

# Pro- and Anti-inflammatory Cytokine Content in Human Peripheral Blood after Its Transcutaneous (*in Vivo*) and Direct (*in Vitro*) Irradiation with Polychromatic Visible and Infrared Light

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## ABSTRACT

**Objective:** The aim of this randomized, placebo-controlled, double-blind trial was to investigate changes in the content of 10 cytokines in the human peripheral blood after transcutaneous and *in vitro* irradiation with polychromatic visible and infrared (IR) polarized light at therapeutic dose. **Background Data:** The role of cytokines in development of anti-inflammatory, immunomodulatory, and wound-healing effects of visible and IR light remains poorly studied. **Methods:** The sacral area of volunteers was exposed (480–3400 nm, 95% polarization, 12 J/cm<sup>2</sup>); in parallel, the blood samples of the same subjects were irradiated *in vitro* (2.4 J/cm<sup>2</sup>). Determination of cytokine content was performed using enzyme-linked immunosorbent assay (ELISA). **Results:** A dramatic decrease in the level of pro-inflammatory cytokines TNF- $\alpha$ , IL-6, and IFN- $\gamma$  was revealed: at 0.5 h after exposure of volunteers (with the initial parameters exceeding the norm), the cytokine contents fell, on average, 34, 12, and 1.5 times. The reduced concentrations of TNF- $\alpha$  and IL-6 were preserved after four daily exposures, whereas levels of IFN- $\gamma$  and IL-12 decreased five and 15 times. At 0.5 h and at later times, the amount of anti-inflammatory cytokines was found to rise: that of IL-10 rose 2.7–3.5 times (in subjects with normal initial parameters) and of TGF- $\beta$ 1 1.4–1.5 times (in the cases of its decreased level). A peculiarity of the light effect was a fast rise of IFN- $\gamma$  at 3.3–4.0 times in subjects with normal initial values. The content of IL-1 $\beta$ , IL-2, IFN- $\alpha$ , and IL-4 did not change. Similar regularities of the light effects were recorded after *in vitro* irradiation of blood, as well as on mixing the irradiated and non-irradiated autologous blood at a volume ratio 1:10 (i.e., at modeling the events in a vascular bed of the exposed person when a small amount of the transcutaneously photomodified blood contacts its main circulating volume). **Conclusion:** Exposure of a small area of the human body to light leads to a fast decrease in the elevated pro-inflammatory cytokine plasma content and to an increase in the anti-inflammatory factor concentration, which may be an important mechanism of the anti-inflammatory effect of phototherapy. These changes result from transcutaneous photomodification of a small volume of blood and a fast transfer of the light-induced changes to the entire pool of circulating blood.

## INTRODUCTION

THE ANTI-INFLAMMATORY, immunomodulating, and wound-healing effects of visible and infrared (IR) light has been widely used in medicine for more than 30 years. However, the mechanism by which irradiation of a small area of the body surface with monochromatic or polychromatic visible and IR

light results in development of the above systemic effects has not yet been elucidated.

Taking into account the relatively deep penetration of visible and IR radiations into the human skin and the possibility of transcutaneous blood photomodification (which is proved by the successful therapy of neonatal hyperbilirubinemia with the blue light<sup>1</sup>), we have proposed that one of the main mecha-

nisms of the systemic effects of optical radiation consists of rapid changes of cells and plasma components of circulating blood.<sup>2</sup> We have shown that, for the first 30–60 min after a local exposure of the volunteers' body surface to polychromatic visible and IR polarized (VIP) light (480–3400 nm) at a therapeutic dose, changes develop in the structural and functional state of all types of peripheral blood cells; the plasma growth factor level rises and, as a consequence, plasma mitogenic (growth-promoting) activity for lymphocytes, keratinocytes, fibroblasts, and endotheliocytes increases.<sup>2–11</sup> The above changes are also recorded after direct irradiation of blood samples of the same volunteers *in vitro* and after mixing the irradiated blood with a much greater volume of non-irradiated autologous blood, which simulates events in the systemic circulation after a local irradiation of the body's surface. Such similarity has allowed us to conclude that the systemic effects developed after exposure to light can be a consequence of transcutaneous photomodification of small amounts of blood and the action of photomodified blood on its entire circulating volume.

The goal of the present work was to study effects of polychromatic VIP light at a therapeutic dose on the content in human blood of the most important 10 pro- and anti-inflammatory cytokines, which, apart from regulation at the local and systemic level of processes of non-specific defense and specific immunity, are known to affect functions of connective and epithelial tissue cells, and to control regeneration, hemopoiesis, and metabolism.<sup>12–14</sup> The available literature on the effect of visible and IR light on the cytokine network is scarce and controversial. With few exceptions, such data were obtained through irradiation of isolated human blood mononuclears or their constant lines, which only allows the evaluation of photoreactivity of immunocytes, rather than the systemic effect of light.

## METHODS

### *Volunteers and blood samples*

Participants in this study were apparently healthy volunteers of both genders aged 18–65 years. They were randomly divided into two groups. The first (experimental) group (43 individuals, 75% women and 25% men, mean age of  $42.2 \pm 1.8$  years) was irradiated daily for 5 days with VIP light (sacral area, diameter of 15 cm), and their blood was drawn as follows: 35 mL before the start of the trials and then 15 mL each at 0.5 and 24 h after the first irradiation and 24 h after four daily sessions (prior to the fifth one), to a total volume of 80 mL during the 5-day-long course. In parallel with the first irradiation of the volunteers, samples of their blood were irradiated under *in vitro* conditions. The second (placebo) group used as control was composed of non-irradiated volunteers (19 individuals, 70% women and 30% men, mean age of  $41.3 \pm 4.3$  years). In this group, sacral area irradiation was imitated (the source of light was shielded with an opaque filter), and the blood for the study was drawn at the same time periods and in the same volumes as in the experimental group. The participants of the trials were not informed as to which group they belonged.

