Interferon System in Women with Genital Papillomavirus Infection Receiving Immunomodulatory Therapy

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The interferon system was studied in women with genital papillomavirus infection. In most patients the interferon system was activated, while the ability of lymphocytes to respond to inductors decreased. Laserotherapy and immunomodulatory therapy with larifan, ridostin, and viferon for 1 month normalized blood interferon concentration (39.4% patients) and interferon-γ production by lymphocytes in response to inductors (87.9% patients). After laser monotherapy these parameters returned to normal only in 13.2 and 7.6% patients, respectively. Correlation and regression analyses showed that changes in the interferon system were synchronized after immunomodulatory therapy. These data indicate that immunomodulatory therapy produces a complex effect on the interferon system. Measurements of blood interferon level can be used to predict the effect of further treatment with interferon-γ inductors.

Key Words: *papillomavirus infection; laser destruction; modulatory therapy; interferon system*

Human papillomaviruses are highly contagious sexually transmitted agents persisting in epithelial tissues for a long time [6]. They are involved in carcinogenesis, e. g. cause cancer of the uterine cervix [7]. It is important to evaluate factors that promote manifestation of papillomavirus infection (PVI) and increase the efficiency of therapy.

Interferons (IFN) play an important role in the protection form viral and bacterial infections (including human PVI). Production of IFN is strictly regulated and reflects functional activity of immunocompetent cells. Inhibition of IFN production in response to inductors of its synthesis reflects the duration and severity of chronic infectious process [2]. By contrast, acute diseases are accompanied by the appearance of IFN in the plasma [1]. There are contradictory data on functional activity of the immune system and efficiency of immunotherapy in women with genital PVI [4,8].

Published data show that immunomodulatory preparations used in combination with standard therapeutical methods increase the efficiency of treatment and decrease the risk for recurrence [3]. It can be suggested that these preparations affect production of IFN with antiviral properties and, therefore, regulate activity of papillomaviruses in tissues and modulate the course of infection [10].

Here we studied the IFN system in women with genital PVI receiving immunomodulatory therapy.

MATERIALS AND METHODS

The IFN system was studied in 104 women with clinical symptoms of genital PVI. The diagnosis was made after colposcopic examination and cytological or histological assay. Highly oncogenic strains 16 and 18 and low oncogenic strains 6 and 11 were detected and typed using polymerase chain reaction.

The patients were randomly divided into 2 groups. Group 1 patients (n=52) were subjected to local destruction of lesions in the uterine cervix, vagina, and vulva with CO_2 laser. Group 2 patients (n=52) received combination therapy, which included laser vaporization and systemic immunomodulatory treat-

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TABLE 1. Distribution of Patients with PVI after Initial Examination of the Interferon System (%, *n*=52)

Parameter, U/ml	Plasma IFN (normal <4 U/ml)		Induced production			
			IFN-α (normal >64 U/ml)		IFN-γ (normal >16 U/ml)	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
4	11.5	13.5	28.8	21.1	69.3	61.6
8	15.4	13.5	32.7	34.7	23.1	30.8
16	38.5	32.7	34.7	38.5	5.7	3.8
32	32.7	36.5	3.8	5.7	1.9	3.8
64	1.9	3.8	0	0	0	0

ment with larifan or ridostin (double-stranded plant DNA, intramuscularly) and viferon (recombinant IFN-α with antioxidant additives, intravaginally) for 10 days. Local laserotherapy was performed 7-10 days after the start of immunomodulatory therapy.

The patients were examined before and 1, 6, and 12 months after the therapy (group 1: *n*=52, 47, 38, and 40, respectively; group 2: *n*=52, 43, 36, and 33, respectively). The data obtained after 1-year observations were processed.

Functional state of the IFN system was determined according to methodical recommendations of the Russian Ministry of Health (Screening Analysis of Whole Blood Interferons in People) [2].

We measured plasma IFN titer and evaluated the intensity of IFN production by leukocytes in response to Newcastle disease virus (IFN-γ inductor) and phytohemagglutinin (IFN-γ inductor).

