# IMMUNOLOGY AND MICROBIOLOGY

# Stimulating Effect of Low-Flow Membrane Oxygenation on Peripheral Blood Immunocompetent Cells

**V. N. Shabalin, I. V. Krivtsova, A. V. Vatazin, Z. R. Karichev, M. E. Vishnevskii**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 8, pp. 179-182, August, 1996
Original article submitted December 1, 1995

Low-flow membrane oxygenation of donor blood stored for 3 days significantly increases the number of T cells (total and active populations) and stimulates the enzyme activity of neutrophils. Clinical trials (a purulent-septic abdominal disorder) show that membrane oxygenation of autologous blood is promising for the treatment of secondary immunodeficiency.

**Key Words:** *membrane oxygenation; lymphocyte; immunodeficiency; peritonitis*

Oxygenators used in clinical practice are intended to replace the lungs in cardiopulmonary bypass during lung or heart transplantation and cardiac surgery. Membrane oxygenators are preferable to bubble ones, in which gas exchange occurs at the blood/gas interface.

Bubble oxygenation is known to produce a number of negative effects, such as increased hemolysis, free hemoglobin content, β-thromboglobulin concentration [6,9], decreased filtration ability of the blood and conformational ability of erythrocytes, and denaturation of immunoglobulins at the blood/gas interface, which leads to the activation of C4a and C3a components of the complement [7,10]. Bubble oxygenation stimulates the formation of microemboli during cardiopulmonary bypass [8].

Oxygenators with true membranes more effectively support pulmonary and cardiopulmonary functions, since blood properties are preserved even during a long-term perfusion [3].

Institute of Clinical and Experimental Immunology, Ministry of Health and Medical Industry, Moscow

Clinical and laboratory studies of the effects of bubble and membrane oxygenation on immunocompetent cells have revealed the activation of T suppressors and increased secretion of lymphokines [12] as well as higher activation of the complement components after bubble oxygenation [10,11,13].

Blood oxygenation during perfusion with small volumes finds new clinical applications [5]. Investigation and clinical use of oxygenation, including the low-flow membrane oxygenation (LFMO), became more available after serial production of low flow rate oxygenators with true membranes (MOST-1903, nominal blood flow rate 500 ml/min according to the American standard, and MOST-1801, 150 ml/min) had been started by Kvant company (Moscow). This is a new area of LFMO application, therefore, the information regarding the effect of LFMO on the immune system is scarce.

We have hypothesized that LFMO produces stimulating effects on the immune system and performed experiments with the use of donors' blood stored for various time periods.

As known, a number of changes occur in the blood during storage [1,2,4]. This changes also con-

0007-4888/96/0008-0811$15.00 

cern immunocompetent cells, manifesting themselves in reduced functional activity of T lymphocytes and phagocytizing cells. This study is an attempt to restore by LFMO the number of active T cells in conserved blood stored for 3 days.

## MATERIALS AND METHODS

Immunological changes in the blood were monitored before oxygenation, immediately after it, and 24 h later. Extracorporeal oxygenation was carried out for 10 min under conditions close to physiological. The volume of conserved blood was 400 ml, and the flow rate in the circuit was 90-110 ml/min.

Blood was obtained from healthy donors aged 20-40 years. Lymphocytes were isolated on a density gradient (1.077 g/ml). Total and active T cell populations were identified in the spontaneous rosette forming test with sheep erythrocytes (E-RFL) by the method of M. Jondal and Smith (1972) with modifications (G. V. Poryadin, 1984). The activity of the lymphocyte receptors was expressed as the morphological affinity index (MAI). For this purpose 100 lymphocytes with different numbers of bound erythrocytes were counted in each blood smear. Group 0 included free lymphocytes, group 1 lymphocytes with 3-5 erythrocytes, group 2 lymphocytes with more than 5 erythrocytes, and group 3 lymphocytes forming full rosettes. The number of groups was multiplied by the number of lymphocytes in the group, and the results were summed and divided by 100. The value thus obtained was the MAI. The number of B cells was determined in the spontaneous rosette formation test with murine erythrocytes (Em-RFL). The concentration of serum immunoglobulins (IgA, IgM, and IgG) was determined by Mancini's radial immunodiffusion. The amount of C1in, C1q, C3, C4, and C5 components was determined by radial immunodiffusion, and the content of circulating immune complexes was measured using 3% and 4% polyethylene glycol 6000 (E. Gashkova, 1978) with our modifications. The phagocytizing activity of neutrophils was assessed from the number of internalized latex particles, and the enzyme activity of neutrophils was determined with tetrazolium nitroblue (G. Frimel', 1987).