The Ethical Committee of the Institute of Cytology of the Russian Academy of Sciences approved this study in 1998; all

participants of the trials gave informed consent to be exposed daily to VIP light for 5 days and to be donors of blood.

### *Irradiation procedure*

As the source of VIP light (480–3400 nm, 95% polarization, power density of 40 mW/cm<sup>2</sup>), a phototherapeutic device, Biotron-2 (Switzerland), was used. At a distance of 20 cm and duration of light exposure of 5 min, the therapeutic dose amounted to 12 J/cm<sup>2</sup>. At blood irradiation under *in vitro* conditions (in Petri dishes), the dose was fivefold lower (2.4 J/cm<sup>2</sup>), as only 20% of the light energy incident onto the body surface was assumed to reach the vascular network.<sup>2</sup>

### *Study of cytokines*

Eight cytokines (IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-10, IL-12, TNF- $\alpha$ , and IFN- $\gamma$ ) were tested in the blood plasma. As anticoagulant, heparin (20 units/mL) was used, though for testing IFN- $\gamma$  the potassium EDTA salt (15%) was used. In the former case, blood was drawn into polypropylene tubes (Elkay, USA); in the latter case, it was drawn into vacutainers (Becton Dickinson, USA). IFN- $\alpha$  and TGF- $\beta$ 1 were determined in the blood serum, without anticoagulant (vacutainers with separation gel; Becton Dickinson), which did not allow methodical *in vitro* irradiation of the whole blood. The cytokines were determined by enzyme-linked immunosorbent assay (ELISA),<sup>15</sup> using test-systems of R&D Systems (USA), while in the case of IFN- $\alpha$ , we used test-systems of PBL Biomedical Laboratories (USA), with subsequent analysis of samples on a SLT-Spectra microplate reader (Austria) at  $\lambda = 450$  nm, with the correction wavelength set at 540 nm. Prior to testing the level of TGF- $\beta$ 1 in blood serum, its latent form was activated, using the recommendations of the manufacturer.

### *Statistical analysis*

Evaluation of statistical significance of changes was performed by the parametric and non-parametric methods for matched (dependent) pairs of results (Student *t*-test and Wilcoxon *U*-criterion). Correlation of effects with the initial parameters and with each other was calculated by the method of Pearson and Spearman.

## RESULTS

### *Cytokine levels in the blood of healthy volunteers*

The total number of the volunteers tested for cytokines was 26–58. Plasma concentration of the studied cytokines showed a very high individual variability. Before the investigation of light effects, we determined what amount of each cytokine tested by reagents of the U.S. company R&D Systems corresponded to the norm in residents of St. Petersburg, as it seemed incorrect to accept the normal values established using reagents of Russian origin or those determined in healthy U.S. residents (i.e., in another population of people). Table 1 presents the upper limit of the norm for the content of 10 cytokines in the blood of healthy U.S. donors (according to data of the test-system manufacturer) and in the north-western

TABLE 1. CYTOKINE CONTENT IN BLOOD PLASMA OF HEALTHY RESIDENTS OF THE UNITED STATES (A) AND RUSSIA (B) ACCORDING TO DATA OF THE TEST-SYSTEM MANUFACTURER (R&D SYSTEMS, USA) AND OUR DATA

Cytokines	Minimum detectable amount, pg/mL	Upper limit in norm, pg/mL	
		A, n = 30–40	B, n = 26–58
IL-1 $\beta$	1	<3.9	<1
IL-2	7	<31.2	<7
IL-4	10	<31.2	<10
IL-6	0.7	<12.5	<10.6
IL-10	3.9	<7.8	<31.0
IL-12	5	<7.8	<10.2
TNF- $\alpha$	4.4	<15.6	<138.4
IFN- $\gamma$	8	<15.6	<60.9
IFN- $\alpha$	10	No data <sup>a</sup>	<165.2
TGF- $\beta$ 1	7	34.7–63.9 <sup>b</sup>	36.0–43.3

<sup>a</sup>Test-systems of PBL Biomedical Laboratories (USA).

<sup>b</sup>Content in serum.

(St. Petersburg) region of Russia (our own data). It is seen that, for six cytokines (IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-12, and TGF- $\beta$ 1), our results are close to the data of R&D Systems, whereas the upper limit of the content of TNF- $\alpha$ , IFN- $\gamma$ , and IL-10 turned out to be significantly higher. This might have resulted from the fact that participants of our trials were, on average, older than the cohort acting as donors (younger than 30 years old). The procedure used to calculate the normal level is described elsewhere.<sup>16</sup>

#### Levels of pro-inflammatory cytokines in the blood plasma of volunteers after irradiation

IL-1 $\beta$  and IL-2 were not detected in the blood plasma of the volunteers before their irradiation. Nor were these cytokines revealed after the single and fourfold daily VIP-irradiations of the body surface, nor after irradiation of blood samples *in vitro* (Table 2A). The IFN- $\alpha$  level remained unchanged (Table 2A). However, the content of this pro-inflammatory cytokine changed significantly (Table 2A). In subjects with normal initial TNF- $\alpha$  level there was a tendency for a decrease (to zero on the 5<sup>th</sup> day). In three subjects characterized by elevated TNF- $\alpha$  level, the content of other pro-inflammatory cytokines dropped 34 times as soon as 0.5 h after the single VIP-irradiation. However, due to the low number of observations and a high variability of the initial parameters, this effect was statistically non-significant ( $p = 0.11$ ).

