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Characteristics of immune response during herpes simplex virus infection in childhood

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Characteristics of immune response during herpes simplex virus infection in childhood

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Introduction: In the main of the infection with the herpes simplex virus (HSV) is the chronic activation of the immune system. IFN-γ and IL-4 are part of the regulatory mechanisms of the immune system's response to recurrent herpes simplex virus.

Methods: The study included 40 children (2-15 years old) with clinical manifestation of herpes simplex virus infection. In patient routine laboratory investigations were performed: white blood cells count, lactate dehydrogenize-LDH, creatinine-kinase-CPK, oxidative stress (nitro-blue tetrazolium) NBT test. Serum levels of IFNγ, IL-4 were measured by ELISA test. Serological test of virus on HSV type I, was positive for all patients.

Results: The high level of LDH, CPK, low ability of NBT reduction were detected. The increase of IFN-γ, IL-4 level, in compared with control group of patients (without of clinical manifestation of herpes virus infection). The patients with the high level of IFN-γ, had the high level of LDH, CPK, GOT, GPT, the low level of Hb, leucopoenia and monocytes. High level of IFN-γ is associated with low level of NBT-test.

Conclusions: During infection of virus herpes simplex, an immune response is activated (lymphocite Th1and Th2 type are stimulated). Different clinical manifestations are based on a certain type of immune response. Our results showed 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 pathogenetic mechanisms and the activity of the immune response in a viral infection. This is all important for prognosis, prevention and therapy.

Key words: herpes simplex virus infection, IFN-γ, IL-4, immune system, children

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Karakteristika imunog odgovora u infekciji virusom herpes simplex kod dece

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Uvod: U osnovi infekcije, virusom herpes simplex (HSV) je hronična aktivacija imunog sistema. IFN-γ i IL-4 su deo regulatornih mehanizama reakcije imunog sistema na rekurentne infekcije virusom herpes simplex.

Metode: Analizirano je 40-oro dece (2-15 godina) sa infekcijom virusom herpes simplex tip I. Kod pacijenata su sprovedena laboratorijska analiza: broj leukocita, nivo laktat dehidrogenize-LDH, kreatinin - kinaze -CPK, oksidativni metabolizam fagocita (sposobnost fagocita da redukuje boju nitro-bluae-tetrazolijum (NBT)). Nivo IL-4 i IFN-γ je određivan pomoću ELISA test. Serološka analiza za HSV tip I bila je pozitivna kod svih pacijenata.

Rezultati: Naši rezultati su pokazivali visok nivo LDH, CPK i niske vrednosti NBT testa. Bile su povećane vrednosti IFN-γ i IL-4. Pacijenti sa visokim vrednostima IFNγ su imali povišene vrednosti LDH,CPK, GOT,GPT, nizak nivo Hb, leukopeniju i monocitozu.

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

Zaključak : Tokom infekcije virusom herpes simplex, aktivira se imuni odgovor (stimulisani su limfociti Th1 i Th2 tipa). Različite kliničke manifestacije se zasnivaju na određenoj vrsti imunog odgovora. Naši rezultati su pokazali dominaciju Th1 tipa odgovora nad Th2 tipom. Proizvodnja IFN gama bila je veća u poređenju sa IL4. Parametri oksidativnog stresa su takođe bili povezani sa dominantnim Th1 tipom imunog odgovora. Važno je prepoznati mehanizme patogeneze i aktivnost imunog odgovora kod virusne infekcije. Ovo je sve važno za prognozu, prevenciju i terapiju

Ključne reči: infekcija herpes simplex virusom, IFNγ, IL-4, imuni sistem, deca

Introduction

Herpes simplex virus type 1 (HSV-1) is DNA virus, member of the Herpes viridians family (25) associated with manifestation like an: 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 <14 years of age have serologic evidence of HSV-1 infection in United States (4)

Primary infection (gingiva stomatitis) with HSV-1 usually arias in children and young adults (3). Studies report that primary infection HSV-1 manifested in two groups of children: first, between 6 months and 5 years of age and secondly in the early adolescent (6,7).

Often the primary infection is asymptomatic (3). Primary infection is manifested with damaged oral mucosa and gingiva accompanied with pyrexia, lethargic, loss of appetite (8,9,3). Also, patients can have irritability headaches and bilateral cervical lymphadenopathy (10,11,12,13).