## RESULTS

Oxygenation of conserved blood increased the total number of E-RFL, restored the number of active E-RFL, enhanced the expression and density of functionally active E-receptors, and stimulated the oxidation-reduction processes in neutrophils (Table 1). These effects lasted 24 h, indicating a stable restoration of the lymphocyte function but not of the enzyme activity of neutrophils. They probably result from reanimation of suppressed or reversibly degraded cells. Oxygenation produced no effect on the content of the complement components, serum immunoglobulins, and circulating immune complexes. Oxygenation of blood stored for 1-2 or 5-6 days had a very low effect, since the parameters of immunocompetent cells remain virtually unchanged after a 1- or 2-day storage, and predominantly active E-RFL respond to oxygenation. After 5-6 days of storage, the parameters of cell-mediated immunity (total and active E-RFL, MAI, and Em-RFL) dropped dramatically and in most cases were not restored by oxygenation.

Donors' blood with changed immunological parameters after a 3-day storage served as a model of secondary immunodeficiency. The positive effect of experimental oxygenation on the immunocompetent cells was a prerequisite of its clinical use for correction of secondary immunodeficiency. It was assumed that oxygenation of autologous blood has a polyvalent effect on the immune system.

In this study we examined the effect of LFMO of autologous blood on immunocompetent cells in secondary immunodeficiency caused by purulent-septic abdominal disorders.

LFMO of autologous blood was performed at the M. F. Vladimirskii Clinical Research Institute.

**TABLE 1.** Dynamics of Cell-Mediated Immunity and Neutrophil Phagocytizing Activity in Donors' Blood Stored for 3 days and Oxygenated by LFMO ($M \pm m$, $n=8$)

| Period | E-RFL, % | | MAI E-RFL | | Em-RFL, % | Phagocytosis, % | |
|---|---|---|---|---|---|---|---|
| | total | active | total | active | | latex test | test with tetrazolium nitroblue |
| Prior to LFMO | 26.38±2.16 | 17.69±1.66 | 0.36±0.05 | 0.22±0.02 | 12.8±1.57 | 29.0±5.7 | 4.9±0.85 |
| After LFMO | 40.7±4.4* | 37.0±3.3* | 0.56±0.07* | 0.51±0.062* | 15.8±3.65 | 42.78±3.5* | 13.28±2.2* |
| 24 h after LFMO | 35.6±6.8 | 45.61±7.5* | 0.57±0.13* | 0.64±0.12* | 15.6±3.75 | 36.0±8.0 | 3.4±1.5 |

**Note.** Here and in Table 2: *$p<0.05$ compared with values prior to LFMO.

TABLE 2. Dynamics of the Parameters of Cell-Mediated Immunity and Phagocytizing Activity of Neutrophils after LFMO in Patients with Purulent-Septic Abdominal Disorders ($M \pm m$, $n$=14)