A fast and statistically significant decrease of IL-6 content after the single exposure to VIP light was revealed (Table 2A, Fig. 1). In volunteers with the initially normal parameters, it decreased at 0.5 h twofold, whereas in individuals with high initial parameters, it rapidly fell 12 times and remained at this level until the end of the course. IL-12 showed a decrease at its normal level ( $p = 0.07$ ) and decreased statistically significantly in individuals with elevated initial content (15 times) after four daily sessions (Table 2A, Fig. 2). In the control (placebo) group, no statistically significant changes were revealed for

both cytokine levels (Fig. 1). A similar decrease was also found for elevated initial IFN- $\gamma$  concentrations (Table 2A, Fig. 3). However, in the case of lower/normal initial IFN- $\gamma$  content, VIP light promoted a marked increase (Table 2A, Fig. 3): immediately after the first phototherapeutic procedure and until the end of the course, the IFN- $\gamma$  level rose 3.3–4 times. In the control group with normal initial parameters, cytokine content decreased statistically significantly (Fig. 3).

The regulatory character of VIP light on the plasma level of the pro-inflammatory cytokines is confirmed by an inverse dependence of the light effect on initial parameters: at all time periods of testing, the correlation coefficient  $r$  values were negative and statistically significant, varying from  $-0.55$  to  $-1.00$  ( $p < 0.05$ ,  $p < 0.01$ ).

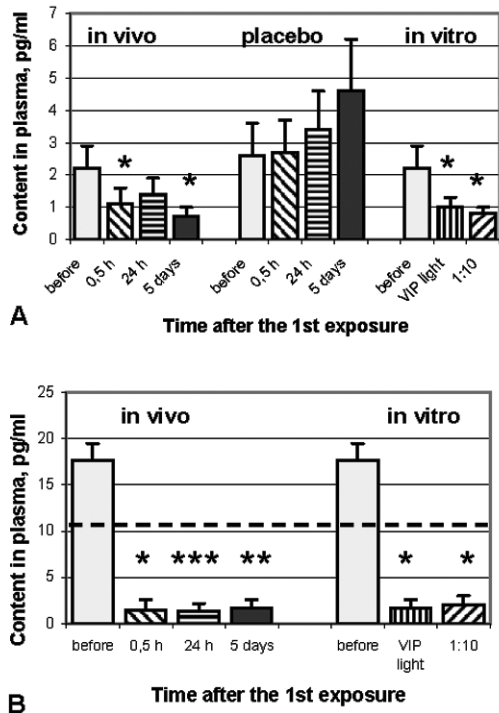
#### Levels of anti-inflammatory cytokines in the blood plasma of volunteers after irradiation

VIP light promotes a rise of the content of anti-inflammatory factors in the blood plasma. Thus, the normal initial IL-10 content increased 0.5 h after irradiation 2.7 times, and later, 3.2 and 3.5 times; the elevated level of the cytokine did not change (Table 2B, Fig. 4) and there was not revealed the inverse dependence of light effects on the initial cytokine levels ( $r = -0.23$ ,  $p > 0.10$ ). Most likely, this was due to effect of exfusions of small amounts of blood for the study, which accompanied the light sessions in our trials. Indeed, 24 h after two drawings of blood (total of 50 mL) in volunteers of the control (placebo) group with the normal initial IL-10 content, a tendency was revealed for its brief increase ( $p = 0.12$ ), whereas in individuals with the elevated cytokine level its amount rose twofold (Fig. 4), these changes of the IL-10 amount correlating positively with its initial level ( $r = 0.52$ – $0.72$ ,  $p < 0.05$ ). Hence, the increase of the blood IL-10 amount in irradiated volunteers with the normal cytokine level only partly was due to the hemoxfusion procedure, whereas the absence of the statistically significant decrease of its amount in subjects with its high ini-

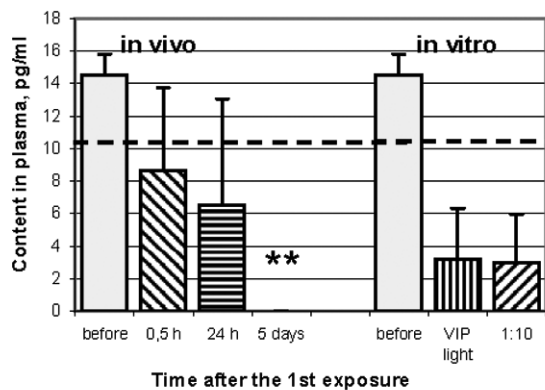
TABLE 2. CONTENT OF PRO-INFLAMMATORY (A) AND ANTI-INFLAMMATORY (B) CYTOKINES IN BLOOD PLASMA OF VOLUNTEERS AFTER FIRST AND FOUR DAILY IRRADIATIONS, AFTER IRRADIATION OF THEIR BLOOD *IN VITRO* AND MIXING IRRADIATED AND NON-IRRADIATED AUTOLOGOUS BLOOD SAMPLES AT A VOLUME RATIO OF 1:10