Shaffer et al point out that HSV can cause lifelong infection, through the spectrum of diseases depending on host factors such as age, immune competence and site of infection (5) Theil et al report that latency infection HSV-1 is a part of inflammatory process where virus factors are a crucial role for of latency (14,15,3)

The interactions between HSV-1 and the host immune system are very complex. Many investigators tried to explain mechanisms of pathogenesis of infection HSV-1. Previous studies demonstrated that monocytes, neutrophils, dendritic cells, macrophages, natural killer (NK) cells and different T-lymphocytes populations all play important roles in control of HSV infections (16,17,18,19).

Usually during virus infection, the immune system is activated with intent to destroy virus. Many studies described that CD4+ and CD8+ T-lymphocytes and their soluble products (cytokines) have a role in control of disorders during HSV-1 infection (20,21)

Mogensen et al demonstrate that the HSV down-modulates the production of proinflammatory cytokines (22). Also, Mogensen et al described about role macrophages during HSV infection (22,23,24). In this study, author and colleagues, demonstrate that HSV-1 suppresses production of cytokines (TNF-α, IL-6, IL-12 and RANTES).

Cytokines regulate cellular survival, may induce antiviral functions in the cell and modulate the development of a local inflammation. Therefore, it is not surprising that targeting cytokines is one strategy of viruses to evade clearance by the immune system (8,9). Many studies try to explain mechanisms of latent infection of HSV. Following primary infection of the skin or mucosa, herpes simplex virus type 1 (HSV-1) invades sensory neurons and is transported by retrograde axonal transport to the neuronal cell bodies in the sensory ganglia. Thus, recurrent disease results from an awakening of latent virus rather than from exogenous re-infection (10). Also, investigators point out main role of cytokines during infection of HSV. The uniform expression of IFN-γ, and TNFα in latently infected suggests persistent stimulation of the cells producing these cytokines. Neurons up-regulation MHC class I expression during HSV-1 infection, but MHC class I expression diminishes to an undetectable level when the virus enters a latent state. This suggests concordant regulation of MHC class I and viral gene expression in neurons. (10).

The aim of this study was to explain immunoregulatory mechanisms (clinical manifestations,

cytokines level-IFN-γ, et IL-4, parameters of oxidative metabolism, enzymes of cell injury, haematological and biochemical parameters) and their consequences concerning clinical disease expression in HSV-1 seropositive children.

Matherials and Methods

The study included 40 children (aged from 2 to 15 years) with signs of herpes virus (herpes simplex virus- HSV) infection and medical history of frequent inflammatory events such as: labial herpes recidivans, urticaria, recurrent respiratory tract infections. Including criteria were age and positive ELISA test for viruses HSV. None of the children were under any treatment before the check-up and blood collection for analysis.

Blood was taken from patients and controls after their verbal consent. In addition, a general consent is also obtained from all patients in this hospital for all investigations as a part of a routine patient processing.

Controls group of patients (20) were normal healthy.

Quantification of haematological and biochemical parameters

White blood cell, haemoglobin level, LDH, CPK, GOT, GPT were measured using micro analyser Culter drift II (Instrumental Laboratory) following the manufacture instructions.

Quantification of NBT test/Tetrazolium reduction test

The Nitro-blue-tetrasolium (NBT)-test of peripheral blood phagocytes was performed according to the method to Park et.al., 1968. The activation of monocyte-macrophage system and the measurement of metabolic oxidative explosion of phagocytes was observed through NBT-test.

Quantification of IFNγ, and IL-4 level in plasma

IFN- γ, and IL-4 serum levels were measured by the ELISA test using kits of Bio Source Europe S.A. (Zoning Industrial B-6220 Fleurs Belgium), stock number KHM0009 following the manufacture instructions.

Serologic survey using ELISA assay

Concentration of antibody (IgM and IgG) for HSV were determined by commercial ELISA test (Humana-Human Gesellschaft fur Biochemical und Diagnostic mbH) following the manufacture instructions.

Statistical analysis

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

Results:

The study included 40 children and control group of healthy children (20).

The children had different clinical manifestations in our study. All of clinical manifestations were very heterogeneous which comprised different organs and systems (Table 1).

In order to point out etiological factors we used an ELISA test to detect the presence of IgG and IgM antibodies against HSV. We found that 52% of the patients were positive for anti-HSV whereas only 4% of the patients were positive for IgM against HSV (Table 2).

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

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

Comparison of interleukins values (IFN-γ and IL-4) with values of LDH, CPK, GOT i GPT is on table 4. These results show that high levels of IFN-γ followed high levels of LDH (72%), CPK (80%), GOT (62%) i GPT (61%). There was no significant deviate at low values of IFN-γ. High values of IL-4 associated with high values of GOT (49%), but low level of IL-4 associated with low values of CPK (25%) (Table 5).

