 10.15789/2220-7619-EOT-1928

EVALUATION OF THE EFFECTS IN THE "IN VITRO" SYSTEM OF SYNTHETIC THYMIC HEXAPEPTIDE ON THE EXPRESSION LEVELS OF NF-kB, IFNα||βR AND CD119 NEUTROPHILIC GRANULOCYTES IN PATIENTS WITH CHRONIC HERPES VIRAL CO-INFECTIONS

Irina Nesterova [a,c],

Evgeniya Khalturina [b],

Vladimir Nelyubin [d],

Sergey Khaidukov [e],

Galina Chudilova. [c]

[a]The Peoples' Friendship University of Russia, Moscow, Russia,

[b] I. M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

[c]Kuban state medical university, Krasnodar, Russia,

[d]Moscow State University of Medicine and Dentistry Moscow, Russia;

[e] Shemyakin-Ovchinnikov Institute of bioorganic chemistry, Moscow, Russia

ОЦЕНКА ВЛИЯНИЯ СИНТЕТИЧЕСКОГО ТИМИЧЕСКОГО ГЕКСАПЕПТИДА В СИСТЕМЕ «IN VITRO» НА УРОВНИ ЭКСПРЕССИИ NF-kB, IFNα||βR И CD119 НЕЙТРОФИЛЬНЫХ ГРАНУЛОЦИТОВ У ПАЦИЕНТОВ С ХРОНИЧЕСКИМИ ГЕРПЕС-ВИРУСНЫМИ КО-ИНФЕКЦИЯМИ

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

Abstract. Background: Strategies used by herpes viruses with human cells are complex and multifaceted. On one hand, inborn defects in antiviral immune defense have been unveiled, which also affect interferon (IFN) system underlying development of chronic recalcitrant relapsing viral infections such as remittent respiratory viral infections, herpesvirus infections, and papillomavirus infections. On the other hand, numerous viruses are able to damage both immune system and IFN network. During inborn and acquired defects in IFN network, inborn or induced mutation in gene products involved in signaling cascades aimed at upregulating gene expression responsible for IFN production are observed. One of the strategies used by diverse viruses is altering some signaling pathways resulting in activated transcription factors including nuclear factor NF-kB. However, antiviral mechanisms executed by neutrophilic granulocytes (NGs), particularly affecting NF-kB expression have not been elucidated.

Aim of the study: to study in vitro features of NF-kB expression and number of neutrophilic granulocytes (NG) expressing membrane IFNα||βR and IFNγR in patients with atypical chronic active herpes virus infections (AChA-HVI), followed by assessing an effect of arginyl-alpha-aspartyl-lysyl-valyl-tyrosyl-arginine – hexapeptide (HP), a synthetic analogue of the active center of the thymopoietin (active substance of drug "Imunofan", Russia), on the expression of NG NF-kB and IFNα||βR and IFNγR.

Methods: We observed 25 patients of both sexes aged 23 to 64 years with AChA-HVI, manifested by chronic fatigue syndrome and cognitive disorders. Study design: stage 1- clinical, ELISA, PCR methods, FC was used. Stage 2- the in vitro experiment: 32 blood samples from 8 healthy adults and 375 blood samples from 25 patients with AChA-HVI were analyzed: % NG expressing NF-kB, IFNα||βR, IFNγR and the relevant MFI levels by using FC before and after incubation with HP.

Results: Our study demonstrated low level (MFI) of NF-kB expression in 100 % NG associated with decreased % of NG expressing IFNα||βR and IFNγR in all patients with AChA-HVI and low serum level for IFNα and IFNγ in comparison

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

with healthy individuals. In the in vitro experiment there was shown that 100 % of NG expressed NF-kB after exposure to HP. However, only 48 % patients (SG2) restored NF-kB expression level (MFI) to normal range and 52% of cases (SG1) had no response. HP increased % of NG expressing IFNα||βR in SG2 and increased % of NG expressing IFNγR in SG 1.

Conclusions: It was shown, that influence of HP "in vitro" has ambiguous effects on the expression of NF-kB, % of NG expressing IFNα||βR and IFNγR in patients with AChA-HVI. We assume that different NF-kB response to HP is associated with inborn or secondary NF-kB deficiency.

Keywords: herpesvirus infections, interferon system, nuclear factor NF-kB, neutrophilic granulocytes, transcription factors, hexapeptide

Резюме. Стратегии взаимодействие герпесвирусов с клетками организма человека весьма сложны и многогранны. С одной стороны существуют врожденные дефекты противовирусной иммунной защиты, в том числе и системы интерферонов, на фоне которых развиваются хронические упорно рецидивирующие вирусные инфекции, такие как повторные респираторные вирусные инфекции, герпесвирусные инфекции, папилломавирусные инфекции. С другой стороны многие вирусы сами способны повреждать как иммунную систему, так и систему интерферонов. При врожденных и приобретенные дефектах системы интерферонов наблюдается врожденная или индуцированная мутация генов молекул, участвующих в сигналлинге, направленном на повышение экспрессии генов, ответственных за синтез IFN. Одной из стратегий вирусов является нарушение ряда клеточных сигнальных путей - факторов транскрипции, в том числе ядерного фактора NF-kB. В настоящее время описана противовирусная активность НГ. При этом механизмы противовирусной защиты НГ и в частности особенности экспрессии NF-kB в доступной нам литературе не освещены.

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

Цель исследования: изучить особенности экспрессии ядерного фактора NF-kB, мембранных рецепторов к IFNά и IFNγ на нейтрофильных гранулоцитах (НГ) у пациентов, страдающих АХА-ГВИ, с последующей оценкой в эксперименте «in vitro» эффектов влияния на них синтетического аналога активного центра гормона тимопоэтина аргинил-альфа-аспартил-лизил-валил-тирозил-аргинин (гексапептид- ГП, «Иммунофан» Россия)

Материалы и методы: под нашим наблюдением находилось 25 пациентов обоих полов в возрасте от 23 до 64 лет, страдающих атипичными хроническими активными герпес-вирусными инфекциями (АХА-ГВИ), манифестирующими синдромом хронической усталости и различными когнитивными расстройствами. Дизайн исследования: этап 1 включал комплекс традиционных методов (сбор анамнеза, методы физикального обследования, ОАК и пр.), дополнительно для детекции герпес- вирусных инфекций использовались методы серодиагностики (определение IgM VCA EBV, IgG VCA EBV, IgM CMV, IgG CMV IgM HSV1/2, IgG HSV1/2 методом ИФА) Для обнаружения генома вирусов в биоматериалах (кровь, слюна, моча, соскоб с миндалин и задней стенки глотки) был использован метод ПЦР-РВ.