IFN concentration in the venous blood plasma was estimated by direct titration. The reciprocal of a dilution, at which IFN inhibited destruction in the monolayer culture of human lung fibroblasts (M-19) infected with mouse encephalomyocarditis virus by 50%, was taken as a unit of activity.

Induced production of IFN was determined by titration of IFN secreted into the medium after incubation of heparinized blood with the corresponding inductor.

Equability of groups was analyzed using χ^2 test. The groups were considered to be different at $p<0.05$. Relationships between individual parameters of the IFN system were evaluated by correlation and regression analyses.

The parameters obtained after titration of IFN were discrete reciprocals of its dilution. This made the analysis of average values and statistical treatment of continuous data difficult. The samples were not characterized by a normal distribution. Therefore, between-group differences were evaluated with nonparametric methods: Man—Whitney *U* test, Wald—Wolfowitz test for independent samples, and Wilcoxon test for related samples. The differences were significant at $p<0.05$. The percentage of patients with different parameters of the IFN system was compared by χ^2 test.

The efficiency of therapy was estimated by the rate of recurrence using χ^2 test. The relative risk (RR) for recurrences was calculated by the contingency table [4,6].

RESULTS

Before the treatment the contents of plasma IFN ($p=0.17$), IFN-α ($p=0.16$), and IFN-γ ($p=0.33$) were similar in patients of both groups.

The initial examination showed that test parameters of the IFN system differed from normal in most patients (Table 1). Plasma IFN concentration was high (>16 U/ml), which indicates that the IFN system underwent pronounced activation in patients of groups 1 and 2. In all patients induced production of IFN-α was suppressed. Induced production of IFN-γ did not differ from normal only in 7.6% patients. These data attested to decreased response of blood cells to inductors.

The patients infected with strains 16/18 (*n*=56) or strains 16/18 and 6/11 (*n*=37) differed from patients infected with strains 6/11 or unidentified strains (*n*=48) in blood IFN content ($p<0.05$). Plasma IFN level surpassed the control in 91.1 and 83.3% patients infected with strains 16/18 and 6/11, respectively.

After the therapy parameters of the IFN system significantly differed in patients of groups 1 and 2. Plasma IFN concentration significantly decreased in group 2 patients examined 1, 6, and 12 months after the therapy (compared to the initial level and group 1 patients, Fig. 1, *a*). The number of patients with maximum content of blood IFN (32 U/ml) decreased in groups 2 (from 33.3 to 6%, by more than 5 times, $p<0.01$) and 1 (from 36.8 to 23.7%, $p<0.01$). Six months after the therapy this parameter reached 32 U/ml in 15.8 and 3% patients of groups 1 and 2, respectively.