Cytokines	Initial cytokine level	n	Cytokine amount prior to exposure, pg/mL (m ± SE)			After irradiation of volunteers			After irradiation of blood in vitro					
			1 <sup>st</sup> irradiation			4 <sup>th</sup> irradiation, at 24 h			VIP light			Mixing, 1:10		
			at 0.5 h	at 24 h	at 24 h	at 24 h	at 24 h	at 24 h	at 24 h	at 24 h	at 24 h	at 24 h	at 24 h	
A	IL-1β	33	0	0	0	0	0	0	0	0	0	0	0	0
	Elevated level	—	—	—	—	—	—	—	—	—	—	—	—	—
	IL-2	17	0	0	0	0	0	0	0	0	0	0	0	0
	Elevated level	—	—	—	—	—	—	—	—	—	—	—	—	—
	TNF-α	25	3.2 ± 1.5	2.6 ± 0.7	0.9 ± 0.7	0	0	0	0.9 ± 0.8	0	0.9 ± 0.8	1.3 ± 1.2	1.3 ± 1.2	1.3 ± 1.2
	Elevated level	3	396.7 ± 167.9	11.7 ± 6.4	0	0	0	0	0	0	0	0	0	0
	IL-6	14	2.2 ± 0.7	1.1 ± 0.5*	1.4 ± 0.5	0.7 ± 0.3*	0.7 ± 0.3*	1.0 ± 0.3*	1.0 ± 0.3*	1.0 ± 0.3*	0.8 ± 0.2*	0.8 ± 0.2*	0.8 ± 0.2*	0.8 ± 0.2*
	Elevated level	4	17.6 ± 1.9	1.5 ± 1.1*	1.4 ± 0.8***	1.7 ± 0.9**	1.7 ± 0.9**	3.6 ± 1.8*	3.6 ± 1.8*	3.6 ± 1.8*	2.0 ± 1.0*	2.0 ± 1.0*	2.0 ± 1.0*	2.0 ± 1.0*
	IL-12	14	1.6 ± 0.7	0.4 ± 0.4	1.4 ± 1.0	0.4 ± 0.4	0.4 ± 0.4	1.4 ± 1.0	1.4 ± 1.0	1.4 ± 1.0	2.0 ± 1.2	2.0 ± 1.2	2.0 ± 1.2	2.0 ± 1.2
	Elevated level	3	14.5 ± 1.3	8.6 ± 5.1	6.5 ± 6.5	0**	0**	3.2 ± 3.2	3.2 ± 3.2	3.2 ± 3.2	3.0 ± 3.0	3.0 ± 3.0	3.0 ± 3.0	3.0 ± 3.0
B	IFN-α	23	7.7 ± 4.5	9.2 ± 4.8	7.1 ± 4.0	8.2 ± 4.2	8.2 ± 4.2	—	—	—	—	—	—	—
	Elevated level	3	209.3 ± 21.3	216.7 ± 13.2	198.0 ± 7.1	237.0 ± 31.5	237.0 ± 31.5	—	—	—	—	—	—	—
	IFN-γ	34	13.4 ± 3.0	44.3 ± 14.3*	45.5 ± 13.9*	53.6 ± 11.4*	53.6 ± 11.4*	54.4 ± 18.8*	54.4 ± 18.8*	54.4 ± 18.8*	70.4 ± 24.3*	70.4 ± 24.3*	70.4 ± 24.3*	
	Elevated level	9	150.4 ± 52.3	97.7 ± 28.9*	46.1 ± 16.6*	30.0 ± 16.4*	30.0 ± 16.4*	123.9 ± 45.4	123.9 ± 45.4	123.9 ± 45.4	69.3 ± 24.0*	69.3 ± 24.0*	69.3 ± 24.0*	
	IL-4	17	0	0	0	0	0	0	0	0	0	0	0	0
	Elevated level	—	—	—	—	—	—	—	—	—	—	—	—	—
	IL-10	31	6.1 ± 1.3	16.4 ± 4.1**	21.5 ± 4.8**	19.3 ± 2.2*	19.3 ± 2.2*	14.2 ± 3.2**	14.2 ± 3.2**	14.2 ± 3.2**	19.0 ± 6.7*	19.0 ± 6.7*	19.0 ± 6.7*	
	Elevated level	3	69.7 ± 18.4	48.1 ± 48.0	55.3 ± 44.3	—	—	24.4 ± 24.3	24.4 ± 24.3	24.4 ± 24.3	29.4 ± 23.6	29.4 ± 23.6	29.4 ± 23.6	
	TGF-β1	7	39.5 ± 1.0	39.4 ± 3.4	45.8 ± 5.9	35.1 ± 2.1	35.1 ± 2.1	—	—	—	—	—	—	—
	Elevated level	7	49.5 ± 3.1	44.1 ± 3.3	54.0 ± 8.6	46.9 ± 3.3	46.9 ± 3.3	—	—	—	—	—	—	—
Low level	5	27.1 ± 2.9	39.5 ± 7.2*	37.2 ± 5.0*	37.3 ± 7.2	37.3 ± 7.2	—	—	—	—	—	—	—	

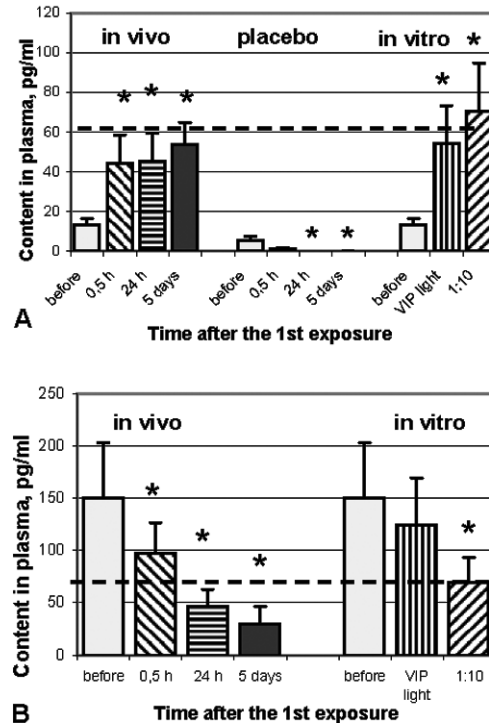
\*\*\*, \*\*\*, \*\*\* Difference from the initial level is statistically significant:  $p \leq 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.



**FIG. 1.** Changes in IL-6 plasma content (mean ± SE) in volunteers with its normal (A) and elevated (B) initial levels after visible and infrared (IR) polarized light (VIP) irradiation of blood *in vivo* (transcutaneously), *in vitro*, after mixing the irradiated and non-irradiated autologous blood samples at a 1:10 ratio and after blood exfusions in the control (placebo) group. \*, \*\*, \*\*\*Difference from the initial level is statistically significant:  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively. Dashed line, the upper limit of norm.



