Analysis of the Low Intensity Terahertz Radiation Influence on Lymphocyte Early Activation Markers

M. V. Duka (Tsurkan)[1], **M. K. Serebriakova**[2, 4], **I. V. Kudryavtsev**[2, 3], **A. S. Trulioff**[2, 4], **and O. A. Smolyankaya**[1]

[1]ITMO University, Russia
[2]Institute of Experimental Medicine of the NorthWest Branch of the Russian Academy of Medical Sciences, Russia
[3]Far Eastern Federal University, Russia
[4]Saint-Petersburg State University, Russia

Abstract— According to the literary data, terahertz radiation has some effects on different blood cells. Our study focuses on the determining the level of some cell surface antigens of lymphocytes when exposed to broadband terahertz range of 0.05–1.70 THz. These data indicate that terahertz radiation with power density of 9.55, 0.63 and 0.03 mW/cm^2 for 1 minute does not alter the functional activity of lymphocytes.

1. INTRODUCTION

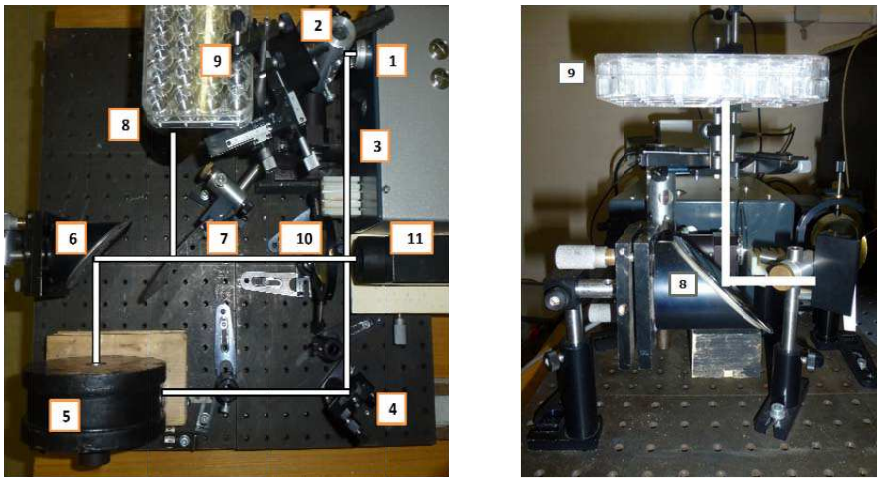
To date, literature sources contain a number of studies on the influence of terahertz (THz) radiation on the functional activity of human blood cells. At various times, the objects of the study were erythrocytes or platelets [1], leukocytes [2], as well as lymphocytes. In the case of the latter, the emphasis was on the role of THz radiation of varying power in regulation of advancing of cells in the cell cycle [3, 4], violation of integrity of the genetic material of cells and DNA stability [4, 5]. However, to evaluate the functional state of cells correctly, one can use a somewhat different approach, based on determining the level of surface antigens that characterize the functional state of cells, i.e., cell activation markers. Lymphocyte activation markers spectrum is very broad. They are traditionally divided into several groups depending on the time of appearance on lymphocyte surface after its activation. During the study, we have selected two antigens — CD38 and CD69, increasing the level of surface expression of which allows to assess the functional state of cells in culture conditions in vitro.

Molecule CD38 — is a transmembrane protein, which is an enzyme that regulates the cytoplasmic calcium concentration — the main mediator in signal transmission from the surface receptors to the cell nucleus. In addition, this enzyme has a number of other properties and can exhibit the activity of adenosine diphosphate-ribosyl cyclase, cyclic adenosine diphosphate ribosyl hydrolase, and NAD-glycohydrolase [6]. CD38 acts as a receptor modulating cell-to-cell cooperation, and can play a role in signal transmission from extracellular space into the cytoplasm of cells. This molecule is widely represented on activated blood cells, which include T-, B-, NK-cells and some other types of cells, and determining the level of expression of this molecule is of important prognostic value while managing the patients with different pathologies [7]. As for the second activation marker selected for our research, CD69 molecule refers to integral membrane proteins. It is believed that CD69 is involved in proliferation of leukocytes, because the level of its expression correlates with an increase in proliferative activity of cells, however, the expression level of this molecule is extremely low on the "resting" — not activated lymphocytes (typically, T- and B-cells) [8].