Этап 2 - эксперимент «in vitro»: изучено 32 образца крови от 8 условно здоровых человека и 375 образцов крови от 25 пациентов с АХА-ГВИ: определен % NG, экспрессирующих NF-kB, IFNα || βR, IFNγ R и уровни их MFI с помощью проточной цитофлюориметрии до и после инкубации с ГП (гексапепетидом).

Результаты: В результате проведенного исследования у пациентов, страдающих АХА-ГВИ, был выявлен низкий уровень экспрессии (MFI) NF-kB у 100% НГ, который сочетался со сниженным % NG, экспрессирующих IFNα || βR и IFNγR, и низким уровнем сывороточных IFNα и IFNγ по сравнению со здоровыми людьми. В эксперименте «in vitro» ГП оказывает неоднозначные вариативные эффекты влияния на экспрессию ядерного фактора NF-kB и мембранных рецепторов IFNα|β и IFNγ НГ пациентов, страдающих АХА-ГВИ. Было показано, что 100% НГ экспрессировали NF-kB после воздействия ГП.

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

Но только 48% пациентов (ГИ2) восстановили уровень экспрессии NF-kB (MFI) до нормального значения, а в 52% случаев (ГИ1) динамики не выявлено. В то же время ГП увеличил % НГ, экспрессирующих IFNα || βR в ГИ2 и увеличил % НГ, экспрессирующих IFNγR в ГИ 1.

Заключение: Было показано, что ГП в эксперименте «in vitro» оказывает неоднозначное влияние на экспрессию NF-kB, % NG экспрессирующих IFNα || βR и IFNγR у пациентов с АХА-ГВИ. Мы предполагаем, что различный ответ на влияние ГП связан с врожденным или вторичным дефицитом NF-kB.

Ключевые слова: герпесвирусные инфекции, система интерферона, ядерный фактор NF-kB, нейтрофильные гранулоциты, факторы транскрипции, гексапептид

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

Introduction: Diseases caused by viral agents are one of the most urgent and difficult to solve in the modern medicine. Large DNA-containing enveloped viruses that can interact with various cells of the human body in several ways. Those viruses are causing the development of both acute infections (lytic pathway) and the formation of chronic, often atypical, active forms of infection. Viral genome integrates in different human cells that lead to the persistence of viruses.

Among those viruses, the most interesting is the Herpesviridae family that includes 8 representatives. The Epstein-Barr virus (EBV) is one of the most striking. The viruses of this family are characterized by the formation of both mono- and mixed infections, often with the addition of bacterial, fungal or mixed nature co-infections. The viral interaction strategies with human cells are very complex and multifaceted. On the one hand, there are congenital defects of the antiviral mechanisms of immune defense, including the interferon system [8,22,26,30]. Those innate mistakes of antiviral immune defense lead to the development of recurrent and persistent viral infections, such as repeated respiratory viral infections, chronic herpes viral infections, papillomavirus infections and so on.

On the other hand, many viruses themselves are capable to damage both the immune system and the interferon system. In both cases of innate or acquired defects of the interferon system, congenital or induced genes' mutation of the molecules involved in signaling pathway is observed. Today well known those genes' mutation: TLR3, interferon-regulating factors 3, 7, interferon receptors, interferon-stimulated genes, NF-kB, etc. On the other hand, many viruses themselves are capable to damage both the immune system and the interferon system. In both cases of innate or acquired defects of the interferon system, congenital or induced genes' mutation of the molecules involved in signaling pathway is observed. Today well known those genes' mutation: TLR3, interferon-regulating factors 3, 7, interferon receptors, interferon-stimulated genes, NF-kB, etc. The existing of innate or secondary genes' mutations leads to a violation of the synthesis of IFN type I: IFNα and IFNβ. One of the strategies of viruses is to disrupt a number of cellular signaling pathways - transcription factors, especially NF-kB [2,4,11,16,25].

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

Transcription factors (TFs) are a large group of proteins that interact with DNA at specific regulatory regions (loci), which entails changing gene transcription (activation or inhibition) using domains transactivation or trans-repression [10,40]. TFs are involved in the immunopathogenesis of a wide range of human diseases. The nuclear factor NF-kB is one of the most important in those protein groups. For the first time in 1986, Sen and Baltimore discovered transcription factors of the NF-kB family as specific for B cells [27]. Later it was shown, that the constitutive activation of NF-kB triggers the expression of a huge array of genes associated with the regulation of the immune response, inflammation, including apoptotic resistance, migration and angiogenesis. In this constitutive activation the NF-kB-sensitive genes TNF, IL-1,6,8 CXC-chemokine ligands are involved [24].

In addition, it is known that the activation of the nuclear factor NF-kB is the main mechanism that implements the antiviral activity of the innate immunity. This mechanism can be triggered by various signals induced by the microenvironment. They activate cellular receptors and induce intracellular signaling, by activating the genes of molecules involved in signaling.

However, it should be noted that some of these activated genes, in turn, can target NF-kB. In this case, there is another mechanism. For example, one of the main activated target genes of NF-kB is Isystem Bα, that blocks the activation of NF-kB. [9,35].

In works Zhang J and Kim JC it was shown experimentally that the HSV-1 UL2 protein and ICP27 can counteract the activation of NF-kB mediated by tumor necrosis factor α (TNF-α) and IkappaBalpha [15,20,33,39]. At the same time, the works of other authors have demonstrated that proteins that are part of the structure of the virion of herpes viruses negatively affect various parts of the NF-kB signaling cascade [1]. Those proteins can act through other mediators and signaling pathways leading to long-term, active expression of NF-kB. According to the data, it has been shown that the insertion of EBV into neutrophilic granulocytes (NG) can induce the transition of NG to apoptosis and multidirectionally activate the intracellular

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

signaling pathways, in particular, the cascade of the nuclear factor NF-kB activation [3]. Currently, the antiviral activity of NG has been described. Upon that, the mechanisms of NG antiviral protection and, in particular, the features of NF-kB expression are not covered in the literature.

At the same time, there is practically no data in the modern scientific literature on the features of NF-kB expression in herpes virus co-infections, including atypical chronic active herpes viral co-infections (AChA-HVI). Taking into account the information given above, there is an urgent need for further studies of an expression features of the nuclear factor NF-kB NG in patients suffering from AChA-HVI co-infections

Purpose of the study: to study in the "in vitro" system the features of the expression of nuclear factor NF-kB and the expression of membrane receptors IFNα||βR and IFNγ (CD119) of neutrophilic granulocytes (NG) in patients suffering from ACHA-HVI, followed by an assessment of the effect of arginyl-alpha-aspartyl-lysyl-valyl-tyrosyl-arginine hexapeptide, a synthetic analogue of the active center of the hormone thymopoietin, on the expression of factor NF-kB and the expression of membrane receptors IFNα||βR and IFNγ (CD119) of NG.

MATERIALS AND METHODS.

