Original article

UDC: 616.98:578.8]-053.2:616-097
doi: 10.5633/amm.2024.0417

CHARACTERISTICS OF IMMUNE RESPONSE DURING HERPES SIMPLEX VIRUS INFECTION IN CHILDHOOD

Hristina Stamenković[1,2], Katarina Harfman-Mihajlović[2,3]

The infection with the herpes simplex virus (HSV) basically implies the chronic activation of the immune system. Interferon gamma (IFN-γ) and Interleukin 4 (IL-4) are part of the mechanisms that control the immune system's response to recurrent herpes simplex virus.

The research included 40 children (2−15 years old) with clinical herpes simplex virus infection. Routine laboratory tests were performed on the patients: leukocyte count, creatinine-kinase-CPK, oxidative stress (nitro-blue tetrazolium), NBT test, lactate-LDH dehydrogenation, IFN-γ, IL-4 levels in serum were measured by ELISA test. The serological test for HSV type I virus was positive in all patients.

A high level of LDH, CPK was detected as well as a low ability to reduce NBT. An increased level of IFN-γ, IL-4 was observed compared to the control group of patients (who did not have clinical manifestations of herpes virus infection). Patients with a high concentration of IFN-γ are associated with a low concentration of NBT-test.

During infection of virus herpes simplex, an immune response is activated (lymphocyte Th1and Th2 type are stimulated). Different clinical manifestations are based on a certain type of immune response. Our results presented the dominance of the Th1 type of response over the Th2 type. The production of IFN gamma was higher compared to IL4. Oxidative stress parameters were also associated with the dominant Th1 type of immune response. This is all important for prognosis, prevention and therapy.

Acta Medica Medianae 2024;63(4):148−154.

Key words: *herpes simplex virus infection, interferon gamma, interleukin 4, immune system, children*

[1]University of Niš, Faculty of Medicine, Department of Pediatrics, Niš, Serbia
[2]University Clinical Center Niš, Pediatric Internal Disease Clinic, Niš, Serbia
[3]University of Niš, Faculty of Medicine, doctoral studies, Niš, Serbia

Contact: Hristina Stamenković
81 dr Zorana Djindjića Blvd., 18000 Niš, Serbia
E-mail: stamenkovic1@yahoo.com

Introduction

Herpes simplex virus type 1 (HSV-1) is a DNA virus, a member of the Herpes viridian's family (25 family members) associated with manifestations like: dermatologic, immunologic, or neurologic disorders (1,2,3). Infection of HSV-1 can manifest as primary, latent and recurrent (3). Xu et al report that 36% of children younger than 14 years of age have serological evidence of HSV-1 infection in the United States (4).

In children and young adults, primary infection (gingival stomatitis) usually occurs with HSV-1 (5). Studies show that primary HSV-1 infection manifests itself in two groups of children: the first group of children, between 6 months and 5 years of age, and the second in adolescence (6,7).

Often the primary infection is asymptomatic. Primary infection is manifested with damaged oral mucosa and gingiva accompanied by pyrexia, lethargy, loss of appetite (8,9). Also, patients can have irritability headaches and bilateral cervical lymphadenopathy (10−13).

Shaffer et al. point out that HSV can cause lifelong infection, through the spectrum of diseases depending on host factors such as immune competence, age and site of infection (5) Theil et al. report that latency infection HSV-1 is a part of the inflammatory process where virus factors are a crucial role for of latency (14,15).

The interactions between the host immune system and HSV-1 are very complex.

Many investigators tried to explain the mechanisms of the pathogenesis of infection HSV-1. Previous studies demonstrated that monocytes, natural killer (NK) cells and different T lymphocytes populations all have important functions in the control of HSV infections (16−19).

Usually during virus infection, the immune system is activated with the intent to destroy the virus. Many studies described that CD4+ and CD8+ T lymphocytes and their soluble products (cytokines) have a function in the control of disorders during HSV-1 infection (20).

Mogensen et al demonstrate that the HSV down-modulates the production of proinflammatory cytokines. Also, Mogensen et al described the role of macrophages during HSV infection (21). In this study, the author and colleagues demonstrate that HSV-1 suppresses the production of cytokines (22).