2. EXPERIMENTAL SETUP

The generation effect of THz radiation is that the laser beam of femtosecond interval creates free charge carriers on semiconductor surface, the motion of which in the magnetic field generates THz radiation. This principle is called a photoconductive antenna generation. In the present research we developed an optical design of the experiment as shown in Fig. 1. Detailed description of the scheme is given in [9].

The generated THz radiation had the frequency band of 0.05–1.7 THz with a maximum signal at 0.5 THz, and the THz pulse duration was 2.5 ps. The samples were irradiated for 1 min. The power of the THz radiation was varied by means of filters, and, allowing for absorption by the plate, was 30, 2 and 0.1 μW. The irradiation area was 3.14 cm^2. The power density was accordingly 9.55, 0.63, and 0.03 μW/cm^2. The experiments were carried out at the temperature of 20°C. Each



(a) (b)

Figure 1: Scheme of terahertz photometer: (a) top view; elements 8 and (b) 9 — a side view: 1 — femtosecond laser (Yb: KYW, 1040 nm, 120 fs, 75 MHz, 1 W); 2, 4 — mirrors; 3 — mechanical modulator; 5 — InAs crystal placed inside a magnet (angle of incidence was 45°); 6, 8 — the parabolic mirror; 7 — beam splitter plate; 9 — an object placed in the wells; 10 — lens; 11 — opto-acoustic detector.

power irradiated by one sample from each donor (18 donors). 18 control wells were not subjected to irradiation.

3. MATERIALS AND METHODS

Venous blood sampling from clinically healthy donors was carried out with heparinized (10 IU/ml) test tubes. The blood was mixed with sterile phosphate-buffered saline (PBS) in the ratio of 1 : 2, and lamination by the density gradient of 1.077 g/mL of Histopaque-1077 (Sigma-Aldrich, USA), and then subject to centrifugation for 30 min at 400 g and at 18–22°C. Upon the completion of centrifugation, the mononuclear cell layer was collected, formed at the phase interface. The resulting cell suspension was washed twice with the complete culture medium (CCM), prepared based on RPMI-1640 ("Biolot", St. Petersburg) with addition of 10% heat-inactivated fetal bovine serum (FBS, "Biolot", St. Petersburg), 50 g/ml gentamicin ("Biolot", St. Petersburg) and 2 mM L-glutamine ("Biolot", St. Petersburg), for 7 minutes while accelerating at 300 g. Then the number of the obtained cells was determined using a hemocytometer. For experimental set up, the wells of 24-well plates ("Sarstedt", Germany) were filled with 200 µl of cell suspension (5×10^6 cells/ml) poured to CCM. After radiation treatment, lymphocytes cultures were added with 250 µl of CCM and incubated at 37°C under 5% CO_2 for 24 hours. Upon completion of incubation, the cells were mixed with cooled PBS containing 2% of FBS, transferred into centrifuge tubes, and washed twice with PBS (300 g for 8 min). The resulting cell suspension was used for performing the experiments described below.

Upon completion of incubation, the lymphocyte suspension was washed twice with PBS at the above described conditions, and then mixed with 100 µl of fresh PBS. To identify the major cell populations the following antibodies were used: to identify the population of T-lymphocytes — CD3-PC7 (Cat. No. 737657), to identify B-lymphocytes — CD19-ECD (A07770), to identify a population of natural killer cells — CD56-PC5.5 (A79388). Antibodies against CD69 (IM1934U) and CD38 (A07778), PE labeled (phycoerythrin) and FITC (fluorescein isothiocyanate-dextran), respectively, were used as activation markers. To correctly identify the lymphocyte population the pan-leucocyte APC labeled (alofikotsianin) marker CD45 (IM2473) was used. Staining with antibodies against surface antigens was carried out in accordance with the manufacturer's recommendations; to assess the level of expression of activation markers the isotype controls were used. At least 20000 lymphocytes were analyzed for each sample by flow cytometer Navios ("Beckman Coulter", USA). Analysis of the results was performed using Kaluza software ("Beckman Coulter", USA).

