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The Ratio of Cytokine Levels in Genital Herpes during Various Phases of Infection

A. V. Shurshalina, V. N. Veryasov, and G. T. Sukhikh

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The concentrations of cytokines (tumor necrosis factor-α and interleukin-10) in cervical scrapes and the percentage of $CD4^+$ lymphocytes producing interferon-γ and interleukin-4 in the peripheral blood were measured in women of reproductive age with typical form of genital herpes. The results suggest that genital herpes is a chronic inflammatory process characterized by high levels of proinflammatory cytokines with predominance of T-helper type 1 over T-helper type 2 cytokines.

Key Words: *genital herpes; cytokines; inflammation*

Cytokines acting as mediators in cell-cell interactions play a central role in the regulation of inflammatory response, including the development of chronic inflammatory reactions [1,2,4]. The ratio of cytokines synthesized by T-helper type 1 (Th1) and T-helper type 2 (Th2) cells in herpetic infection is now widely discussed [4,6-8]. A. Heiligenhaus *et al.* [5] showed that in mice infected with herpes simplex virus (HSV) the concentrations of interleukin-2 (IL-2) and interferon-γ (IFN-γ) (Th1 cytokines) in damaged tissues markedly increased on days 1-2 after infection, while IL-4 (Th2 cytokine) was detected only on days 7-14 and in far lower concentrations. M. Baker *et al.* [3] studied local cytokine concentrations in ophthalmic herpes and detected a clear-cut relationship between high level of proinflammatory cytokine IL-6 and reactivation of HSV-1 infection. The authors came to a conclusion that ophthalmic herpes is a chronic inflammatory process characterized by high levels of cytokines (inflammation regulators).

We measured the concentration of tumor necrosis factor-α (TNF-α), a monocyte-macrophage derived proinflammatory cytokine, and its antagonist IL-10 in cervical scrapes. Intracellular measurement of proinflammatory cytokine IFN-γ produced by Th1 and IL-4, a product of Th2, in $CD4^+$ lymphocytes allowed us to evaluate the ratio of Th1/Th2 cytokines.

Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Thirty-six women with the typical form of genital herpes (GH) during relapse and 67 women during remission were examined. Control group consisted of 10 healthy women without serum antibodies to HSV-1 and HSV-2, DNA of HSV or other sexually transmitted infections.

For stimulation of cytokine production, peripheral blood mononuclear cells resuspended in RPMI-1640 with 5% ETS and 20 mM L-glutamine (10^6 cells/ml) were treated with ionomycin (1 μg/ml, Sigma) and phorbol myristate acetate (50 ng/ml, ISN) in the presence of Bretelgin A (10 μg/ml) blocking excretion of synthesized cytokines at 37°C and 5% CO_2 for 6 h. After incubation the cells were precipitated by centrifugation (400*g*, 10 min), washed twice with phosphate buffered saline (PBS), incubated with FITC-labeled monoclonal antibodies to CD4 for 30 min at 4°C. Labeled cells were washed with PBS, fixed for 15 min, and permeabilized at 18-20°C for 15 min (Fix&Peam Kit, Caltag).

Cytokines were intracellularly labeled with phycoerythrin-conjugated monoclonal antibodies to IL-4 or IFN-γ. The percentage of cells with two markers (external CD4 and internal IL-4 or IFN-γ) was evaluated by flow cytofluorimetry. Phenotyping of peripheral blood mononuclears and analysis of intracellular synthesis of IL-4 and IFN-γ in $CD4^+$ lymphocytes were carried out by flow cytofluorimetry on a Bryte HS flow cytofluorimeter (Bio-Rad).

The concentrations of TNF-α and IL-10 were measured in the cervical mucus and cells collected with a sterile probe. The probe was inserted in the cervical canal to a depth of 1 cm and rotated (360°) in both directions (total exposure not less than 10 sec). This method allows to collect not only the mucus, but also mucosa cells. The probe was promptly transferred into a tube with 10 mM Tris-buffer (pH 9.8) containing 1% bovine serum albumin and 5 μM EDTA; 5 μM phenylmethylsulfonylfluoride (protease inhibitor) was added to the buffer immediately before collecting the material. After 10-min incubation the sample was centrifuged at 5000 rpm for 5 min and the supernatant was stored at -20°C.

The concentrations of TNF-α and IL-10 were measured by ELISA with Cytelisa (Cytimmune) kits in accordance with the manufacturer's instructions. The results were recorded on a Model 150 photometer (Bio-Rad) at 450 nm.