Domination of low values of NBT (spontaneous and stimulation) in patients with high values of IFNγ (Table 6).

Table 1: Clinically manifestations

clinical manifestations	n	(%)
Labial HSV infection	7	17,5
Urticaria recidivans	6	15
BHR	5	12,5
Atopic dermatitis	5	12,5
Stomatits aftosa	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, IFNγ 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%

Disscusion

The spectrum of disease caused by HSV includes primary and recurrent infections of mucous membranes (e.g. gingivitis, herpes labials, and genital HSV infections), keratoconjunctivitis, neonatal HSV infections of the immune compromised host, encephalitis, Kaposi like of varicella eruption, and an reminds with erythema multiform (2). Disease symptoms (HSV-1) can range from mucocutaneous lesions to life-threatening encephalitis or systemic disease involving

multiple organ system (3). Leaute-Labreze et.al. (2000) conclude that childhood erythema multiform is related to herpes infection, as recurrent erythema multiform.

Our examination of patients pointed to alteration of herpesvirus infection and parameters of stimulation of immune system, clinically manifestation and parameters of activation of phagocytes. In this study, patients with different clinical manifestations (labial HSV-17,5%, urticaria-15%, BHR-12,5%, atopic dermatitis-12,5%, stomatitis aphtosa 12,5%, laryngitis-10%,) are indicated in Table 1.

Herpes simplex virus types 1 (HSV-1) associated with variety of diseases, depending on the portal of entry, the immune state of the host, and whether the infection is initial or recurrent (12)

Seroprevalence studies indicate that the incidence of primarry HSV-1 infection is greatest during childhood. Results from researchers show**:** symptomatic primary oral HSV-1 infection has accounted for 10% of cases of sore throat in college studies (35) From 20% to 40% of the population have had episodes od herpes labialis (35). The frequency of recurrent episodes is extremly variable, ranging from rare outbreaks every 5-10 years to >12 episodes yearly(35).

In our study, all mothers of children had herpes labialis recidivans.

Erythema multiform is an acute inflammatory disease of the skin and mucous membranes (13). Many investigations analysed clinical manifestations during infection with HSV. “Typical” erythema multiform, where <10% of the total body surface area is involved (13), is frequently associated with a history of recent HSV infection. The etiological connection with HSV infection has been strengthened by the demonstration of HSV within such lesions by culture, immunofluorescence, and, more recently, PCR-based assays; HSV DNA was detected in ~70% of samples by PCR-based assays in 4 studies (14,15,16,17). The pathogenesis of erythema multiforme and its relationship to HSV remain incompletely defined. An association with the concept of HSV being involved in the etiology of most cases of erythema multiform is strengthened by studies of response to treatment. In an observational study of continuous oral acyclovir twice daily for 6 months (16), this treatment completely suppressed recurrent erythema multiforme in15 of 37 patients, whereas partial suppression was seen in 8 of 37 patients.

Eczema herpetic, also referred to as Kaposi’s varicelliform eruption, is due to HSV infection in persons with atopic eczema or other preexisting skin disease (13). Most cases represent primary HSV infections, although recurrent cases of eczema herpeticum have been described (13, 19). If the disease remains undiagnosed or not treated with systemic acyclovir, it can be fatal. More aggressive forms of HSV infection, involving multiple cutaneous and mucosal sites but not 120% of the total body surface area, are also more commonly seen in atopic eczema.

Atopic eczema appears to be characterized by exaggerated Th2 responses to common allergens, and such responses may be involved in the pathogenesis of eczema herpetic.
This is a rare condition (annual incidence, ~1.5 cases per 1 million population) (19).

In our study we didnt have aggressive form of HSV infection on the skin. ELISA test showed positive test at patients (IgG antibody on HSV) 52%. Positive IgM antibody on HSV was 4% in patients.

Our results show high level of IL-4 with were connected low level of Hb (55%) and high level of monocytes (35%). Otherwise, high levels of IFN-γ were connected with low level of Hb (62), leukopenia (74%) and high level of monocytosis (82%). Higher values of monocytes can indicate a possible infection of virus, while low level of Hb is a part of defense of cell injury during disorders of immune regulation. Middle level of IFN-γ, were significant low ($p<0,01$).

The function of IFN-γ in the HSV infection has been examined. The test results showed that the viral clearance is dependent upon IFN-γ activation, as shown in studies with knock-out mice and with neutralizing anti-IFN-γ antibodies (20).