4. RESULTS AND DISCUSSION

Results were expressed as percentage of cells bearing either one of activation markers (CD38+CD69− and CD38−CD69+), both markers simultaneously (CD38+CD69+), or bearing no of them (CD38−CD69−). These markers were examined for populations of T-lymphocytes (responsible for implementation of cell responses of the acquired immunity), B-lymphocytes (the main function of which is to participate in humoral responses of the acquired immunity), as well as natural killer (NK) cells, and NKT-cells — peripheral blood lymphocyte populations involved in antiviral and antitumor body responses. Processing of the results was performed using PASW Statistics 18 software package (IBM, USA). The results are summarized in the Table 1.

Table 1: The percentage distribution of the cells by the presence of these activation markers ($X \pm s$, $n = 18$, X — average, s — error, n — number of observations for one point).

Power density, $\mu W/cm^2$	T-lymphocytes, %				B-lymphocytes, %			
	CD69+ CD38−	CD69+ CD38+	CD69− CD38+	CD69− CD38−	CD69+ CD38−	CD69+ CD38+	CD69− CD38+	CD69− CD38−
control	5.0 ± 0.8	8.8 ± 1.4	52.2 ± 2.9	34.0 ± 2.6	9.6 ± 1.0	27.7 ± 1.8	49.5 ± 2.0	13.1 ± 2.2
0.03	5.0 ± 0.7	9.1 ± 1.9	52.9 ± 2.9	33.0 ± 2.5	9.5 ± 1.2	29.0 ± 2.0	49.2 ± 2.2	12.2 ± 1.8
0.63	5.2 ± 0.7	9.3 ± 1.6	52.1 ± 2.8	33.5 ± 2.6	9.8 ± 1.2	29.9 ± 1.7	48.6 ± 2.0	11.7 ± 1.7
9.55	5.1 ± 0.5	9.1 ± 1.5	52.2 ± 2.6	33.6 ± 2.7	9.4 ± 1.0	29.0 ± 1.7	49.6 ± 1.8	12.1 ± 1.7
Power density, $\mu W/cm^2$	NK, %				NKT, %			
	CD69+ CD38−	CD69+ CD38+	CD69− CD38+	CD69− CD38−	CD69+ CD38−	CD69+ CD38+	CD69− CD38+	CD69− CD38−
control	1.5 ± 0.3	72.7 ± 5.0	23.5 ± 4.8	2.3 ± 0.5	18.2 ± 2.6	15.6 ± 0.7	32.5 ± 3.9	33.7 ± 3.2
0.03	1.8 ± 0.4	72.0 ± 4.7	23.8 ± 4.5	2.4 ± 0.6	18.7 ± 2.9	16.6 ± 1.5	32.2 ± 3.9	32.5 ± 3.5
0.63	1.7 ± 0.3	72.4 ± 4.7	23.8 ± 4.5	2.1 ± 0.5	18.7 ± 2.6	16.7 ± 1.3	32.8 ± 3.9	31.8 ± 3.3
9.55	1.8 ± 0.3	72.2 ± 4.6	23.8 ± 4.3	2.2 ± 0.5	19.6 ± 2.7	16.7 ± 1.5	32.2 ± 3.5	31.4 ± 2.9