We observed 25 patients of both sexes aged 23 to 64 years suffering from atypical chronic active herpes virus infections (ACHA-HVI), manifested by chronic fatigue syndrome and various cognitive disorders (the main study group is MSG). This group of patients is characterized by a certain symptom complex. To assess the severity of clinical symptoms of CFS, we used a 5-point scale developed by us. The presence or absence of symptoms, depending on the severity of their manifestation, was evaluated in points from 0 to 4, where: 0 points - absence of symptoms; 1 point - minimal symptoms; 2 points - average severity of symptoms; 3 points - severe degree; 4 points - very severe degree. The control group (CG) consisted of 8 practically healthy individuals corresponding to gender and age.

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

STUDY DESIGN

Stage 1. In the complex of the study, in addition to traditional methods (collection of anamnesis, methods of physical examination, CBC, etc.), serodiagnostic methods were used to detect herpes virus infections (IgM VCA EBV, IgG VCA EBV, IgM CMV, IgG CMV IgM HSV1/2, IgG HSV1/2) using ELISA test systems NPO "Diagnostic Systems" (Russia). To detect the genome of viruses in biomaterials (blood, saliva, urine, scraping from the tonsils and the posterior pharyngeal wall), the PCR method of the «AmpliSens» test system (Russia) was used.

Stage 2. In the in vitro system, 32 blood samples from 8 apparently healthy adults and 375 blood samples from 25 patients with AChA-HVI were examined. The amount (%) of peripheral blood NG expressing the nuclear factor NF-kB, membrane receptors for IFNα||βR, IFNγ (CD119) and the intensity of their expression according to MFI were estimated by flow cytometry using an FC 500 flow cytometer (Beckman Coulter, USA) (value of fluorescence intensity) before and after incubation with hexapeptide (name of the substance according to the nomenclature of international non-proprietary names - INN, ATX code: L03AX).

The study was approved by the Ethics Commission, and informed consent was obtained from all patients to participate in the study and to process personal data in accordance with the World Medical Association's Declaration of Helsinki (WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects, 2013).

For statistical processing of the data obtained, Microsoft Excel computer programs were used. The results were presented as the median (upper and lower quartile) Me [Q1; Q3], Mann-Whitney and Wilcoxon tests. The significance of the difference was determined at $p < 0.05$.

RESULTS:

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

When analyzing the clinical material, it was found that all patients of the main study group suffered from mixed AChA-HVI in 100% of cases. The dominant combinations were: EBV + CMV + HHV6 –52%, EBV + HSV1 - 36%; EBV + CMV –12% of cases. It is important to note that EBV was the predominant virus found in all patient's groups. A number of clinical features of mixed AChA-HVI has been identified: a prolonged feeling of severe weakness, chronic fatigue, in addition, patients worried about sweating, intermittent pain in the throat, muscles and joints (fibromyalgia and arthralgia), headaches, low-grade fever, lymphadenopathy, sleep disturbance, decreased memory, attention, intelligence, less often - psychogenic depression. Often patients suffered from virus-associated recurrent ARVI, chronic repeated herpes-viral infections (HSV1, HSV2), chronic CMV and HHV6 infections, chronic bacterial and fungal infections. Diseases associated with AChA-HVI were characterized by a recurrent course.

All these symptoms were assessed according to our 5-point scale (Tab. 1). The severity of symptoms on this scale was Me [Q1; Q3] - 44.5 [37.5; 51.5].

Tab.1. Assessment scale of clinical symptom severity for post-viral chronic fatigue syndrome

The diagnosis of AChA-HVI was confirmed by serodiagnostic methods, molecular genetic methods (PCR); in addition, violations of the induced IFNα production in 100,0% and a deficiency of the induced IFNγ production in 76,0% of cases were found. The patients of the main study group had a pronounced decrease in the induced production of IFNα to 85 [50; 120] ME/ml and IFNγ to 16 [4; 28] ME/ml.

Analysis of the data obtained showed that in conditionally healthy individuals (control group), the number of NGs expressing nuclear factor NF-kB was 100%, while MFI, assessing the level of expression of nuclear factor NF-kB, was 8.9 [8,7; 10.1]. In addition, it was shown that in the main study group (MG), as in the control group, 100% of NG expressed the nuclear factor NF-kB. However, in comparison

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

with CG, a significant decrease in the level of expression of NF-kB according to MFI was revealed to 5.1 [4.5; 6.5] ($p < 0.05$). (Fig. 1).

Fig. 1. Expression levels of nuclear factor NF-kB in neutrophilic granulocytes of patients suffering from AChA-HVI and in control group (conditionally healthy individuals) according to MFI distribution.

In addition, it was found that in patients of the control group, the number of NGs expressing membrane IFNα||βR was 4.55 [2.3; 7.2] % with MFI 1.19 [1.15; 1.22], and membrane CD119 (IFNγR) - 19.9 [14.3; 27.6]% with MFI 1.48 [1.1; 2.2]. In the main study group (MSG), the number of NGs expressing IFNα||βR was significantly reduced to 1.0 [0.6; 1.9] % ($p < 0.05$) with MFI 1.71 [1.61; 1.91], and the number of NG expressing CD119 (IFNγR) had an insignificant upward trend and amounted to 39.5 [28.7; 48.6] % with MFI 1.48 [1.35; 1.75] (Tab.2).

Tab. 2. Comparative characteristics of the expressed nuclear factor NF-kB, membrane IFNα|βR and CD119 (IFNγR) neutrophilic granulocytes in apparently healthy individuals and patients with AChA-HVI.

An in vitro experiment was carried out in which the effect of HP on the expression of the nuclear factor NF-kB and the number of NGs expressing IFNα||βR and IFNγ was assessed in apparently healthy individuals and patients suffering from AChA-HVI.

It was found that under the influence of a hexapeptide (HP) in the MSG, the population of NG expressing the nuclear factor NF-kB is divided into two subgroups: Study Group 1 (SG 1) and Study Group 2 (SG 2). The levels of NF-kB expression were significantly differ in SG 1 and SG 2. In SG 2 a more high level of MFI NF-kB – 7.5[6.9; 8.0] was detected than in SG 1, in which the level of MFI NF-kB was only 5.5[5.4; 5.6] ($p < 0.01$). After HP influence the level of NF-kB NG expression according to MFI was 5.5 [5.4;7.5] in the SG 1 and did not significantly differ from the decreased level of MFI NF-kB in the MG before HP exposure - MFI 5.1 [4.5; 6.5] ($p \geq 0.01$). Moreover, the level of MFI NF-kB NG expression in SG 2 increased after HP influence from 5.1 [4.5; 6.5] to 7.5 [6.9; 8.0] ($p < 0.01$). At the

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

same time, it was significantly higher than it was been in SG1 -5.5 [5.4; 5.6] ($p < 0.05$) and didn't significantly change from the level of MFI NF-kB in the CG – 8.9 [8.7; 10.1] ($p < 0.05$) (Fig 2).