| Parameter | Prior to LFMO | Duration of LFMO, h | | | | After LFMO | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 24 h | 3rd-5th day | 7th-9th day |
| E-RFL | | | | | | | | |
| total, % | 43±4.5 | 46.2±3.0 | 54.9±3.8* | 57±3.72* | 58.25±4.3* | 52.6±6.1 | 49±2.9 | 50.3±5.46 |
| abs. | 0.51±0.073 | 0.5±0.075 | 0.6±0.1 | 0.66±0.13 | 0.61±0.06 | 1.24±0.32* | 0.79±0.15* | 1.33±0.5* |
| E-RFL | | | | | | | | |
| active, % | 23.15±3.24 | 38.3±6.17* | 39.1±5.54* | 35.16±5.5 | 42.25±8.4* | 31.8±4.1 | 27.57±2.0 | 36.3±4.3* |
| abs. | 0.25±0.034 | 0.44±0.08* | 0.4±0.07* | 0.36±0.08* | 0.37±0.08* | 0.655±0.17* | 0.48±0.09* | 1.42±0.32* |
| MAI E-RFL | | | | | | | | |
| total | 0.63±0.088 | 0.66±0.42 | 0.818±0.09 | 0.925±0.1* | 0.86±0.08* | 0.84±0.13 | 0.76±0.046 | 0.8±0.1 |
| active | 0.29±0.04 | 0.55±0.09* | 0.58±0.12* | 0.54±0.12* | 0.64±0.12* | 0.45±0.08 | 0.38±0.037 | 0.56±0.1* |
| Em-RFL | | | | | | | | |
| % | 11.53±1.81 | 15.9±4.5 | 13.9±3.0 | 13.2±2.77 | 13.7±2.24 | 14.88±3.2 | 14.3±2.5 | 11.6±1.7 |
| abs. | 0.126±0.029 | 0.14±0.03 | 0.13±0.024 | 0.14±0.039 | 0.11±0.019 | 0.3±0.13 | 0.27±0.09 | 0.19±0.07 |

Patients aged 18-50 years with peritonitis, pancreatic necrosis, and other purulent-septic abdominal disorders were treated with LFMO. The procedure was carried out for 1-4 h using a MOST oxygenator (blood flow rate 90-110 ml/min, oxygen flow rate 500-1000 ml/min) and a Gambro apparatus for plasma filtration. Immunological parameters were measured prior to oxygenation, each hour during the procedure, 24 h, and on days 3-5 and 7-9 after it. The initial parameters of immunocompetent cells were below the baseline level. The state of lymphocytes was assessed using the above-mentioned tests.

From analysis of the results obtained it can be concluded that LFMO of autologous blood stimulates the immunocompetent cells. The number of circulating cells increased, reaching the baseline level by the second hour of procedure (Table 2). The stimulating effect was rather stable: it was preserved for 24 h and slightly decreased by the 3rd-5th day. The dynamics of the content of active T cells (cells with high metabolic activity) was different. In contrast to the total T-cell content, the number of active E-RFL increased 1.6-fold during the first hour of oxygenation and remained at this level during the entire procedure and 24 h after it, slightly declining by the 3rd-5th day. The content of B cells did not increase significantly after LFMO.

Several mechanisms may be responsible for the development of the immunostimulating effect within several hours. First, rapid metabolization of oxygen by blood cells is provided by the small intermembrane distance (not more than 150 μ), which ensures rapid and homogeneous oxygen transfer. This activates lymphocytes which were inert before oxygenation. Second, membrane oxygenation alters rheological and microcirculatory properties of the blood and enhances the release of lymphocytes from depots. Third, oxygen stimulates the major organs of the immune, endocrine, and nervous systems.

Thus, our results indicate that:

1. LFMO enhances the expression and increases the density of functionally active lymphocyte E-receptors, increases the number of total and active E-RFL, and stimulates the oxygen-dependent mechanisms of phagocytosis.

2. Changes in the functional activity of immunocompetent cells depend on the time of storage of conserved blood. Oxygenation is most effective within the first three days of storage, providing the highest immunostimulating effect.

3. LFMO of autologous blood is recommended as an effective means for stimulating the immunocompetent cells during treatment of purulent-septic pathologies.

## REFERENCES


1. O. K. Gavrilov, *Manual on Blood Transfusion* [in Russian], Moscow (1980), p. 19.
2. A. M. Kostyuchenko and A. N. Tulupov, *Probl. Gematol.*, No. 1, 21-25 (1982).
3. G. I. Lukomskii, M. E. Alekseeva, L. A. Vaisberg, *et al.*, *Anest. Reanimatol.*, No. 4, 6 (1990).
4. B. V. Petrovskii, in: *Manual on General and Clinical Transfusiology* [in Russian], Moscow (1979), pp. 82, 158.
5. A. A. Pisarevskii, A. B. Karasev, Z. R. Karichev, and A. S. Raetskii, *Med. Tekhnika*, No. 5, 47-50 (1987).
6. M. Benedetti, R. De Caterina, A. Bionda, *et al.*, *Int. J. Artif. Organs*, **13**, No. 8, 488-497 (1990).
7. A. Hakoshima, H. Goto, K. Abe, *et al.*, *J. Cardiothorac. Anesth.*, **3**, No. 2, 189-192 (1989).