Cytokines control cell survival. Antiviral functions are triggered by cytokines and lead to the activation of inflammatory factors. Therefore, it is not surprising that one of the strategies of viruses is to target cytokines to avoid removal by the immune system (23). Many studies try to explain the mechanisms of latent infection of HSV. Herpes simplex virus type 1 (HSV-1) after a primary infection of the skin or mucous membrane, attacks sensory neurons and is transmitted by in sensory ganglia. Therefore, remittent illness is not the result of exogenous reinfection, but the awakening of a latent virus (24). Also, investigators point out the main role of cytokines during infection of HSV (25).

The purpose of this study was to explain the physiology of immunoregulation (parameters of oxidative metabolism, clinical manifestations, haematological and biochemical parameters, cell injury enzymes) and their effect on the clinical manifestation of disease in HSV-1 seropositive children.

Matherial and Methods

Fourty children were involved in the study(from 2 to 15 years old) with signals of herpes virus (HSV) herpes simplex virus infection and medical history of frequent inflammatory reactions such arelapse of labial herpes, urticaria, remittent respiratory tract infections. Inclusive measures were positive ELISA test and age for viruses HSV. None of the children were under any treatment before blood collection for analysis.

Blood was taken after their verbal agreement from patients and controls. There are 20 healthy patients in the control group.

Quantification of NBT test/Tetrazolium reduction test

The Nitro-blue-tetrasolium (NBT) test was performed according to the method to Park et.al., 1968.

Serologic survey using ELISA assay

Level of IgM and IgG antibody for HSV were determined by ELISA test.

Statistical analysis

Data are reported as mean x, ± SD and as a percentage of certain parameters. The statistical significance of differences was estimated by using Student's t-test. Microsoft program SSPS version 7,5 was used for statistical calculation of data.

Results

Control group had healthy children (20) and 40 children.

There are very heterogeneous clinical manifestations which comprised different organs and systems (Table 1).

52% of the patients were positive for anti-HSV whereas only 4% of the patients were positive for IgM against HSV (Table 2).

Means levels of IFN-γ and IL-4 were high values in relation to control group (Table 3). Results showed significantly low values of mean level of IFN-γ ($p < 0,01$) − Student t-test. There was no normal level of IFN-γ. Values of IL-4 were significantly high and low ($p < 0,01$). A higher value of INF-γ was present in about 75% of patients and high value of IL4 was present in about 46% of patients. High values of IFN-γ followed change in haematologic parametars as low values of leukopenia (74%), Hb (62%), monocytosis (82%).

A high concentration of IFN-γ followed leukopenia (74%). We used low levels of haemoglobin about 80-90 g/lit, in relation to the age of the children, in our study. Monocytosis (35%) and low values of Hb (55%) were associated with high values of IL-4 (Table 4).

There was no significant deviation at low values of IFN-γ. High values of IL-4 are associated with high values of GOT (49%), but low level of IL-4 are associated with low values of CPK (25%) (Table 5).

Domination of low levels of NBT in patients with high levels of IFN-γ (Table6).

Table 1: Clinically manifestations

Clinical manifestations	n	(%)
Labial HSV infection	7	17,5
Urticariarecidivans	6	15
BHR	5	12,5
Atopic dermatitis	5	12,5
Stomatitsaftosa	5	12,5
Laryngitis	4	10
Erythema anulare	4	10
Pneumonia	3	7,5
Encephalitis	1	2.5

Table 2: Positive ELISA test for HSV in patients

HSV	
IgM +	IgG +
4%	52%

Table 3: Mean values of IFN-γ, IL-4

	IFN-γ (IU/l)	IL-4 (pgr/ml)
Study group(n=40)	0.59 ± 0.21	1.42 ± 0.42
Control group (n=40)	0.25 ± 0.41	1.20 ± 0.21

Results show as mean values ± SD; n-number of patients

Table 4: Comparision between haematological parametars and IFN-γ, IL-4. Interleukins, and low levels of Hb, leukopenia, monocytosis

	Low Hb	Leukopenia	Monocytosis
high IFN-γ	62%	74%	82%
high IL-4	55%		35%

Table 5: Comparision between enzymes, IFN-γ and IL-4. Interleukins (IFN-γ, IL-4) and enzymes (LDH, CPK, GOT, GPT)