**FIG. 2.** Changes in IL-12 plasma content (mean ± SE) in volunteers with its elevated initial level after visible and infrared (IR) polarized light (VIP) irradiation of blood *in vivo* (transcutaneously), *in vitro*, after mixing the irradiated and non-irradiated autologous blood samples at a 1:10 ratio and after blood exfusions in the control (placebo) group. Other designations are the same as in Figure 1.



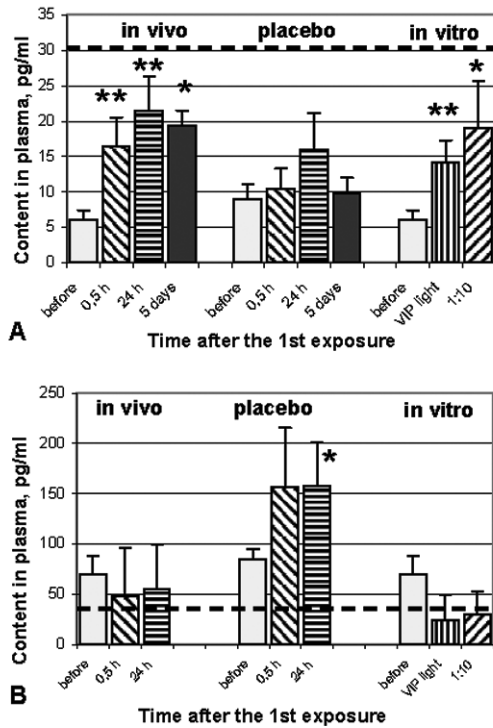
**FIG. 3.** Changes in IFN- $\gamma$  plasma content in volunteers with its normal (A) and elevated (B) initial level after visible and infrared (IR) polarized light (VIP) irradiation of blood *in vivo* (transcutaneously), *in vitro*, after mixing the irradiated and non-irradiated autologous blood samples at a 1:10 ratio and after blood exfusions in the control (placebo) group. Other designations are the same as in Figure 1.

tial level was a consequence of combined action of two factors—hemoexfusions and VIP-irradiation—with the former promoting a twofold rise of the IL-10 level, and the latter inducing the opposite effect.

Study of the content of other anti-inflammatory cytokines has shown no effect of VIP-sessions on the IL-4 level (Table 2) and a statistically significant elevation (almost 1.5 times) of the TGF- $\beta$ 1 content in subjects with its low initial amount (Table 2B, Fig. 5). The regulatory character of the effect of phototherapeutic sessions on the TGF- $\beta$ 1 level is confirmed by statistically significant negative correlation coefficients, with their values varying from  $-0.49$  to  $-0.60$  ( $p < 0.05$ ).

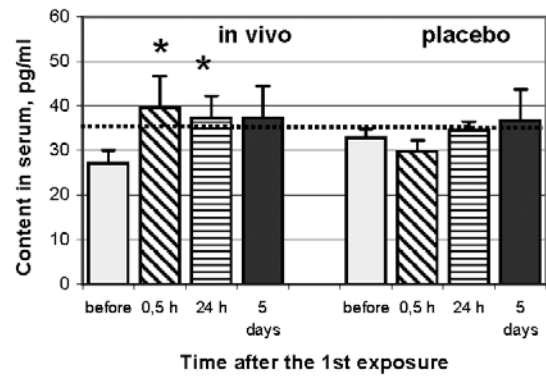
*Levels of pro- and anti-inflammatory cytokines in plasma after irradiation of blood in vitro*

To find out to which degree the fast cytokine level changes in systemic circulation were a consequence of the transcutaneous photomodification of blood, in parallel experiments the blood of the same volunteers was irradiated *in vitro* and, besides, samples of the irradiated and non-irradiated autologous blood were mixed in the volume ratio 1:10 to model thereby the events in the vascular bed after the local body surface irradiation, when a small amount of the irradiated blood contacts a significantly larger volume of circulating blood.



**FIG. 4.** Changes in IL-10 plasma content (mean  $\pm$  SE) in volunteers with its normal (A) and elevated (B) initial level after visible and infrared (IR) polarized light (VIP) irradiation of blood *in vivo* (transcutaneously), *in vitro*, after mixing the irradiated and non-irradiated autologous blood samples at a 1:10 ratio and after blood exfusions in the control (placebo) group. Other designations are the same as in Figure 1.

As seen in Table 2 and Figures 1, 3, and 4, direction and degree of changes have turned out to be very similar to those after the single and fourfold VIP-irradiations of the volunteers' body surface. This similarity was confirmed by high positive correlation coefficients of the effects in these experimental series



**FIG. 5.** Changes in TGF- $\beta$ 1 serum content (mean  $\pm$  SE) in volunteers with its low initial level after visible and infrared (IR) polarized light (VIP) irradiation of blood *in vivo* (transcutaneously) and after blood exfusions in the control (placebo) group. Dotted line, the low limit of norm; other designations are the same as in Figure 1.

(Table 3). Only the IL-10 level changes weakly correlated with those under conditions *in vivo*, which, as indicated above, might be a consequence of effects of hemoexfusions accompanying the volunteers' exposures. These results allow associating the fast changes of the cytokine network under conditions *in vivo* with direct effect of light on blood in superficial skin blood vessels. The experiments with modeling the situation *in vivo* by mixing the irradiated and non-irradiated autologous blood samples indicated that after contact of photomodified blood with the 10-fold volume of the non-irradiated blood the changes not only did not disappear (due to a dilution effect), but even were enhanced and preserved a great similarity with effects of the light itself, both *in vitro* and *in vivo* (Table 2, Figs. 1–4). The identity of the effects produced, on one hand, by the light and, on the other hand, by the light-modified blood *in vitro* and *in vivo* is indicated by the positive correlation of the effects (Table 3). Such similarity was also observed in the case of IL-