8. J. F. Liu, Z. K. Su, W. X. Ding, *et al.*, *Ann. Thorac. Surg.*, **54**, No. 6, 1196-1202 (1992).
9. L. Nilsson, L. Bagge, and S. O. Nystrom, *Scand. J. Thorac. Cardiovasc. Surg.*, **24**, No. 1, 65-69 (1990).
10. K. Oda, *Nippon Kyobu Geka Gakkai Zasshi*, **39**, No. 9, 1703-1711 (1991).
11. T. Tamiva, M. Yamasaki, Y. Maeo, *et al.*, *Ann. Thorac. Surg.*, **46**, No. 1, 47-57 (1988).
12. M. Tatke, S. K. Khanna, V. Malhotra, *et al.*, *Indian Heart J.*, **43**, No. 1, 31-34 (1991).
13. M. Yamasaki, *Nippon Kyobu Geka Gakkai Zasshi*, **37**, No. 2, 234-241 (1989).

# Isolation of Cytotoxic Proteins from Human Platelets

**T. S. Golubeva, O. Yu. Chertov, M. V. Kiselevskii, and A. R. Tuguz**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 8, pp. 183-185, August, 1996
Original article submitted December 5, 1995

Three protein fractions with cytotoxic activity towards lung adenocarcinoma cells are isolated from human platelets. Molecular weights of proteins from two fractions are determined. Three proteins with molecular weights of 14.5 and 22 kD are isolated from the first fraction and proteins with molecular weights of 28, 35, 44, and 65 kD from the second.

**Key Words:** *platelet; cytotoxic protein*

Recent data indicates that platelets participate not only in thrombus formation. They can be activated and involved as effector cells in various immunological responses to infection [7,10]. Platelets also have a role in the reaction to malignant tumor growth [1,2,8, 11,12]. Specifically, in patients with different tumor localization, increased intracellular granulation is the typical reaction of the megalokaryocyte-platelet system [2]. In addition, a tendency towards increased platelet aggregation has been observed [2,11, 12]. Platelets are known to stimulate metastasizing and endovascular invasion of tumor cells [12].

Platelets are activated by platelet-activating factor (PAF) which induces the release of biologically active substances from secretory granules [3,4]. This markedly enhances platelet cytotoxicity [5]. Similarly to T cells and monocytes, platelets exhibit killer activity towards malignant cells. It was shown that platelets lyse leukemia cells [13]. Platelets from patients with lung cancer lyse freshly isolated autologous tumor cells and allogenic ACL cells (human lung adenocarcinoma) [1]. Platelets from oncological patients display cytotoxic activity towards HeLa, K-562, and Mel-1 cells. The ACL strain proved to be the most sensitive target. Incubation of platelets from both healthy donors and oncological patients with PAF (10 pM-100 nM) markedly increases killer activity of platelets [5]. Platelet cytotoxic activity is influenced by various proteases [12]. The mechanisms underlying this activity remain obscure. Electron microscopy studies showed that platelets adsorb onto the target cells, after which ultrastructural changes typical of periodical secretion (hypertrophy of the Golgi apparatus, enlargement of secretory granules, and their orientation towards the contact zone) occur [5]. These changes are similar to those observed in T lymphocytes contacting with tumor cells [6]. From these findings it can be hypothesized that cytotoxic activity of platelets is due to their ability to secrete cytotoxic proteins with lytic activity towards tumor cells. Our aim was to isolate these proteins.

## MATERIALS AND METHODS

Heparin (15 μl/ml) was added to peripheral venous blood (400 ml) of healthy donors and centrifuged for 10 min at 1550 rpm. Platelet-rich plasma was centrifuged for 10 min at 3000 rpm. Sedimented plate-

Oncology Research Center, Russian Academy of Medical Sciences; M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow

0007-4888/96/0008-0814$15.00 