Fig. 2 Comparison of the expression levels (MFI) for NF-kB in neutrophilic granulocytes from patients with AChA-HVI before and after exposure to HP in in vitro experimental system.

Under the influence of hexapeptide (HP), the NG population in the MSG was divided into two groups (SG1 and SG2) according to the number of NGs expressing membrane IFNα||βR and IFNγ (CD119) (Fig. 3).

Fig. 3. Count of NG expressing membrane receptors IFNα||βR and IFNγ (CD119) before and after HP exposure in patients suffering from AChA-HVI

After influence of HP in SG 1 (52% of cases) an insignificant increasing of NG number (%) expressing membrane IFNα||βR from 1.0 [0.6; 1.9] to 1.65 [1.5; 1.8]% was revealed in comparison with the MG ($p > 0.05$). The expression level of surface membrane IFNα||βR NG according MFI did not change in comparison with the MG too ($p > 0.05$). Meanwhile there was a significant increasing in the number of level NG, expressing membrane CD119 (IFNγR) from 39.5[28.7; 48.6] % to 56.0 [49.6; 58.2]% ($p < 0.05$) after exposure of HP. This fact indicates that the number NG, expressing membrane CD119 (IFNγR) was increased by 1.42 times or by 41.7%. The expression level of surface membrane CD119 (IFNγR) NG according MFI data did not change ($p > 0.05$).

At the same time after exposure of HP an ambiguous effect of HP on the levels of NG expressing membrane IFNα||βR and CD119 (IFNγR) was revealed in the SG 2 (48% of cases). HP has influenced on the level of NG, expressing membrane IFNα||βR in SG2, significantly increasing its number from 1,00 [0.6;1.9]% in MG to 3.81 [3.8;4.2]% in SG2 ($p<0.05$) and reached the NG level of CG ($p > 0.05$). At the same time after influence of HP the expression level according to MFI data of membrane IFNα||βR NG in SG2 did not change in comparison with group CG and MG ($p1,2 > 0.05$).

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

There was an insignificant decreasing in comparison with MSG in the number of the NG (%), expressing membrane CD119 (IFNγR) from 39.5 [28.7; 48.6]% to 32.3 [30.2; 48.1]% ($p > 0.05$). Meanwhile there was a significant increasing in the level of NG, expressing membrane CD119 (IFNγR), from 19.9 [14.3; 27.6] % to 32.3 [30.2; 48.1] %, in comparison with CG ($p > 0.05$). After influence of HP the expression level according to MFI data of membrane CD119 (IFNγR) NG in SG2 did not change in comparison with group CG and MSG ($p_{1,2} > 0.05$).

Discussion:

The problem of treating patients with chronic active herpes virus infections is still very far from being solved. Taking into account that EBV is present in all identified combinations of herpes-viral co-infections and is the dominant infection in the patients included in this study (AHI). Also it's important to consider its negative effect on the nuclear factor NF-kB and membrane receptors IFNα||βR NG, CD119 (IFNγR) expressing by NG.

According to the literature, EBV BGLF2 protein inhibits two key proteins STAT1 and STAT2, which are involved in the stage 2 signaling of type I IFN synthesis. In addition, BGLF2 recruits host cell enzymes to remove the phosphate group from STAT1, thereby inactivating its activity and redirecting STAT2 to degradation. It leads to defective ISG expression and disruption of type I IFN synthesis, and, consequently, to a decrease in IFN type I antiviral and immunomodulatory activity [6,7,13,14,18,19,28,29, 32,33,34,37]. These data confirm the damaging effect of EBV, that causes the occurrence of secondary defects in the expression of not only NF-kB, but also membrane receptors IFNα||βR NG, and do not contradict the results obtained by us during the present study.

It should be noted that earlier in the works of foreign authors the presence of congenital errors of immunity such as primary immunodeficiencies caused by mutations in the genes STAT1 / STAT2, TLR3, UNC93B1, TICAM1, TBK1, IRF3, IRF7, IFNAR1, IFNAR2, which explains the deficiency of spontaneous and induced production of IFN I type was shown [17,21,23,31,36,38]. In this regard, the likelihood of congenital disorders of the interferon system in patients with AChA-

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

HVI (SG1) is not excluded. It is confirmed by the lack of an adequate NF-kB response to the effect of HP in the in vitro system and explains the occurrence of atypical herpes-viral co-infections.

On the other hand, according to our data and according to the literature, autoimmune diseases in parallel with atypical chronic active EBV infection can manifest in patients with a genetic predisposition. There is also evidence that chronic EBV infection can lead to an increase in the expression of the nuclear factor NF-kB, which, in turn, can provoke the development of autoimmune diseases and tumor processes [5,12]. It should be emphasized that we did not observe autoimmune disorders and tumor processes in patients of MG. At the same time we noted the leading syndrome of chronic fatigue, myalgia, arthralgia and the syndrome of minor cognitive disorders that did not exclude the presence of neuroinflammatory changes.

In conclusion, we would like to note that the results obtained in this study allow us to clarify the immunopathogenesis of atypical chronic active herpes virus co-infections associated with the prevalence of EBV infection. The data obtained on the positive effect of in vitro HP on the restoration of the nuclear factor NF-kB expression level, as well as the expression of membrane receptors IFNα||βR NG in, presumably, secondary defects of the interferon system, accompanied by deficiency of type I and II IFNs. These results can serve as a basis for further development of the strategy and tactics of immunotherapy with using active substance HP ("Imunofan", Russia) for restoration of the level of expression of NF-kB NG with further reconstruction of secondary defects of interferon system. This immunomodulatory drug based on the active substance HP may be used in future in clinical practice.

Conclusion

The data obtained made it possible to come to the main conclusions:

1. There is a violation of the nuclear factor NF-kB expression associated with a decrease in the level of number NG expressing membrane receptors IFNα||βR and CD119 (IFNγR) in all patients suffering from AChA-HVI with the deficiencies of a serum IFNα and IFNγ.

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

 10.15789/2220-7619-EOT-1928

2. In the “in vitro” experiment, HP exhibited different effects of influences on the levels of nuclear factor NF-kB expression (MFI) and the level of number NG expressing membrane IFNα||βR NG and membrane IFNγR in patients with AChA-HVI:

- there were a significant restoration of the nuclear factor NF-kB NG expression to the level of healthy individuals in NG of 48% patients with AChA-HVI (SG2) and a significant increasing in the level of number NG expressing membrane IFNα||βR, while the level of number NG expressing IFNγR has not changed;

- in 52% of AChA-HVI (SG1) patients the level of NF-kB NG expression and the level of number NG expressing IFNα||βR NG has not significantly changed, while there was a significant increasing in the level of number NG expressing of membrane IFNγR.