**TABLE 3.** SIMILARITY OF CHANGES OF CYTOKINE CONTENT IN BLOOD OF VOLUNTEERS AFTER (A) VIP IRRADIATION OF BLOOD *IN VITRO* AND MIXING THE IRRADIATED AND NON-IRRADIATED AUTOLOGOUS BLOOD AT A VOLUME RATIO OF 1:10, (B) AT 0.5 AND 24 h AFTER THE FIRST IRRADIATION OF BLOOD *IN VIVO* (TRANSCUTANEOUSLY) AND *IN VITRO*, (C) AT 24 h AFTER FOUR DAILY IRRADIATIONS OF BLOOD *IN VIVO* (AT THE 5<sup>TH</sup> DAY) AND *IN VITRO*

Variants compared	Effects compared	Values of the correlation coefficient $r$				
		TNF- $\alpha$	IL-6	IL-12	IFN- $\gamma$	IL-10
A	VIP light—1:10	1.00**	0.99**	0.61*	0.56**	0.58**
B	0.5 h—VIP light	0.99**	0.89**	0.72**	0.81**	0.73**
	0.5 h—1:10	0.89**	0.91**	0.58	0.73**	0.69**
	24 h—VIP light	1.00**	0.91**	0.80**	0.82**	0.32
	24 h—1:10	1.00**	0.80**	0.66**	0.73**	0.39*
C	5 <sup>th</sup> day—VIP light	1.00**	0.94**	0.79**	0.94**	0.03
	5 <sup>th</sup> day—1:10	1.00**	0.98**	0.82**	0.91**	0.78**

\*,\*: Statistically significant  $r$  values:  $p < 0.05$  and  $p < 0.01$ , respectively.

10, which indicates the leading role of the light treatment (rather than hemoexfusions) in changes of this cytokine.

Under conditions *in vitro* the effects of light and the irradiated blood were of regulatory character, similarly to the effect of light on blood *in vivo* ( $r$  varied from  $-0.56$  to  $-1.00$ ,  $p < 0.01$ ). The same regularity is to be noted for changes of IL-10 under conditions *in vitro* (i.e., in the absence of hemoexfusions, the light and irradiated blood promoted a rise of the cytokine level at its low initial content and its decrease at the high initial level) (Table 2, Fig. 4).

## DISCUSSION

Data from the literature on the effects of visible and IR light on production of immunocytokines are scarce and controversial. Most papers report an increase of levels of pro-inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and IFN- $\gamma$ ) in the culture medium after *in vitro* irradiation of human blood mononuclears and their cell lines.<sup>17,18,19–25</sup> Rossano et al.<sup>26</sup> suggest that the effect of IR light on the production of IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 by donor blood monocytes is comparable to mitogen influence. However, other authors found no up-regulatory effect of visible and IR radiation on spontaneous synthesis of pro-inflammatory cytokines.<sup>17,27,28</sup> Moreover, a decrease of spontaneous TNF- $\alpha$  production by cells of the human monocyte line was revealed.<sup>29</sup> Similarly controversial are data on mitogen-stimulated cytokine production.<sup>20,22,23,28,30,31</sup>

According to the data of the present study, the elevated plasma content of some pro-inflammatory cytokines (TNF- $\alpha$ , IL-6) dramatically fell as early as 30 min after the first exposure of volunteers and was preserved at this low level until the end of the 4-day phototherapeutic course, whereas the elevated IL-12 and IFN- $\gamma$  parameters gradually decreased and reached normal values by the 5<sup>th</sup> day. No IL-1 $\beta$ , IL-2, and IL-4 were revealed in the blood of volunteers before or after irradiation. These results, on the whole, are in a good agreement with observations of the authors who, without studying the possibilities of rapid changes of the cytokine network, recorded a decrease of elevated IL-1 $\beta$  and TNF- $\alpha$  concentrations in blood plasma of patients with purulent-inflammatory, oncological, and cardiovascular diseases after a long course of percutaneous or intravascular blood irradiation with IR and red laser light,<sup>32–34</sup> as well as in animals with experimental arthropathy.<sup>35</sup>

An important result of the present study is establishment of the fact that VIP-irradiation simultaneously induces a rise of the amount of anti-inflammatory cytokines, IL-10 and TGF- $\beta$ 1. It is known that IL-10 restricts inflammation processes in the organism; it suppresses synthesis by monocytes/macrophages and granulocytes of pro-inflammatory cytokines and chemokines, promotes release of their natural antagonists (antagonist of IL-1 receptor and soluble subunit of TNF- $\alpha$  receptor), and reduces production of oxygen radicals. At the same time, IL-10 enhances proliferation, differentiation, and cytotoxicity of CD8<sup>+</sup> T-lymphocytes and NK-cells, which is believed to be associated with its activating effect on antitumor immunity. By promoting proliferation and differentiation of B-lymphocytes, their protection from apoptosis, and an increase of expression of MHC II antigens on these cells, IL-10 contributes the maintenance of an active state of humoral immu-

nity.<sup>12–14</sup> There is evidence<sup>36</sup> in favor of synthesis of IL-10 in the situation associated with cellular stress, specifically under the effect of glucocorticoids, catecholamines, UV radiation, and reactive oxygen species (ROS). An increase of the IL-10 content is also reported at stress reactions of the organisms, such as traumas and surgical interventions.<sup>37</sup> The combination of these circumstances may be responsible for an increase of the IL-10 level in the placebo group, in which the 3–4-fold hemoexfusions are accompanied by alterations of skin cells and blood vessels, blood losses, and certain pain and emotional stress. The anti-inflammatory TGF- $\beta$ 1 effect also is associated with down-regulation of the monocyte/macrophage and granulocyte activity (synthesis of pro-inflammatory cytokines, production of ROS and nitric radicals), and suppression of lymphocyte proliferation.<sup>12–14</sup>

All the above suggests that one of the major mechanisms of the anti-inflammatory effect of visible and IR light consists of a fast and sufficiently steady decrease in blood plasma of the high content of the pro-inflammatory cytokines and of a rapid rise of the amount of mediators with alternative properties.