	High LDH	High CPK	High GOT	High GPT
High IFN-γ	72%	80%	62%	61%
High IL-4	Normal	Normal	49%	Normal
Low IL-4	Normal	25%	Normal	Normal

Table 6: Comparision between NBT test, IFN-γ, and IL4. Interleukine IFN-γ, IL4 and NBT-test

	Low spont. NBT test	Low. stim. NBT-test
High IFN-γ	35%	72%
High IL4	17%	47%

Discussion

Children are infected primarily with orolabial HSV by 5 years of age, with infection rates of 33% in populations that are of lower socioeconomic status and 20% in those who have improved socioeconomic status. By adulthood, HSV affects 70% to 80% people of the lower socioeconomic population and 40% to 60% of the higher socioeconomic population (26).

This study showed that herpes virus infection leads to activation of the immune system and phagocytes.

Patients with other evidence of the disease in this study, (labial HSV:17,5%, urticaria:15%, BHR:12,5%, atopic dermatitis:12,5%, stomatitis aphtosa:12,5%, laryngitis:10%,) are indicated in Table 1.

All mothers of children included in the study had herpes labialis.

HSV infection in persons with eczema or other skin diseases causes eczema herpetic (27).

Exaggerated Th2 responses to common allergens are characterized for atopic eczema and such answers may be showed in the physiology of eczema herpetic (28).

In our study we did not have aggressive form of HSV infection on the skin. In 52% of patients ELISA test was positive (IgG antibody on HSV). Positive IgM antibody on HSV was 4% in patients.

Our results show high concentrations of IL-4 were connected to a low percent of Hb (55%) and high concentration of monocytes (35%). Otherwise, high concentrations of IFN-γ were connected to a high percent of monocytosis (82%) and low percent of Hb (62%), leukopenia (74%).

During HSV-1 infection many investigations analysed the roles of subpopulations (CD4+ and CD8+ T lymphocytes) (21). CD4+ T cells are sufficient to clear the infection from both peripheral and neuronal sites in the absence of CD8+ T cells, (21). CD4+ T cells have been suggested to be responsible for the inflammatory response in HSV keratitis proved by investigation (29).

The cytokines IL-4, IL-5, IL-10 and IL-13 express Th2 cell lineage and are involved in the activation of B cells. In HSV-1 infection IL-4 and IL-10 have been detected, but some of these data are controversial, probably due to different mouse models and virus strains used (25). Increased IL-4 levels correlated with increased HSV-1 replication in the eye indicate that IL-4 might function by down-regulating IL-2 in the HSV-1 infection. When IL-2 is down producing, HSV-1 titers increase (30).

In our investigation values of IFN-γ, IL-4, were compared with values of LDH, CPK, GOT, GPT (Table 5). Patients with high levels of IFN-γ had high level of CPK (80%), LDH (72%), GOT (62%) and GPT (61%). The presence of risen levels of this immune modulation cytokine in insistent infection proposes that when the immune system is unable to mediate viral clearance it may contribute to injury of hepatocyte (31). Patients with low levels of IL-4 had high levels of CPK (25%), while patients with high levels of IL-4 had high levels of GOT (49%). The presence of risen values of CPK may contribute to cell injury during herpes virus reactivation.

IL-4 mRNA and protein were detected at days 7 through 14 after HSV-1 infection; In a study by Heilinhenhaus et al. compared to IL-2 and IFNgama, IL-4 staining intensities were lower (32).

In resistance to HSV infection IFN-γ may be critical. HSV-specific IFN-γ production by cultured peripheral blood mononuclear cells (PBMC) is lacking in some patients who have frequent episodes of herpes labialis (33). A recent study (29) compared titers of antibody to HSV and cytokine production by cultured PBMC for seropositive patients with or without a history of herpes labialis.

Research has shown that activation of lymphocytes causes HSV-1 keratitis, then type 1 cells release IL-2 and IFN-γ. Type 2 cytokines play a role in better disease prognosis (34).

