Specific features of γ-interferon system in patient with frequent recurrences of herpes simplex (from medical practice)

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Abstract

Among the group of patients with frequent recurrences of herpetic infection (FRH, HSV I, II) with different localization one patient was revealed torpid to standard therapy. Examining his γ-interferon system (IFNγ) we determined that IFNγ production and expression of IFNγ a-chain receptor were normal but a peripheral blood cell response to the given cytokine estimated by Interleukin 12 production (IL12p70) was reduced. It may due to impaired signaling pathway of IFNγ receptor, caused by HSV and could play an important role in development of chronic infection and resistance to therapy.

Key words: *lymphocytes, interferon γ, interleukin 12, herpes simplex virus*

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Introduction

During examination and treatment of herpetic patients (n=128) with frequent recurrences and different localization (HSV I, II, over 10 episodes annually) we revealed the following specific features of IFNγ production: a cytokine concentration curve in blood sera was of undulating character with its peak directly before recurrence; two weeks after recurrence $CD3^+IFN\gamma^+$-cell volumes in peripheral blood were 10% lower (6.7±0.81, M±σ) as compared to normal 15.0±3.84% (M±σ).

The standard therapy of FRH patients followed recurrences (Valtrex, tablet (Glaxo Wellcome) – 5 g/course, «Viferon-III» suppositories (Feron OOO) – 5 mln U/course, total 6 courses) resulted in absence of recurrences for one year among 59% patients, and reduction up to 2 episodes annually among 41% patients. The episodes were characterized by obliterate character, three-day duration, and absence of general symptoms. However one patient of the group (patient P., age 55, herpetic infection of genital localization) showed no improvement after conducted therapy, in other words, the character, frequency, and duration of recurrences did not change.

The patient's immune status study (estimation of phagocytic and bacteriocytic activities of peripheral blood leucocytes, IgA, IgG, IgM levels, lymphocyte subpopulations, and proliferative activity) did not show evidences of immune deficiency, $CD3^+IFN\gamma^+$-cell volumes were 23%, and IFNγ concentration in blood sera was stable high.

Hence, we observed a case of permanent recurring herpetic infection resistant to standard therapy and developing at normal $CD3^+IFN\gamma^+$-cell volumes in peripheral blood with normal IFNγ production. Our aim was to investigate IFNγ system of the patient P. since HSV is known to suppress cellular response to the given cytokine [4] resulting in development of chronic infection.

Materials and methods

The object of our study was a group of healthy volunteers, n=15, age from 18 to 50 (M±σ, 29,0±7,1) and patient P. age 55, chronic herpetic infection of genital localization.

Estimation of CD119 expression

In order to estimate the expression of IFNγ-receptor α-chain we used anti-CD119-PE (Caltag), isotypic control

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of antibodies – mouse IgG1-PE (Caltag). The probes were analyzed in "FACSCalibur" flow cytometer (Beckton Dickinson) with argon laser (488 nm) using "CellQuest" program on FL2 canal (585 nm) for lymphocyte and monocyte clouds. Expression coefficient calculated in the following way: Geomean(test)/Geomean(control).

Determination of $CD3^+IFN\gamma^+$ - *cell volumes*

Mononuclear cells (MNC) were isolated by centrifuging on Ficoll-Paque gradient (Pharmacia), p=1.077. IFNγ was induced with phorbol 12-myristate 13-acetate (PMA) (Sigma) – 100 ng/ml and ionomicin (Sigma) – 1 μM with brefeldin A (Sigma) – 5 μg/ml in whole cultural medium (10% fetal calf serum (ICN), 2 mM glutamin (Sigma), RPMI 1640 (Sigma), 40 mg/ml gentamicin (KRKA)) for 18 hours at 37°C 5% CO_2. Then the cells were washed out and pretreated with fixating permeable solution (4% paraform (Sigma), 0,1% saponin (Sigma)) for 30 min. at +4°C. Then cells stained with anti-CD3-FITC (Caltag) and anti-IFNγ-PE (Caltag) in 0.5% BSA (Sigma) for 30 min. at 4°C. The probes were analyzed in FACSCalibur flow cytometer using "CellQuest" program in FL1 (535 nm) and FL2 (585 nm) canals counting up double-labeled cells. For isotypic control we used: mouse IgG1-FITC (Caltag) and mouse IgG1-PE (Caltag). In controls the double-labeled cell percentage was determined within 0,5%.

IFNγ, IL12p40 and IL12p70 productions

The whole blood was dissolved with sterile RPMI in ratio 1:2 (blood/medium). To induce IFNγ we used the following stimulators: phytohaemagglutinin (PHA) (Sigma) – 5 μg/ml, for IL12p70, p40 –LPS *E.coli* K-235 (Sigma) – 200 ng/ml and LPS 200 ng/ml + IFNγ 1000 U/ml (Pharmingen). 72 hours later the supernatants were taken off and frozen at –20°C. Cytokine concentration was estimated with ELISA using commercial OptEIA human IFN-gamma Set, IL12p40 Set and IL12 p70 Set (Pharmingen) and following the attached protocols. Cellular capacity for IFNγ response was estimated by difference between IL12p70 production in combined response to LPS/IFNγ and to LPS only.

