB. In situ neutralization in Boc-chemistry solid phase peptide synthesis. Rapid, high yield assembly of difficult sequences. Int J Peptide Protein Res. 1992; 40: 180-193. DOI: 10.1111/j.1399-3011.1992.tb00291.
38. Taga T, Kishimoto T, Signaling mechanisms through cytokine receptors that share signal transducing receptor components. Curr Opin Immunol. 1995; 7: 17-23. DOI: 10.1016/0952-7915(95)80024-7
39. Trinchieri G, Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat. Rev. Immunol. 2003; 3: 133-146. DOI: 10.1038/nri1001
40. Van der Fits, L Mourits, S Voerman, JS Kant, M Boon, L Laman, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J. Immunol. 2009; 182: 5836-5845. DOI: 10.4049/jimmunol.0802999
41. Wojno EDT, Christopher A Hunter CA, Stumhofer JS. The Immunobiology of the Interleukin-12 Family: Room for Discovery. Immunity. 2019; 50: 851-870. DOI: 10.1016/j.immuni.2019.03.011
42. Zwicky P, Ingelfinger F, Silva de Melo BM, Ruchti F, Schärli S, Puertas N, et al. IL-12 regulates type 3 immunity through interfollicular keratinocytes in psoriasiform inflammation. Sci. Immunol. 2021; 6(64): eabg9012.