Lekstrom-Himes et al. suggest that IFN-γ plays two roles in HSV-1 infection. First, it prevents acute disease and limits the quantity of virus amenable to ganglionic latency; second, it limits the spread of the virus once reactivated.

Viruses trigger an oxidative metabolism and function of phagocytes. In our investigation, most patients with high concentraions of IFN-γ,had low NBT test (35).

Conclusion

During HSV infection, an immune response is activated that goes in the direction of Th1 and

Th2 type of response. Different clinical manifestations are based on a certain type of immune response. Our results determined the dominance of the Th1 type of response over the Th2 type. The production of IFN gamma was higher compared to IL4. Oxidative stress parameters were also associated with the dominant Th1 type of immune response. It is important to recognize the mechanisms and the activity of the immune answer in a viral infection. This is all important for prognosis, prevention and therapy.

References

1. Arena A, Bisignano C, Stassi G, Filocamo A, Mandalari G.Almond Skin Inhibits HSV-2 Replication in Peripheral Blood Mononuclear Cells by Modulating the Cytokine Network Molecules 2015; 20: 8816-22. [CrossRef] [PubMed]
2. Whitley R, Roizman B Herpes simplex viruses: is a vaccine tenable? J. Clin. Invest 2002; 109:145-51. [CrossRef] [PubMed]
3. Spear P, Manoj Sh, Yoon M, Jogger C, Zago A, Myscofski D. Different receptors binding to distinct interfaces on herpes simplex virus gD can trigger events leading to cell fusion and viral entry Virology 2006; 344(1):17-24. [CrossRef] [PubMed]
4. Leaute-Labreze C, Lamireau T, Chawki D, Maleville J, TaiebA.Diagnosis, classification, and management of erythema multiforme and Steves-Johnson syndrome Arch Dis Child 2000;83(4):347-52. [CrossRef] [PubMed]
5. Kuo Y, Lin C. Recurrent herpes simplex virus type 1 infection precipitated by the impaired production of interleukin-2, a-interferon, and cell mediated immunity. J Med Virol 1990; 31(3):183–9. [CrossRef] [PubMed]
6. Green JA, Yeh TJ, Overall JC. Sequential production of IFN-a and immunespecific IFN-□by human mononuclear leukocytes exposed to herpes simplex virus. J Immunol 1981; 127(3):1192–6. [CrossRef] [PubMed]
7. Atedzoe BN, Menezes J, Addario MD, Xu J, Ongradi J, Ahmad A. Modulatory effects of human herpes virus-7 on cytokine synthesis and cell proliferation in human peripheral blood mononuclear cell cultures. J Leukoc Biol 1999; 66(5):822-28. [CrossRef] [PubMed]
8. Mullick J, Kadam A, Sahu A Herpes and pox viral complement control proteins: "the mask of self". Trends Immunol 2003;24(9):500-7. [CrossRef] [PubMed]
9. Meinl E, Fickensher H, Thome M, Tschoopp J, Fleckenstein B. Anti-apoptotic strategies of lymphotropic viruses. Immunol Today 1998;19(10):474-9. [CrossRef] [PubMed]
10. Khanna K.M, Lepisto A.J, Hendricks R.L. Immunity to latent viral infection: many skirmishes but few fatalities Trends in Immunology 2004;25(5):230-4. [CrossRef] [PubMed]
11. Park B, Fikrig S, Smitwick E. Infection and nitrobluetetrasolium reduction by neutrophils Lancet 1968;2(7567):532-4. [CrossRef] [PubMed]
12. Lawrence R. Stanberry, Anthony L. Cunningham, Adrian Mindel, Laurie L. Scott, Spotswood L. Spruance, Fred Y. Aoki, and Charles J. Lacey. Prospects for control of Herpes Simplex Virus Disease thorough Immunization Clinical Infection Disease 2000;30(3):549-66. [CrossRef] [PubMed]
13. Bastuji-Garin S, Rzany B, Stern RS, et al. Clinical classification of cases of toxic epidermal necrolysis: Stevens-Johnson syndrome and erythema multiforme. Arch Dermatol 1993; 129(1):92–6. [CrossRef] [PubMed]
14. Brice SL, Krzemien D, Weston WL, Huff JC. Detection of herpes simplex virus DNA in cutaneous lesions of erythema multiforme. J Invest Dermatol 1989; 93(1):183–7. [CrossRef] [PubMed]
15. Aslanadenh J, Helm KF, Espy MJ, Muller SA, Smith TF. Detection of HSV-specific DNA in biopsy tissue of patients with erythema multiforme by polymerase chain reaction. Br J Dermatol 1993; 126(1):19-23. [CrossRef] [PubMed]
16. Schofield JK, Tatnall FM, Leigh IM. Recurrent erythema multiforme: clinicalfeatures and treatment in a large series of patients. Br J Dermatol 1993; 128(5):542–5. [CrossRef] [PubMed]
17. Brice SL, Leahy MA, Ong L, et al. Examination of non-involved skin, previously involved skin, and peripheral blood for herpes simplex virus DNA in patients with recurrent herpes-associated erythema multiforme. J CutanPathol 1994; 21(5):408–12. [CrossRef] [PubMed]
18. Khalil I, Lepage V, Douay C, et al. HLA-DBQB1*0301 allele is involved in susceptibility to erythema multiforme. J Invest Dermatol 1991; 97(4): 697–700. [CrossRef] [PubMed]
19. Bork K, Bruninger W. Increasing incidence of eczema herpeticum: analysis of seventy-five cases. J Am AcadDermatol 1988; 19:1024–9. [CrossRef] [PubMed]
20. Corry DB, Kheradmand F, Luong A, Pandit L. Immunological mechanisms of airway diseases and pathways to therapy. In: Rich RR, editor. Clinical Immunology: principles and practice. 4th ed. Philadelphia: Elsevier Saunders; 2013. [CrossRef]
21. Mogensen TH, Melchjorsen J, Malmgaard L, Casola A, Paludan SR. Suppression of proinflammatory cytokine expression by herpes simplex virus type 1. J. Virol 2004; 78(11): 5883–90. [CrossRef] [PubMed]
22. Cunningham AL, Merigan TC. Interferon production appears to predict time of recurrence of herpes