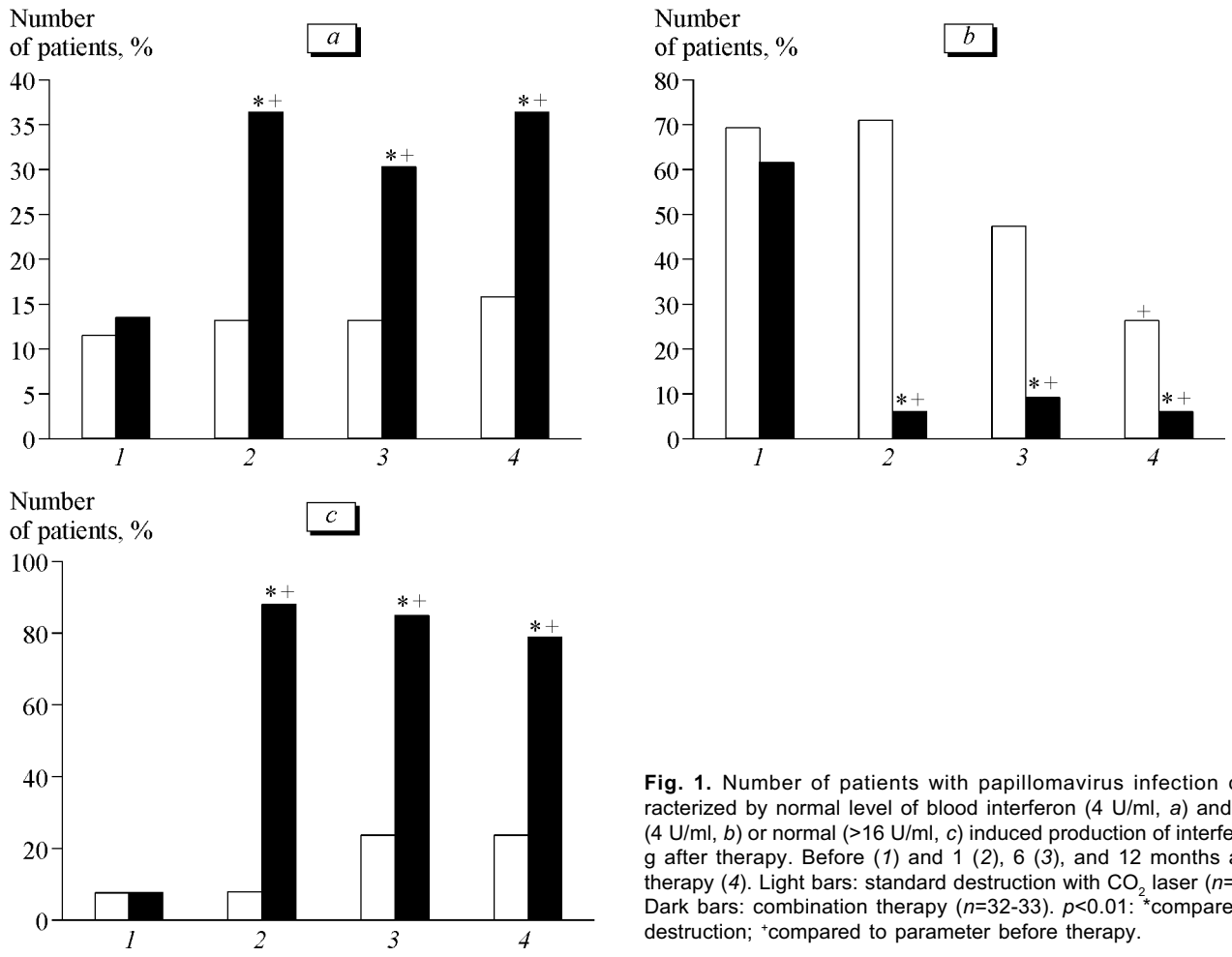

Fig. 1. Number of patients with papillomavirus infection characterized by normal level of blood interferon (4 U/ml, *a*) and low (4 U/ml, *b*) or normal (>16 U/ml, *c*) induced production of interferon-g after therapy. Before (*1*) and 1 (*2*), 6 (*3*), and 12 months after therapy (*4*). Light bars: standard destruction with CO_2 laser (n=38). Dark bars: combination therapy (n=32-33). p<0.01: *compared to destruction; +compared to parameter before therapy.

Twelve months after the therapy blood IFN level was 32 U/ml in 18.4 and 6% patients of these groups, respectively (p<0.01). The number of patients with plasma IFN concentrations of 8 and 16 U/ml changed insignificantly.

Induced production of IFN-α remained practically unchanged after the therapy. In the control we did not reveal between-group differences in this parameter. It should be emphasized that the number of group 1 patients with the minimum intensity of induced IFN-α production increased to 57.9% one month after the therapy (28.8% in the control, p<0.01), but decreased to 47.3 (p<0.05) and 39.5% 6 and 12 months after treatment, respectively. These data indicate that the IFN system was suppressed after destructive monotherapy without immunomodulators and then slowly recovered over 1 year.