3. Restoration of the expression of NF-kB NG in 48% patients suffering from AChA-HVI under the influence of HP in the experiment may indicate secondary damage to the expression of NF-kB that occurred under the damaging influences of herpes viral co-infections. The absence of an effect of HP on the level of expression NF-kB in 52% patients with AChA-HVI evidences about deeper damages of NF-kB expression, possibly due to congenital disorders expression of NF-kB. However these assumptions require carrying out of further research.

4. These results may serve as a basis for further development of the strategy and tactics of immunomodulate therapy with using active substance HP of drug "Imunofan" (Russia) for restoration of secondary defects of expression of NF-kB, the level of number NG, expressing membrane IFNα||βR, IFNγR and for the reconstruction of normal functioning of the interferon system in patients with AChA-HVI

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

ТАБЛИЦЫ

Tab.1. Assessment scale of clinical symptom severity for post-viral chronic fatigue syndrome

Symptoms	Score Me [Q1;Q3]
• Long term low grade fever;	4,0 [3,5;4,5]
• Throat pain and discomfort;	4,0 [3,5; 4,5]
• Increased sweatiness, sensitivity to cold;	3,5 [2,5; 4,5]
• Headache, migraine;	4,0 [3,5; 4,5]
• regional lymphadenopathy;	4,5 [4,0; 5,0]
• increased fatigue, a significant decrease in efficiency;	5,0 [5,0; 5,0]
• neurological disorders(paraesthesia, synaesthesia, sensitivity disorders, low muscle tone, etc.);	4,5 [4,0; 5,0]
• decrease in memory processes , difficulty concentrating;	3,0 [1,5; 4,5]
• headaches, joint pain, myalgia	3,5 [2,5; 4,5]
• sleep disorders (insomnia orincreased drowsiness);	4,0 [3,5; 4,5]
• panic attacks, mood disorders; emotional lability; psychogenic depression etc	4,5 [4,0; 5,0]
• Total Score	44,5 [37,5;51,5]

Tab. 2. Comparative characteristics of the expressed nuclear factor NF-kB, membrane IFNα|βR and CD119 (IFNγR) neutrophilic granulocytes in apparently healthy individuals and patients with AChA-HVI.

Before the in vitro influence of a hexapeptide						
	CD 119 Me (Q1;Q2)		**IFNα||βR** Me (Q1;Q2)		**NF- kB** Me (Q1;Q2)	
Control group n=6	**% NG**	**MFI**	**% NG**	**MFI**	**%NG**	**MFI**
	19,9 (14,3;27,6)	1,48 (1,1;2,2)	4,55 (2,3;7,2)	1,19 (1,15;1,22)	100	8,9 (8,7;10,1)
Main study group n=25	39,5* (28,7;48,6)	1,48 (1,35;1,75)	1* (0,6;1,9)	1,71* (1,61;1,91)	100	5,1* (4,5;6,5)
Under the in vitro influence of a hexapeptide						

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

	CD 119		IFNα||βR		NF- kB	
Study group 1 n=13	56,0*♦ (49,6;58,2)	1,68 (1,5; 1,9)	1,65* (1,5;1,8)	1,7 (1,6;2,0)	100	5,5* (5,4; 5,6)
Study group 2 n=12	32,3*^ (30,2; 48,1)	1,5 (1,3; 1,6)	3,81♦^ (3,8; 4,2)	1,7 (1,6; 2,0)	100	7,5*♦^ (6,9; 8,0)

** differences from control group*

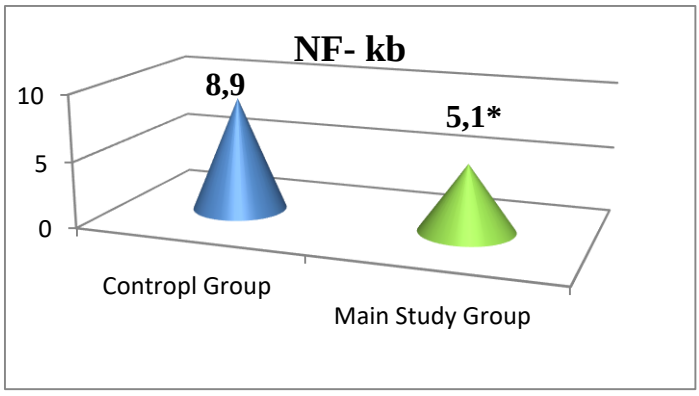
♦ differences from MSG (main study group)

^ differences SG1 and SG2 (study group1 and study group2)

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

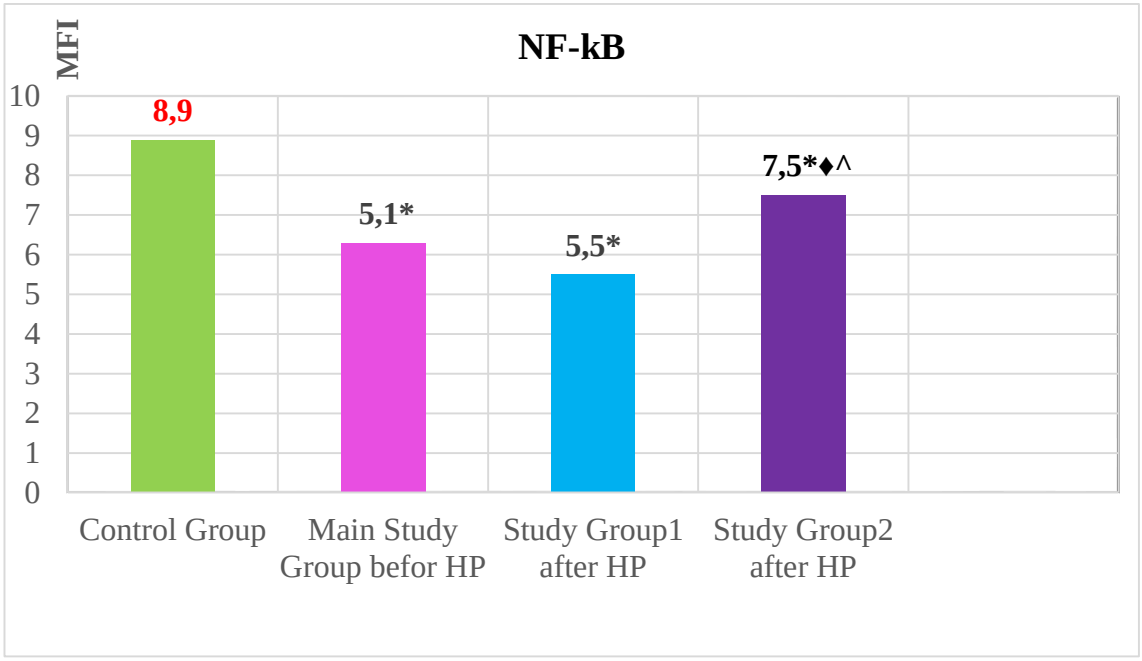
10.15789/2220-7619-EOT-1928

РИСУНКИ



**differences from control group*

Fig. 1. Expression levels of nuclear factor NF-kB in neutrophilic granulocytes of patients suffering from AChA-HVI and in control group (conditionally healthy individuals) according to MFI distribution.