Results and discussion

In the peripheral blood of patient P., with recurring herpes resistant to complex therapy we determined normal $CD3^+IFN\gamma^+$-cell volumes capable for IFNγ production (see table 1). Moreover, 2 weeks after the recurrence or 7-10 before the following attack its level was within high normal meaning. We did not reveal impaired IFNγ receptor expression, since CD119 (IFNγ receptor α-chain, responsible for cytokine-binding) expression coefficient on lymphocytes and monocytes was comparable to control.

Table 1. IFNγ-system indices in patient P. and among healthy donors

Patient P. - age 55, with frequent recurrences of herpes infection, genital localization.
Healthy donors - n=15, age - from 18 to50 (M±σ, 29.0±7.1).

Index		Donors (M±σ)	Patient P.
$CD3^+IFN\gamma^+$, %	Spontaneous production	0.5±0.32	0
	Induced production	15.0±3.8	23
Interferon-gamma, pg/ml MNC	Spontaneous production	60.0±82.9	39.6
	PHA	2052.0±912.8	3402.9
	LPS	149.0±133.2	30.7
Interferon-gamma, pg/ml 30%-whole blood	Spontaneous production	61.0±41.4	397.5
	PHA	2658.0±810.9	3701.8
	LPS	420.0±279.3	849.6
Interleukin 12, p70 pg/ml MNC	Spontaneous production	22.0±24.8	5.2
	LPS	29.0±35.8	0
	LPS+IFNγ	57.0±43.0	0
Interleukin 12, p70 pg/ml 30%-whole blood	Spontaneous production	39.0±21.2	24.2
	LPS	83.0±54.0	25.5
	LPS+IFNγ	151.0±81.4	25.4
Interleukin 12 p40, pg/ml MNC	Spontaneous production	389.0±219.3	25.3
	LPS	809.0±269.3	1107.7
Interleukin 12 p40, pg/ml 30%-whole blood	Spontaneous production	453.0±145.3	859.7
	LPS	1046.0±531.5	1623.9
CD119, expression coefficient	lymphocytes	1.7±0.31	1.2
	monocytes	3.4±1.82	4.9

We previously showed that patients with chronic mycobacterial infections revealed significantly different IFNγ-productions and -responses in whole blood and MNC [2]. In order to define possible impaired cytokine production in patient P. both IFNγ and IL12 productions were measured in a test using dissolved whole peripheral blood and mononuclear cells.

We examined the production of IL12 subunit, molecular mass – 40 kD (IL12p40) as well as IL12p70 intact molecule. According to publications [1] bacterial effect results in the production of IL12p40 subunit mainly. IL12p70 could be as well synthesized by bacterial products, though IFNγ mediates a significantly increased IL12p70 production [1].

PHA cell-mediated IFNγ production in both 30% whole blood and MNC approached high normal limit.

Spontaneous IFNγ production in 30% whole blood was significantly higher as compared to normal, the latter could be referred to stimulating factors present in plasma and maintaining permanent cell activation.

However, in patient P. LPS-induced IFNγ production in MNC culture was at spontaneous level whereas in the whole blood it was even higher than normal. It is possible that plasma proteins involved in LPS- induced signaling in monocytes and B-cells (LPS-binding protein, CD14 soluble form) mediate TNFα, IL6, IL12p70 productions. The latter in turn stimulate lymphocytes for IFNγ production. If autoplasma components are removed from MNC culture the LPS-induced signaling is probably performed by surface cell molecules (TLR4, CD14), not maintaining a sufficient cytokine-inductors of IFNγ production.

IFNγ-mediated IL12p70 production in the patient's MNC was estimated insufficient. Spontaneous and LPS--induced IL12p70 productions in both 30% whole blood cells and MNC were within normal, but LPS/IFNγ-induced IL12p70 were low. IL12p40 production was within normal.

According to publications, both synthesis of p35-subunit and formation of intact IL12p70 molecule are IFNγ--mediated. In patient P. we did not reveal recombinant IFNγ--mediated increase of IL12p70 production at normal IL12p40. The latter could be referred to impaired signaling of IFNγ receptor on monocytes, caused by HSV. Yokota et al. [4] showed a significantly reduced phosphorylation of main molecules of STAT1 and Jak 1,2 interferon pathways in U937 cells infected with HSV1. Singh et al. [3] also reported possible suppression of cellular response to IFNγ in patients with genital herpes.

The submitted data indicates that a reliable estimation of immune system in herpetic patients with frequent recurrences (over 10 recurrences annually) requires a detailed investigation of IFNγ system. CD3+IFNγ$^+$ cell volumes in peripheral blood, IFNγ$^+$-production in PBC and cellular response to IFNγ estimated by IL12p70 production could be used as major criteria to estimate IFNγ system.

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