The results were statistically processed using Student's *t* test.

RESULTS

The percentage of $CD4^+$-cells synthesizing IFN-γ in patients with GH during remission was 15.80±1.05, which was significantly higher than in the control (10.3±0.5%, $p<0.05$). During relapse the percentage of cells producing IFN-γ increased to 23.7±4.4% (*i.e.* by 50% in comparison with the corresponding parameter during remission) and considerably surpassed the control value ($p<0.03$, Fig. 1).

The percentage of IL-4-synthesizing $CD4^+$ lymphocytes underwent less pronounced changes. During remission this parameter was 7.47±0.60%, which did not differ statistically from the control ($p<0.05$). During relapse the percentage of IL-4-synthesizing cells increased by 7% but the differences from the control and remission were insignificant ($p>0.05$).

The IFN-γ/IL-4 (Th1/Th2) ratio during remission considerably surpassed the control value ($p<0.03$) and was similar during GH relapse and remission (Fig. 1).

The mean concentration of proinflammatory cytokine TNF-α during remission was 80.00±5.09 pg/ml, which significantly differed from the control ($p<0.001$, Fig. 2). During GH relapse the concentration of TNF-α increased 2-fold and was significantly higher than during remission and in the control ($p<0.02$).

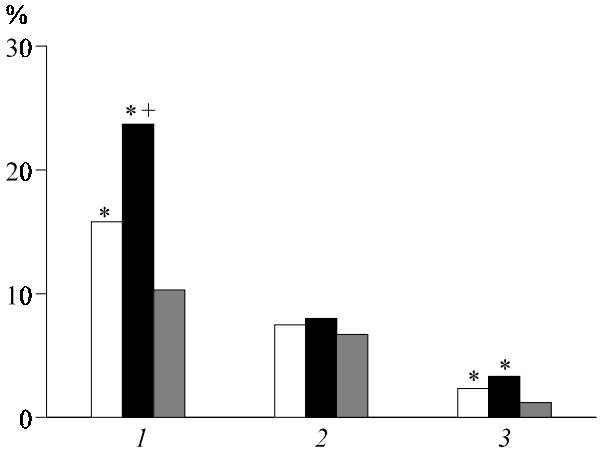

Fig. 1. Percentage of $CD4^+$ lymphocytes synthesizing IFN-γ (*1*) and IL-4 (*2*) and their ratio (*3*) during remission (light bars) and relapse (dark bars) of genital herpes. Here and in Fig. 2: $p<0.05$ *compared to the control (shaded bars); +compared to remission.

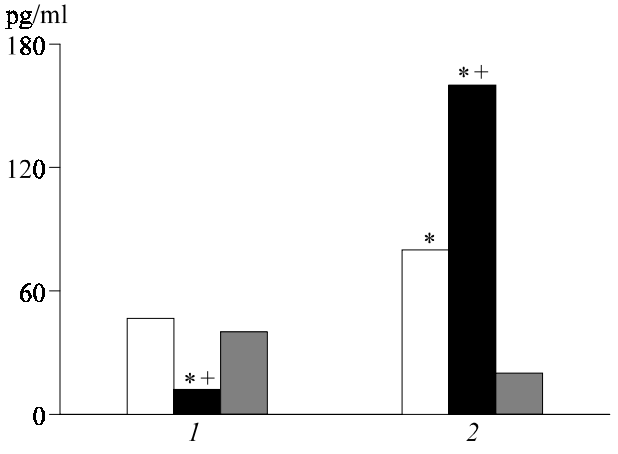

Fig. 2. Concentrations of IL-10 (*1*) and TNF-α (*2*) in cervical scrapes during remission (light bars) and relapse (dark bars) of genital herpes; shaded bars: control.

IL-10 exhibits antiinflammatory activity. During remission the mean concentration of IL-10 in the cervical mucosa was 46.6±3.6 pg/ml, which did not differ significantly from the control ($p>0.05$). During GH relapse the concentration of this cytokine decreased by 74% in comparison with remission and differed significantly from the corresponding values during remission and from the control ($p<0.05$, Fig. 2).

These findings suggest that GH is a chronic inflammatory process associated with high levels of proinflammatory cytokines (TNF-α) and predominance of Th1 cytokines (TNF-α) over Th2 cytokines (IL-4).

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