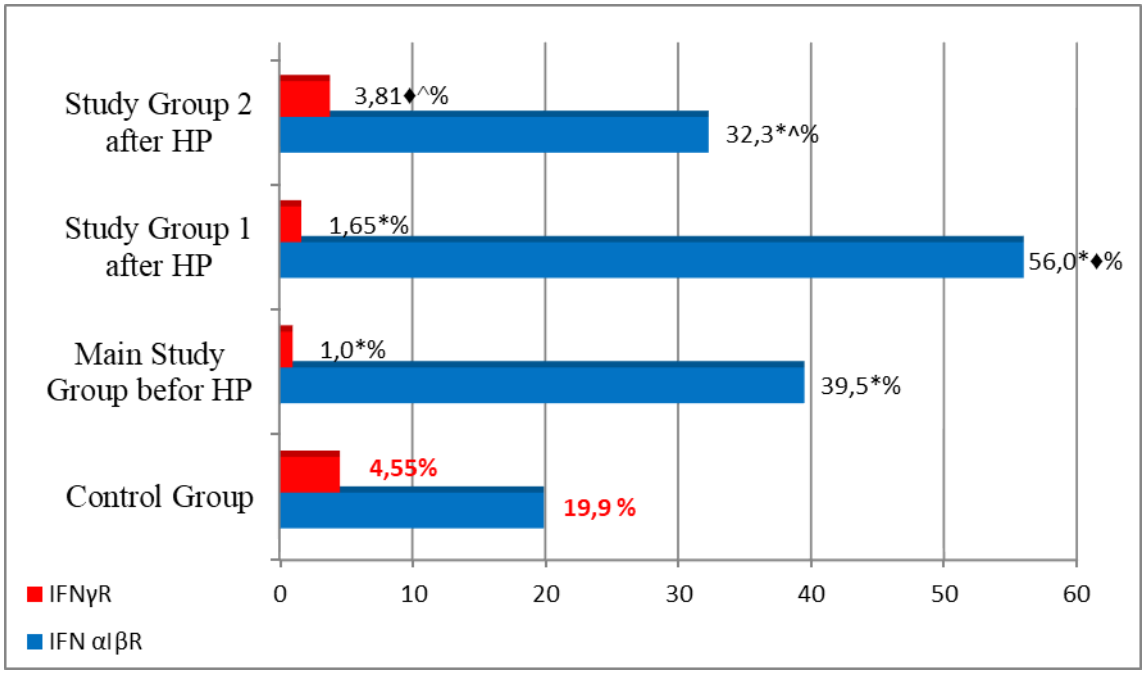


** differences from control group*
♦ differences from MSG (main study group)

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928
^ differences SG1 and SG2 (study group1 and study group2)

Fig. 2 Comparison of the expression levels (MFI) for NF-kB in neutrophilic granulocytes from patients with AChA-HVI before and after exposure to HP in in vitro experimental system.



** differences from control group*
♦ differences from MSG (main study group)
^ differences SG1 and SG2 (study group1 and study group2)

Fig. 3. Count of NG expressing membrane receptors IFNα||βR and IFNγ (CD119) before and after HP exposure in patients suffering from AChA-HVI

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

Блок 1. Информация об авторе ответственном за переписку

Халтурина Евгения Олеговна, кандидат медицинских наук, доцент, доцент кафедры микробиологии, вирусологии и иммунологии имени А.А. Воробьева Сеченовского университета; ORCID: 0000-0002-8948-8983; SCOPUS ID 57196080380; SPIN: 2813-4952; Web of Science ResearcherID AAW-9175-2021, +7(916)650-15-14; jane_k@inbox.ru

Khalturina Evgeniya Olegovna PhD, Department of Microbiology, Virology and Immunology named after A.A. Vorobiev, Sechenov University; ORCID: 0000-0002-8948-8983; SCOPUS ID 57196080380; SPIN: 2813-4952; Web of Science ResearcherID AAW-9175-2021, +7(916)650-15-14; jane_k@inbox.ru

ФГАОУ ВО Первый МГМУ им. И. М. Сеченова Минздрава России (Сеченовский университет) 125009, Москва, ул. Моховая, д. 11, стр. 10
I.M. Sechenov First Moscow State Medical University (Sechenov University) 125009, Moscow, Mohovaya Street, 11, bild.10

Блок 2. Информация об авторах

Халтурина Евгения Олеговна, кандидат медицинских наук, доцент, доцент кафедры микробиологии, вирусологии и иммунологии имени А.А. Воробьева Сеченовского университета; ORCID: 0000-0002-8948-8983; SCOPUS ID 57196080380; SPIN: 2813-4952; Web of Science ResearcherID AAW-9175-2021, +7(916)650-15-14; jane_k@inbox.ru

Khalturina Evgeniya Olegovna PhD, Department of Microbiology, Virology and Immunology named after A.A. Vorobiev, Sechenov University; ORCID: 0000-

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

0002-8948-8983; SCOPUS ID 57196080380; SPIN: 2813-4952; Web of Science ResearcherID AAW-9175-2021, +7(916)650-15-14; jane_k@inbox.ru

Нестерова Ирина Вадимовна, доктор медицинских наук, профессор – профессор кафедры аллергологии и иммунологии ФГАОУ ВО Российский университет дружбы народов, Минздрава РФ

Nesterova Irina Vadimovna- PhD, MD (Medicine), Professor, Department of Allergology and Immunology, Faculty of Continuing Medical Education, Peoples' Friendship University of Russia, Moscow, Russian Federation

Чудилова Галина Анатольевна - доктор биологических наук, доцент кафедры клинической иммунологи, аллергологии и лабораторной диагностики ФПК и ППС ФГБОУ ВО Кубанский государственный медицинский университет, Краснодар, Россия

Chudilova Galina Anatolievna- PhD, MD (Biology), Associate Professor Department of Clinical Immunology, Allergology and Laboratory Diagnostics, Faculty of Continuing Medical Education, Kuban State Medical University, Krasnodar, Russia

Нелюбин Владимир Николаевич- доктор медицинских наук, профессор Научно-исследовательский медико-стоматологический институт Московского государственного медико-стоматологического университета имени А.И. Евдокимова, Москва, Россия

Nelubin Vladimir Nikolaevich- PhD, MD (Medicine), Professor, Research Medical and Dental Institute of A.I. Evdokimov Moscow State Medical and Dental University, Moscow, Russia

Хайдуков Сергей Валерьевич- доктор биологических наук, старший научный сотрудник лаборатории углеводов ФНЦ ФГБУН Институт биоорганической химии им. Ак. М.М. Шемякина и Ю.А. Овчинникова РАН, Москва, Россия