In the course of this study, no increase in the level of surface expression of CD38 on any of the cell types studied, which include T-, B-, NK- and NKT-cells, was noted. However, literature study indicates that the significant increase in CD38 level is reported in culture conditions of lymphocytes in vitro in response to the introduction of standard immunological stimulants such as phorbol myristyl acetate, as well as IL-4 and anti-CD3 or M surface immunoglobulins, respectively [10]. When using terahertz radiation with the power density of 9.55, 0.63 and 0.03 mcW/cm^2 no activation of any of the above cell populations was the case. Similar results were obtained when assessing the level of expression of CD69 of major lymphocyte populations, changes in the expression level of which may be associated with proliferative activity of lymphocytes. As mentioned above, this molecule is nearly absent on inactivated cells (T- and B-lymphocytes in particular). However, during the transfer of lymphocytes to the activated state on RNA level — using polymerase chain reaction — a significant increase in CD69 gene expression is observed 3–4 hours after the activation of cells in vitro [11]. This is precisely why the CD69 marker is traditionally treated as "early" lymphocyte activation marker. As for the populations of cytotoxic cells (NK- and NKT-cells), the expression level of CD69 on them is continuously higher. Whereas additional stimulation of cells is accompanied by an additional increase in the level of both CD69, and CD38 [7, 12]. It's worth mentioning that the latter antigen — CD38− in case of B-lymphocytes and natural killer cells — is treated by most researchers as a "differentiation" or maturation marker characterized by the "maturity degree" of cells, rather than their activation status.

Literature analysis showed that there is no effect on the kinetics of the cell cycle, micronucleus formation, proliferation index and the induction of micronuclei cells with cytogenesis block when irradiating human peripheral blood lymphocytes with free-electron laser at 0.12 and 0.13 THz for 20 min, and average power of 1 mW and 0.6 mW, respectively [3].

In work [4] it was shown that using the radiation of 0.13 THz (20 min) in the power range of 0.15–5.00 mW/cm^2, other researchers have also confirmed no effect on cell cycle kinetics of lymphocytes. They also noted that the THz radiation causes no chromosome damage (MN-analysis).

Impact of THz radiation (0.1 THz, 31 mW/cm^2) lasting for 60, 120 and 1440 min causes genome instability in lymphocytes [5]. However, according to G. J. Wilmink, these results should be treated

with caution [13].

All the above studies used continuous radiation sources. Also, power density of THz radiation and duration of irradiation of the above works is significantly higher than those used in our experiment (9.55, 0.63 and 0.03 mW/cm^2, for 1 minute). Only work [5] is comparable to ours in regard to power density (31 mW/cm^2). However, a large number of publications concerning the effects of weak fields show that a low-intensity radiation can cause biological effects, which are not observed during the radiation of a greater power. So the main possible mechanisms regarding the millimeter and submillimeter wave (terahertz) range are presented in [14]. We should also note that our earlier experiments on nerve cells [15] showed that the reduction of broadband terahertz radiation power density leads to stimulation of cell growth. Based on the above, we have chosen the power density data.

Due to the fact that the main practical interest of the use of THz radiation in medicine today is aimed to develop diagnostic terahertz instruments, to diagnose using these instruments it makes sense, to our opinion, to define the duration of exposure based on the duration of the study procedure. Since the scanning takes a few minutes, then the exposure duration at this stage was chosen as 1 min.

In our study, terahertz radiation with the power density of 9.55; 0.63 and 0.03 mW/cm^2 for 1 minute did not alter the functional activity of lymphocytes. It is possible that an increase in exposure duration may affect the activation of cells. Variation of irradiation parameters will be the next stage of our research.

5. CONCLUSION

Analysis of the level of expression of cell activation markers (CD38 and CD69) lymphocytes (T-lymphocytes, B-lymphocytes, natural killer cells and NKT-cells) showed that the terahertz pulse irradiation with the frequency range of 0.05–1.7 THz and power density of 9.55; 0.63; 0.03 mW/cm^2 for 1 minute does not result in the reliable increase in number of lymphocytes containing these markers.

ACKNOWLEDGMENT

This work was financially supported by Government of Russian Federation, Grant 074-U01.

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