A peculiarity of the VIP light effect on the cytokine network is an almost immediate promotion of the plasma IFN- $\gamma$  concentration in subjects with normal initial parameters. Such results agree well with data<sup>19</sup> that show a strong induction of early interferon (IFN- $\gamma$  and IFN- $\alpha$  of a high titer), as early as 3 h after irradiation of human blood mononuclears with He-Ne laser. Taking into account that IFN- $\gamma$  is a physiological antagonist of IL-10, we calculated their ratio (IFN- $\gamma$ /IL-10). Prior to exposure, it amounted, on average, to 2.4 and changed insignificantly at 0.5 and 24 h after the first light session, but rose almost twice (to 4.6) after four daily irradiations. Since an increase of this ratio (to 5.6) also occurred after the blood irradiation *in vitro* (whereas in the placebo group the opposite tendency was revealed), it can be concluded that a peculiarity of the VIP light effect is a more significant increase of the IFN- $\gamma$  than of the IL-10 content. Since the hemoexfusions accompanying the volunteers' exposures to light promote elevation of the IL-10 content and down-regulate the IFN- $\gamma$  level, it can be suggested that, in the absence of blood collection (at performance of the phototherapeutic course), the release of IFN- $\gamma$  into blood will be more pronounced, while that of IL-10, decreased. As a result, the IFN- $\gamma$ /IL-10 ratio will shift even more towards IFN- $\gamma$ , and the IL-10 inhibitory effect on monocytes/macrophages activity will decrease.

An increase in the systemic circulation of IFN- $\gamma$ —a most important stimulator of cell-mediated immune response—is accompanied by a rise of microbiocidal, antiviral, and antitumor properties of monocytes/macrophages, an increase of their production of superoxide and nitric radicals, by an enhancement of cytotoxicity of NK- and CD8<sup>+</sup> T-cells and of expression of MHC II molecules on cells of many tissues.<sup>12,14</sup> We already reported some of these effects of VIP light.<sup>2,8,10,11,38</sup> A fast increase of the IFN- $\gamma$  level in circulation seems to be one of mechanisms of the therapeutic effect of visible and IR light in some viral infections (acute respiratory tract infections, influenza, herpes), intracellular invasions (tuberculosis), onco-pathology (in the complex of pre- and post-surgery immunorehabilitation).<sup>39–41</sup> Besides, taking into account the use for recent years of anti-proliferative properties of IFN- $\gamma$  preparations for prevention and therapy of postsurgery hyperproduc-

tion of connective tissue cells,<sup>42,43</sup> it can be suggested that application for the same purposes of visible and IR radiations from laser and non-laser sources is based to a significant degree on the effect of rise of the IFN- $\gamma$  content in blood.

Of principal significance was establishment of the fact that the direction, degree, and regulatory character of changes of cytokine levels in human blood after single and fourfold VIP-irradiations of the body surface resembled very much those after the direct (*in vitro*) irradiation of blood and after mixing the irradiated and non-irradiated autologous blood samples at a volume ratio of 1:10. This means that the main mechanism of changes of the peripheral blood cytokine network under conditions *in vivo* is associated with a transcutaneous photo-modification of small amounts of blood and “translation” (transferring) of the light-induced effects to the whole pool of circulating blood. In other words, the mechanisms responsible for the fast and gradual changes in circulation of the levels of the studied cytokines after the light exposure of the body surface operate first of all in the blood itself. These changes can be due to changes of secretion of cytokines by blood cells or expression of cytokine receptors on them, an increase or decrease of cytokine binding to plasma soluble receptors (sR), changes of stability of the cytokine binding to their transporting protein ( $\alpha$ 2-macroglobulin [ $\alpha$ 2M]), and finally to photo-modification of structure of the cytokines themselves, which does not allow their identification by ELISA. Other mechanisms also cannot be ruled out.

Monocytes and neutrophils are known to be the main producers of pro-inflammatory cytokines in human blood.<sup>12,14</sup> Since after the light-induced fall of the TNF- $\alpha$  and IL-6 levels the cytokine content is not restored, the VIP light can be hypothesized to block synthesis these acute phase of pro-inflammatory cytokines and most likely, it simultaneously “switches on” in these cells the mechanism of synthesis of anti-inflammatory cytokines. However, the astonishingly high rate and synchronicity of the “disappearance” of some cytokines from blood plasma and the “appearance” of some others suggests their level to be determined not only by processes of synthesis, but also by some other light-induced events.

Thus, it cannot be ruled out that, for instance, at the light treatment, like at mitogen stimulation, in mononuclears, due to a partial proteolysis of membrane receptors there occurs shedding of their extracellular subunits, which leads to an increase in the blood plasma of pool of highly specific soluble receptors (sR) that bind their ligands and become endogenous inhibitors of free cytokines. At present, sR to TNF- $\alpha$  and - $\beta$ , IL-1 $\beta$ , IL-4, IL-5, IL-6, IL-7, IFN- $\gamma$ , IL-2, and some growth factors are identified.<sup>44</sup> The desorption of glycoproteins from the donor blood mononuclear surface soon after their activation with UV and visible light was revealed earlier.<sup>45-47</sup> Binding of pro-inflammatory cytokines by the corresponding soluble receptors can form a mechanism of fast elimination of these mediators (TNF- $\alpha$  and IL-6) from blood and prevent elevation of concentration of others (for instance, IL-2 and IL-4).