Khaidukov Sergey Valerievich- PhD, MD (Biology), researcher at the carbohydrate laboratory of the Federal Research Center of the Federal State

 10.15789/2220-7619-EOT-1928

Budgetary Institution Institute of Bioorganic Chemistry named after M.M. Shemyakina and Yu.A. Ovchinnikova RAS, Moscow, Russia

1. И.В. Нестерова[1,3], Е.О. Халтурина[2], В.Н. Нелюбин[4], С.В. Хайдуков[5], Г.А. Чудилова[3]

[1]ФГАБОУ ВО «Российский университет дружбы народов» Министерства образования и науки России, Москва, Россия

[2]ФГАОУ ВО Первый МГМУ имени И.М. Сеченова Минздрава России (Сеченовский Университет), Россия,

[3]ЦНИЛ ФГБОУ ВО «Кубанский государственный медицинский университет» Минздрава России, Краснодар, Россия

[4]ФГБОУ ВО Московский государственный медико-стоматологический университет им. А.И. Евдокимова Минздрава России, Москва, Россия

[5]ФНЦ ФГБУН Институт биоорганической химии им. Ак. М.М. Шемякина и Ю.А. Овчинникова

Nesterova I.V.[1,3], Khalturina E.O [2], Nelyubin V.N [4], Khaidukov S.V. [5],Chudilova G.A. [3]

[1]The Peoples' Friendship University of Russia, Moscow, Russia,

[2] I. M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

[3]Kuban state medical university, Krasnodar, Russia,

[4]Moscow State University of Medicine and Dentistry Moscow, Russia;

[5]Shemyakin-Ovchinnikov Institute of bioorganic chemistry, Moscow, Russia

 ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

Блок 3. Метаданные статьи

Сокращенное название статьи для верхнего колонтитула:

Evaluation of thymic hexapeptide

Оценка влияния гексапептида

Keywords: herpesvirus infections, interferon system, nuclear factor NF-kB, neutrophilic granulocytes, trancription factors, hexapeptide

Ключевые слова: герпесвирусные инфекции, система интерферона, ядерный фактор NF-kB, нейтрофильные гранулоциты, факторы транскрипции, гексапептид

Оригинальная статья

Количество страниц текста – 10, количество таблиц – 2, количество рисунков –3.

16.04.2022

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

СПИСОК ЛИТЕРАТУРЫ

П/ссылки	Авторы, название публикации и источника, где она опубликована, выходные данные	ФИО, название публикации и источника на английском	Полный интернет-адрес (URL) цитируемой статьи и/или
1.	Amici C, Belardo G, Rossi A, Santoro MG Activation of I kappa b kinase by herpes simplex virus type 1. A novel target for anti-herpetic therapy. J Biol Chem. 2001 Aug 3; 276(31):28759-66	-	[PubMed] [Ref list]
2	Caselli E, et al. Human herpesvirus 8 acute infection of endothelial cells induces monocyte chemoattractant protein 1–dependent capillary-like structure formation: role of the IKK/NF-κB pathway. Blood. 2007; 109(7):2718–26	-	CAS PubMed Article PubMed Central Google Scholar
3	Charostad, J., Nakhaie, M., Dehghani, A. *et al.* The interplay between EBV and KSHV viral products and NF-κB pathway in oncogenesis. *Infect Agents Cancer* 15**,** 62 (2020)	-	https://doi.org/10.1186/s13027-020-00317-4

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

4	Chew T, Taylor KE, Mossman KL. Innate and adaptive immune responses to herpes simplex virus. Viruses. 2009;1:979–1002. doi: 10.3390/v1030979.	-	DOI - PMC - PubMed
5	de Jesus AA, Hou Y, Brooks S. Distinct interferon signatures and cytokine patterns define additional systemic autoinflammatory diseases. J Clin Invest. 2020 Apr 1; 130(4):1669-1682. doi: 10.1172/JCI129301.	-	PMID: 31874111
6	de Oliveira DE, Ballon G, Cesarman E. NF-κB signaling modulation by EBV and KSHV. Trends Microbiol. 2010;18(6):248–57.	-	PubMed Article CAS PubMed Central Google Scholar]
7	Dell'Oste V, Gatti D, Giorgio AG, Gariglio M, Landolfo S, De Andrea M. The interferon-inducible DNA-sensor protein IFI16: a key player in the antiviral response. New Microbiol. 2015 Jan;38(1):5-20. Epub 2015 Jan 1.	-	PMID: 25742143.
8	Ehrlich ES, et al. KSHV RTA abolishes NFκB responsive gene expression during lytic reactivation by targeting vFLIP for degradation via the proteasome. PLoS One. 2014; 9(3):e91359.	-	PubMed PubMed Central Article CAS Google Scholar

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

9	Gianni T, Leoni V, Campadelli-Fiume G . Type I interferon and NF-kappaB activation elicited by herpes simplex virus gH/gL via alphavbeta3 integrin in epithelial and neuronal cell lines. *J Virol* 2013; 87: 13911–13916	-	CAS Article Google Scholar
10	Hatada EN, Krappmann D, Scheidereit C. NF-kappaB and the innate immune response. Curr Opin Immunol. 2000;12:52–58. doi: 10.1016/S0952-7915(99)00050-3	-	DOI - PubMed
11	Hiscott J. Convergence of the NF-kappaB and IRF pathways in the regulation of the innate antiviral response. *Cytokine Growth Factor Rev* 2007; 18: 483–490.	-	CAS Article Google Scholar
12	Jiang J, Zhao M, Chang C, Wu H, Lu Q. Type I Interferons in the Pathogenesis and Treatment of Autoimmune Diseases. Clin Rev Allergy Immunol. 2020 Oct; 59(2):248-272. doi: 10.1007/s12016-020-08798-2.	-	PMID: 32557263 Review.
13	Kalamvoki M, Roizman B. HSV-1 degrades, stabilizes, requires, or is stung by STING depending on ICP0, the US3 protein kinase, and cell derivation. Proc Natl Acad Sci U S A. 2014; 111:E611–617. doi: 10.1073/pnas.1323414111	-	DOI - PMC - PubMed

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

14	Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol. 2005; 6:981–988. doi: 10.1038/ni1243		DOI - PubMed
15	Kim JC, Lee SY, Kim SY, Kim JK, Kim HJ, Lee HM *et al*. HSV-1 ICP27 suppresses NF-kappaB activity by stabilizing IkappaBalpha. *FEBS Lett* 2008; 582: 2371–2376		CAS Article Google Scholar
16	Le Negrate G. Viral interference with innate immunity by preventing NF-kappaB activity. *Cell Microbiol* 2012; 14: 168–181		CAS Article Google Scholar
17	Lepelley A, Martin-Niclós MJ, Le Bihan M, Mutations in COPA lead to abnormal trafficking of STING to the Golgi and interferon signaling. J Exp Med. 2020 Nov 2; 217(11):e20200600. doi: 10.1084/jem.20200600.		PMID: 32725128
18	Low-Calle A M, Prada-Arismendy J, Castellanos JE. Study of interferon-beta antiviral activity against Herpes simplex virus type 1 in neuron-enriched trigeminal ganglia cultures. Virus Res. 2014;180:49–58. doi: 10.1016/j.virusres.2013.12.022		DOI - PubMed