The ability of cells to produce IFN-γ in response to inductors underwent most pronounced changes (Fig. 1, *b*, *c*). We found that 1, 6, and 12 months after the therapy, the intensity of induced IFN-γ production in group 2 patients far surpassed the initial level (p<0.01) and the corresponding parameter in group 1 patients (p<0.01). The percent of group 2 patients with normal level of IFN-γ markedly increased (Fig. 1, *c*). In group 1 improvement of this parameter was observed only after 6 months (p<0.05).

We evaluated whether the IFN system was characterized by a "complex" recovery after treatment. The rates of simultaneous normalization for 2 or 3 parameters and probability of this event (product of the rates of normalization for each parameter) were compared. Plasma IFN level and induced production of IFN-γ simultaneously returned to normal more frequently (p=0.4) than it could be expected (p=0.35). This theoretical value was determined from the assumption that normalization of each parameter is the independent event. During the initial examination blood IFN concentration weakly and negatively correlated with induced production of IFN-γ in blood cells (r=-0.17). One month after the therapy we found significant correlations between these parameters in group 2 patients (r=-0.43, p<0.05). Our results indicate that changes in the IFN system were synchronized and, therefore, test parameters more often simultaneously returned to normal.

We analyzed the dependence of the expected amount of IFN-γ (x) on plasma IFN level ($\mu_{y|x}$). The regression equation appeared as $\mu_{y|x}=14.19-0.13x$. For group 2 patients $\mu_{y|x}=14.5-0.3x$.

Group 2 patients were characterized by a greater regression slope (slope coefficient β=-0.3) and higher correlation coefficient than group 1 patients. These data indicate that immunomodulatory therapy was followed by the appearance of significant inverse relationships between blood IFN level and reaction of leukocytes to IFN-γ inductors.

In group 2 patients 20% reactions to IFN-γ inductors could be predicted from plasma IFN content (R^2=0.20). Therefore, the measurements of blood IFN level in group 2 patients after immunomodulatory therapy reflected the expected reaction to IFN-γ inductors with a probability of 20%. For group 1 patients this probability was 0.9%. However, the probability of expected reactions to inductors of IFN-γ estimated from its plasma content is too low for the use in clinical practice. As differentiated from R^2, the standard error of regression analysis allows us to estimate the limits for IFN production by leukocytes with a probability of 95%. The smaller is the standard error, the narrower is this interval. This analysis more precisely predicts the reaction of organisms to immunomodulators [9].

In group 1 patients the standard error was greater than in group 2 patients (Table 2). When after the therapy blood IFN level in group 2 patients is 4 U/ml, induced production of IFN-γ does not differ from normal with a probability of 95%. In group 1 patients with normal level of plasma IFN the intensity of induced IFN-γ production can undergo considerable variations (4-32 U/ml). Therefore, IFN inductors will be low effective in these patients. Correlation and regression analyses indicate that it is possible to select the scheme of immunomodulatory therapy to increase the efficiency of treatment of genital PVI in women.

The effects of combination therapy and standard method for destruction of lesions associated with human PVI were compared in patients with genital PVI. Groups 1 and 2 included 135 and 132 women, respectively.

The rate of recurrence of PVI over 12 months reflected the efficiency of therapy. The incidence of recurrence in patients of groups 1 and 2 was 46.3 and 26.7%, respectively. Recurrent diseases of the uterine cervix associated with PVI were revealed in 10.3 and 4.6% patients of groups 1 and 2, respectively. RR for recurrence of PVI in group 2 patients was 0.56 (compared to group 1 patients, confidence interval 0.24-0.88). These data show that adjuvant immunomodulators and destructive treatment produce a grater clinical effect than standard monotherapy with destruction of genital lesions associated with human PVI.

Our results indicate that immunomodulators hold much promise for combination therapy of patients with PVI.

TABLE 2. Regression Analysis for the Dependence of Induced IFN-γ Production on Blood IFN Level in Patients with PVI 1 Month after Therapy

Parameter of regression analysis	Group 1 (n=38)	Group 2 (n=33)
R^2	0.0093	0.2020
Standard error	8.75	5.87
Coefficient for regression slope (b)	-0.13	-0.30

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