Considered as another endogenous inhibitor of free cytokines in the blood plasma can be their carrier plasma protein –  $\alpha$ 2-macroglobulin ( $\alpha$ 2M). Under conditions *in vivo*, many cytokines and growth factors – TNF- $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-10, IFN- $\gamma$ , TGF- $\beta$ 1 and - $\beta$ 2, EGF, bFGF, PDGF, NGF – are

bound to  $\alpha$ 2M non-covalently with preservation of specific activity of ligands.<sup>48-52</sup> Complexes with  $\alpha$ 2M provide not only transport of active protein-peptide molecules, but also their protection from destruction by proteinases, fast elimination from circulation, and a similarly fast increase of their content in blood plasma. In particular, in complex with  $\alpha$ 2M, IL-2 preserves the proliferative potential with respect to IL-2-dependent T-lymphocytes.<sup>51</sup>

Strength and affinity of binding cytokines and growth factors to  $\alpha$ 2M, the degree of preservation of their activity in complexes as well as affinity of the complexes to  $\alpha$ 2M receptors on cells responsible for their clearance depend on the structural  $\alpha$ 2M state that is very sensitive to changes of microenvironment and to effect of various factors. Thus, at a slight shift of pH to acid direction, complexes with  $\alpha$ 2M are dissociated, and cytokines (IL-2, IL-10, and IL-4) are released.<sup>51,52</sup> According to Wu et al.,<sup>53</sup> oxidants, such as neutrophil-derived hypochlorite, alter the ability of  $\alpha$ 2M to bind to cytokines and growth factors and the oxidized  $\alpha$ 2M increases binding to pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, and IL-2) and decreases complex formation with anti-inflammatory cytokines (TGF- $\beta$ 1, TGF- $\beta$ 2) and growth factors (PDGF, bFGF, and NGF). The authors propose that oxidation of  $\alpha$ 2M serves as a switch mechanism that down-regulates the progression of acute inflammation by sequestering pro-inflammatory cytokines, and up-regulates the development of tissue repair processes by releasing anti-inflammatory and growth factors from their complexes with  $\alpha$ 2M. If to take into account that visible and IR light stimulate the production of ROS and nitric oxide (NO) by many cells, and blood cells, in particular,<sup>17,54-57</sup> it becomes obvious that oxidation of  $\alpha$ 2M can be a real mechanism of the fast decrease in blood irradiated *in vivo* and *in vitro* of excessive and normal amounts of pro-inflammatory cytokines and of the simultaneous increase of levels of anti-inflammatory cytokines and growth factors. By the way, an elevation in the blood of VIP-irradiated volunteers of the PDGF and TGF- $\beta$ 1 content, which is accompanied by an enhancement of the plasma growth-promoting activity for keratinocytes, endothelial cells, and fibroblasts have been shown in our recent works.<sup>5,6,9</sup>

In summarizing all the above, it is to be noted that due to a possibility of the fast binding of pro-inflammatory cytokines by soluble receptors and oxidized  $\alpha$ 2M, it does not seem possible at present to make final conclusion of inhibition of their production and activity with VIP light.

Much more certain seems stimulation with light of synthesis of IFN- $\gamma$ , IL-10, and TGF- $\beta$ 1, whose contents in peripheral blood rise during VIP- course. The comparatively fast production of these cytokines can occur owing to participation of cells of the first wave defense (natural killers, monocytes, and possibly neutrophils), whose activation mechanism is “simplified” as compared with lymphocytes. Participation of CD8<sup>+</sup> T-lymphocytes able to synthesize all three above-mentioned cytokines, especially rapidly in the case of IFN- $\gamma$ ,<sup>12,14</sup> cannot be ruled out. According to our data, VIP light enhances expression of CD8 markers, promotes an increase of the content of CD8<sup>+</sup> cells, and probably stimulates spontaneous synthesis of their DNA.<sup>8,10</sup> As mentioned above, the source of the fast appearance in the blood of IL-10, TGF- $\beta$ 1, and IFN- $\gamma$  can be their

complexes with oxidized  $\alpha 2M$ . Beside, an important role in the release of IFN- $\gamma$  is believed to be played by platelets, whose membrane receptors bind and transport the significant IFN- $\gamma$  amounts.<sup>58</sup> It seems that VIP light producing a fast deaggregation of platelets<sup>7</sup> may shed of the extracellular part of the IFN- $\gamma$  receptors and cytokine will be revealed in plasma by ELISA.

Taking into account all the said above, our results allow estimating only the general direction of changes of the cytokine network after irradiation of the human body surface with visible + IR light at therapeutic doses. In future, it would be worth studying effect of light on the cytokine network of patients with diseases of different etiopathogenesis by using not only ELISA, but also methods of evaluation of the intracellular content of cytokines and their mRNA as well as highly specific biotests.

To conclude it should be noted the VIP light spectral range (480–3400 nm) as well as the energy ratio of its visible and IR components are similar to those of the terrestrial Solar radiation without its UV and blue rays. This means that exposures to Sun may result in human blood: a fast and gradual decrease of the elevated amount of pro-inflammatory mediators, and a significant increase of IFN- $\gamma$ . As to the role of polarization in the biostimulating effects of the laser light, it has already being discussed for many decades,<sup>18,59–62</sup> but our studies<sup>11,38,63</sup> indicate that with equal power and energy density the polarized and non-polarized VIP lights induce rather similar effects. At the same time, the minor component of solar radiation – UV light – due to its lower penetration into the skin affects predominantly the upper skin structural elements (Langerhans cells, dendritic cells, keratinocytes) and, accordingly, the modification of cytokine profile of these cells. Numerous studies indicate a pronounced local and systemic immunosuppressive effects of UV radiation – production of IL-1, TNF- $\alpha$ , IL-6, IL-8, IL-10, and PGE2, which is combined with suppression of synthesis of IL-12, IL-2, and IFN- $\gamma$  as well as with inhibition of IFN- $\gamma$  signal transduction.<sup>64–68</sup> The goal of future studies consists in elucidating the rations and interaction possibilities of immunological effects of UV, visible, and IR solar radiations in various latitudes, taking into account seasonal and circadian changes of their energy.

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