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

19	Ma F, Li B, Liu SY, Iyer SS, Yu Y, Wu A, Cheng G. Positive feedback regulation of type I IFN production by the IFN-inducible DNA sensor cGAS. J Immunol. 2015; 194:1545–1554. doi: 10.4049/jimmunol.1402066		DOI - PMC - PubMed
20	Marino-Merlo F, Papaianni E, Frezza C, et al. NF-κB-Dependent Production of ROS and Restriction of HSV-1 Infection in U937 Monocytic Cells. *Viruses*. 2019;11(5):428. Published 2019 May 10.		doi:10.3390/v11050428
21	Michalska A, Blaszczyk K, Wesoly J, Bluyssen A Positive Feedback Amplifier Circuit That Regulates Interferon (IFN)-Stimulated Gene Expression and Controls Type I and Type II IFN Responses. Front Immunol. 2018 May 28; 9:1135.		doi: 10.3389/fimmu.2018.01135. eCollection 2018. PMID: 29892288. Review.
22	O'Neill LA, Bowie AG. Sensing and signaling in antiviral innate immunity. Curr Biol. 2010; 20:R328–333. doi: 10.1016/j.cub.2010.01.044.		DOI - PubMed
23	Okada S, Asano T, Moriya K, Boisson-Dupuis S. Human STAT1 Gain-of-Function Heterozygous Mutations: Chronic Mucocutaneous Candidiasis and Type I Interferonopathy. J Clin Immunol. 2020 Nov;40(8):1065-1081.		doi: 10.1007/s10875-020-00847-x. Epub 2020 Aug 27. PMID: 32852681

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

24	Poma P. NF-κB and Disease. Int J Mol Sci. 2020 Dec 2; 21(23):9181.		doi: 10.3390/ijms21239181. PMID: 33276434; PMCID: PMC7730361.
25	Roizman B, Whitley RJ. An inquiry into the molecular basis of HSV latency and reactivation. Annu Rev Microbiol. 2013;67:355–374.		doi: 10.1146/annurev-micro-092412-155654. - DOI - PubMed
26	Santoro MG, Rossi A, Amici C. NF-kappaB and virus infection: who controls whom. *EMBO J* 2003; 22: 2552–2560		CAS Article Google Scholar
27	Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell. 1986;46(5):705–16		

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

28	Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science. 2013;339:786–791. doi: 10.1126/science.1232458.		DOI - PMC - PubMed
29	Swiecki M, Omattage NS, Brett TJ. BST-2/tetherin: structural biology, viral antagonism, and immunobiology of a potent host antiviral factor. Mol Immunol. 2013;54:132–139. doi: 10.1016/j.molimm.2012.11.008.		DOI - PubMed
30	Unterholzner L. The interferon response to intracellular DNA: why so many receptors? Immunobiology. 2013; 218:1312–1321. doi: 10.1016/j.imbio.2013.07.007.		DOI - PubMed
31	Vahed H, Agrawal A, Srivastava R, et al. Unique Type I Interferon, Expansion/Survival Cytokines, and JAK/STAT Gene Signatures of Multifunctional Herpes Simplex Virus-Specific Effector Memory $CD8^+$ T_{EM} Cells Are Associated with Asymptomatic Herpes in Humans. *J Virol*. 2019;93(4):e01882-18. Published 2019 Feb 5		doi:10.1128/JVI.01882-18

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

32	Valentine R, et al. Epstein-Barr virus-encoded EBNA1 inhibits the canonical NF-κB pathway in carcinoma cells by inhibiting IKK phosphorylation. Mol Cancer. 2010;9(1):1.		PubMed PubMed Central Article CAS Google Scholar
34	van Lint AL, Murawski MR, Goodbody RE, Severa M, Fitzgerald KA, Finberg RW, et al. Herpes simplex virus immediate-early ICP0 protein inhibits Toll-like receptor 2-dependent inflammatory responses and NF-kappaB signaling. J Virol. 2010;84:10802–10811. doi: 10.1128/JVI.00063-10.		DOI - PMC - PubMed
35	Wang K, Ni L, Wang S, Zheng C. Herpes simplex virus 1 protein kinase US3 hyperphosphorylates p65/RelA and dampens NF-kappaB activation. J Virol. 2014; 88:7941–7951. doi: 10.1128/JVI.03394-13.		DOI - PMC - PubMed
36	1. Wei H, et al. Methylation of NF-κB and its Role in Gene Regulation. Gene Expr Regulation Mamm Cells. 2018:291		
37	Wu DX, Fu XY, Gong GZ, Sun KW, Gong HY, Novel HBV mutations and their value in predicting efficacy of conventional interferon. Hepatobiliary Pancreat Dis Int. 2017 Apr; 16(2):189-196.		doi: 10.1016/s1499-3872(16)60184-4. PMID: 28381384

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

38	Xing J, Ni L, Wang S, Wang K, Lin R, Zheng C. Herpes simplex virus 1-encoded tegument protein VP16 abrogates the production of beta interferon (IFN) by inhibiting NF-kappaB activation and blocking IFN regulatory factor 3 to recruit its coactivator CBP. J Virol. 2013; 87:9788–9801. doi: 10.1128/JVI.01440-13		DOI - PMC - PubMed
39	Yamashiro LH, Wilson SC, Morrison HM. Interferon-independent STING signaling promotes resistance to HSV-1 in vivo. Nat Commun. 2020 Jul 7;11(1):3382		doi: 10.1038/s41467-020-17156-x. PMID: 32636381
40	Zhang J, Wang S, Wang K, Zheng C. Herpes simplex virus 1 DNA polymerase processivity factor UL42 inhibits TNF-alpha-induced NF-kappaB activation by interacting with p65/RelA and p50/NF-kappaB1. *Med Microbiol Immunol* 2013; 202: 313–325.		
41	Zhang Q, Lenardo MJ, Baltimore D. 30 Years of NF-κB: A Blossoming of Relevance to Human Pathobiology. Cell. 2017 Jan 12; 168(1-2):37-57. doi: 10.1016/j.cell.2016.12.012. Epub 2017 Jan 12.		PMID: 28086098; PMCID: PMC5268070.

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)

10.15789/2220-7619-EOT-1928

ISSN 2220-7619 (Print)
ISSN 2313-7398 (Online)