Many investigations were analysed roles of subpopulation (CD4+ and CD8+ T lymphocytes) during HSV-1 infection (21)**.** In the absence of CD8+ T cells, CD4+ T cells are sufficient to clear the infection from both peripheral and neuronal sites (21). Also, investigations indicate that CD4+ T cells have been suggested to be responsible for the inflammatory response in HSV keratitis (21).

It has been previously shown that CD4+ T-lymphocytes are critical mediators in HSV-1 stromal keratitis (HSK). CD4+ T cell subpopulations (type 1, type 2) can be defined by their capabilities of producing different sets of cytokines (**3**).

The Th2 cell lineage expresses the cytokines IL-4, IL-5, IL-10 and IL-13, and is involved in the activation of B cells. Both IL-4 and IL-10 have been detected in HSV-1 infection, but some of these data are controversial, probably due to different mouse models and virus strains used (25). Increased IL-4 levels have been 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 (25).

In our investigation levels of IFN-γ, IL-4, were compared with levels of LDH, CPK, GOT, GPT (Table 5). Patients with high level of IFNγ had high level of LDH (72%), CPK (80%), GOT (62%) and GPT (61%). The presence of elevated levels of this immune modulation cytokine in persistent infection suggests that when the immune system is unable to mediate viral clearance it may contribute to injury of hepatocyte (22). Patients with high level of IL-4 had high level of GOT (49%) while patients with low level of IL-4 had high level of CPK (25%). The presence of elevated levels of CPK may contribute cell injury during herpes virus reactivation.

IL-4 mRNA and protein were detected at days 7 through 14 after HSV-1 infection; compared to IL-2 and IFNgama, IL-4 staining intensities were lower in study of Heilinhenhaus et al. (34).

Studies comparing infected persons who do and do not have recurrences suggest that alterations in cytokine production may correlate with the development of recurrent infection (23, 24). IFN-γ may be critical in resistance to HSV infection. HSV-specific IFN-γ production by cultured peripheral blood mononuclear cells (PBMC) is lacking in some patients who have frequent episodes of herpes labialis (27,28). For patients with recurrent infections who produced IFN-γ, there was a positive correlation between the concentration of IFN-γ in lesion vesicle fluid or in the culture supernatants of unstimulated or viral antigen–stimulated PBMC collected during a recurrence and the time to the next recurrence (24). 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. Those patients with a history of frequent recurrences were found to have higher median titers of serum antibody to HSV and trends for lower levels of HSVspecific IFN-γ and IL-2 production by PBMC. The findings suggest that a Th1-like cytokine response (IFN-γ and IL-2 production) may be associated with resistance to recurrences of herpes labialis.

These studies (Lekstrom-Himes et al) demonstrate that IFN-γ protects mice from fatal HSV-1 encephalitis yet has minimal to no effect on viral replication or neuroinvasiveness achieve this outcome, it was necessary to select among diverse host Reactivation of HSV-1 from latency may be closely tied to the immune response to systemic stress. UV irradiation, a common

inducer of HSV-1 reactivation, alters Th1 and Th2 cytokine production in affected tissues. It reduces production of IL-2 and IFN-γ and augments levels of the Th2 cytokines IL-4 and IL-5 (29).

The findings suggest that the lymphocytic infiltration during the development of HSV-1 keratitis is predominantly composed of type 1 cells expressing IL-2 and IFN-gama. Type 2 cytokines participate in the late stage of inflammation and might be useful to improve the course of the disease (30,34).

Lekstrom-Himes et al. suggest that IFN-γ plays two roles in HSV-1 infection. First, it suppresses acute disease and limits the quantity of virus amenable to ganglionic latency; second, it limits the spread of virus once reactivated so that the recurrent infection will be less evident clinically or less severe (29).

Oxidative metabolism and function of phagocytes are activated by virus. Activation of macrophages is the principal and result of cell-mediated immunity and priming for an enhanced respiratory burst is an essential component of that process (31). Activated immune competent cells and some their mediators (IL-1, IL-2, INF-γ, TNFα), arachidonic acid metabolites like an PGE2 have direct or indirect effects on oxidative burst of peripheral blood phagocytes (32,33).

In our study the majority of patients with high level of IFNγ, had low NBT-test (spontaneous, stimulation).

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 showed 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 pathogenesis mechanisms and the activity of the immune response in a viral infection. This is all important for prognosis, prevention and therapy.

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