labialis. J Immunol 1983; 130(5):2397–9. [CrossRef] [PubMed]
23. Shillitoe EJ, Wilton JMA, Lehner T. Sequential changes in cell-mediated immune responses to herpes simplex virus after recurrent herpetic infection in humans. Infect Immun 1977; 18:130–7. [CrossRef] [PubMed]
24. O'Reilly RJ, Chibbaro A, Anger E, Lopez C. Cell-mediated immune responses in patients with recurrent herpes simplex infections. J Immunol 1977; 118(3):1095–102. [CrossRef] [PubMed]
25. Stamenkovic H, et al. Immune System Behavior during Herpesvirus Infection in Childhood. J Infect Dis Ther 2014; 2:5. [CrossRef]
26. Green JA, Weiss PN, Yeh TJ, Spruance SL. Immune specific interferon production by peripheral blood mononuclear leukocytes from patients with primary and recurrent oral-labial herpes simplex virus infections. J Med Virol 1985; 16(3):297–305. [CrossRef] [PubMed]
27. Spruance SL, Evans TG, McKeough MB, et al. Th1/Th2-like immunity and resistance to herpes simplex labialis. Antiviral Res 1995; 28(1):39–55. [CrossRef] [PubMed]
28. Mikloska Z, Kesson AM, Penfold ME, Cunningham AL. Herpes simplex virus protein targets for CD4 and CD8 lymphocyte cytotoxicity in cultured epidermal keratinocytes treated with interferon-g. J Infect Dis 1996; 173(1):7–17. [CrossRef] [PubMed]
29. James SH, Whitley RJ. Treatment of herpes simplex virus infections in pediatric patients: current status and future needs Clin Pharmacol 2010;88(5):720-4. [CrossRef] [PubMed]
30. Johnston RB, Kitagawa S. Molecular basis for the enhanced respiratory burst of activated macrophages. Fed Proc1985; 44(14):2927-32. [PubMed]
31. Boulanger MJ, Chow DC, Breynova E, Martick M, Stanford G, Nocholas J, Garcia KC. Molecular mechanisms for viral mimicry of a human cytokine: Activation of gp 130 by HHV-8 interleukin-6. J Mol Biol 2004; 335(2): 641–54. [CrossRef] [PubMed]
32. Heiligenhaus A, Bauer D, Zheng M, Mrzyk S, Steuhl KP.CD4+ T-cell type 1 and type 2 cytokines in the HSV-1 infected cornea. Graefes Arch Clin Exp Ophthalmol. 1999;237(5):399-406. [CrossRef] [PubMed]
33. Rasmussen SB, Sørensen LN, Malmgaard L, Ank N, Baines JD, Chen ZJ, et al. Type I interferon production during herpes simplex virus infection is controlled by cell-type-specific viral recognition through Toll-like receptor 9, the mitochondrial antiviral signaling protein pathway, and novel recognition systems. J Virol. 2007; 81(24): 13315–24. [CrossRef] [PubMed]
34. Jie Zhang, Huan Liu, Bin Wei. Immune response of T cells during simplex virus type 1 (HSV-1) infection. J. ZhejlangUniv-Sci B 2017;18(4):277-88. [CrossRef] [PubMed]
35. Lekstrom-Himes JA, Leblanc RA, PesnicakL,Godleski M, Straus SEGamma Interferon Impedes the Establishment of Herpes Simplex Virus Type 1 Latent Infection but Has No Impact on Its Maintenance or Reactivation in Mice. J Virol 2000;74(14): 6680–3. [CrossRef] [PubMed]

Originalni rad **UDC: 616.98:578.8]-053.2:616-097**
doi: 10.5633/amm.2024.0417

KARAKTERISTIKE IMUNSKOG ODGOVORA U INFEKCIJI HERPES SIMPLEKS VIRUSOM KOD DECE

Hristina Stamenković[1,2], Katarina Harfman Mihajlović[2,3]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za pedijatriju, Niš, Srbija
[2]Univerzitetski klinički centar Niš, Klinika za pedijatriju, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, Srbija

Kontakt: Hristina Stamenković
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: stamenkovic1@yahoo.com

U osnovi infekcije herpes simpleks virusom (HSV) jeste hronična aktivacija imunosistema. Interferon gama (IFN-γ) i interleukin 4 (IL-4) čine deo regulatornih mehanizama reakcije imunosistema na rekurentne infekcije herpes simpleks virusom.

Analizirano je četrdesetoro dece (starosti 2–5) sa infekcijom herpes simpleks virusom tipa 1. Urađena je laboratorijska analiza bolesnika kojom su određeni broj leukocita, nivo laktat dehidrogenize (LDH), kreatinin kinaze (CPK) i oksidativni metabolizam fagocita (sposobnost fagocita da redukuje boju *nitro-blue-tetrazolium* – NBT). Nivo IL-4 i IFN-γ određivan je pomoću ELISA testa. Serološka analiza za HSV tipa 1 bila je pozitivna kod svih bolesnika.

Naši rezultati su pokazali visok nivo LDH, CPK i niske vrednosti NBT testa. Bile su povećane vrednosti IFN-γ i IL-4. Kod bolesnika sa visokim vrednostima IFN-γ bile su povišene vrednosti LDH, CPK, GOT, GPT, a zabeleženi su i nizak nivo hemoglobina (Hb), leukopenija i monocitoza.

Povišene vrednosti IFN-γ udružene su sa nižim vrednostima NBT testa.

U toku infekcije herpes simpleks virusom aktivira se imunski odgovor (stimulisani su limfociti Th1 i Th2 tipa). Različite kliničke manifestacije zasnivaju se na određenoj vrsti imunskog odgovora. Naši rezultati pokazali su dominaciju Th1 tipa odgovora nad Th2 tipom odgovora. Proizvodnja IFN-γ bila je veća nego proizvodnja IL-4. Parametri oksidativnog stresa takođe su bili povezani sa dominantnim Th1 tipom imunskog odgovora. Važno je prepoznati mehanizme patogeneze i aktivnost imunskog odgovora kod virusne infekcije. Sve ovo je važno za prognozu, prevenciju i terapiju.

Acta Medica Medianae 2024; 63(4):148–154.

***Ključne reči**: infekcija virusom herpes simpleks, interferon gama, interleukin 4, imunosistem, deca*