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The content of leukocytes in the blood of recipient rabbits after allogeneic transfusion of packed red blood cells

M. O. Malyuk, O. V. Yehorov, I. O. Kharkevych, V. V. Klymchuk, T. L. Savchuk

National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

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National University of Life
and Environmental Sciences
of Ukraine, Heroiv Oborony
st., 15, Kyiv, 03041, Ukraine.
Tel.: +38-096-214-99-31.
E-mail:
nikolai_malyuk@ukr.net

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Researching how the immune system reacts to stimuli such as blood transfusion of alloantigens on models of different species of laboratory animals, in particular rabbits, is important for understanding the mechanisms through which post-transfusion reactions develop. In this study, we identified the absolute and relative contents of white blood cells in blood of the recipient rabbits, and also their subpopulations after allogeneic transfusion of packed red blood cells (pRBCs). Allogeneic transfusion of pRBCs without leukoreduction was conducted once, in the calculation of 5.5 mL/kg of body mass of the experimental animals. The material for the studies were samples of blood serum, collected from 5 rabbits on the 3rd, 7th, and 23rd days after pRBC administration. Allogeneic transfusion of pRBCs to the recipient rabbits caused development of leukocytosis in their blood: compared with the initial condition, the absolute content of leukocytes in the blood of the experimental animals increased 27.0% on the 3rd day after transfusion, equaling $8.94 \cdot 10^9/L$; 40.1% on the 7th day, accounting for $9.86 \cdot 10^9/L$; and 34.1% on the 23rd day, measuring $9.44 \cdot 10^9/L$. Leukocytosis resulted from increased content of granulocytes in the blood compared with the initial state: the absolute content of granulocytes surged 66.9% on the 3rd day after transfusion, measuring $4.54 \cdot 10^9/L$, 2.21-fold on the 7th day, equaling $6.02 \cdot 10^9/L$, and 1.87-fold on the 23rd day, equaling $5.08 \cdot 10^9/L$. Allogeneic transfusion of pRBCs to the recipient rabbits caused decrease in the relative content of T-lymphocytes and increase in the content of B-lymphocytes in their blood. Compared with the initial condition, the relative content of T-lymphocytes in the blood of the experimental animals declined 20.6% on the 3rd day of the study, accounting for 58.0%; 30.1% on the 7th day, equaling 51.0%; and 25.8% on the 23rd day, equaling 54.2%. Compared with the initial state, the relative content of B-lymphocytes spiked 94.7% on the 3rd day of the study, measuring 22.2%, 2.23-fold on the 7th day, accounting for 25.4%, and 2.51-fold on the 23rd day, equaling 28.6%.

Keywords: packed red blood cells; allogeneic transfusion; absolute content of white blood cells; relative content of white blood cells; T lymphocytes; B lymphocytes.

Introduction

Transfusion of whole blood to save lives of people and animals has been practiced with varying success for several centuries, since the British doctor Richard Lower in 1665, for the first time in the global history, conducted blood transfusion from animal to animal and from animal to human (Fastag et al., 2013). Thanks to introduction of novel methods and equipment, developed in the second half of the 20th century, blood transfusion in human and veterinary medicine became more efficient (Cotter, 1991; Davidow et al., 2013). Blood transfusion has achieved great progress in veterinary medicine, with blood transfusion to animals becoming an integral part of practical veterinary medicine, which provides a potentially life-saving treatment to animals with various pathological processes and conditions (Rozanski & de Laforcade, 2004; Tocci & Ewing, 2013). Emergence of methods of separating blood into components allowed the practitioner veterinarians to use not only whole blood, but also its components for the needs of a treated animal, thus expanding the practical possibilities of blood transfusion even more. Also, improvements were made in methods of screening, testing of animals' blood groups, and *in vitro* studies for blood matching of a donor animal and recipient (Kumar, 2017). Considering this, blood-transfusion therapy is more complex and requires a veterinary doctor who is familiar with preparation of a donor animal for blood donation, recipient animal for receiving blood, and also its components to transfusion, and is prepared to mitigate all risks that can emerge in the process of blood transfusion or right after it. Besides positive therapeutic effects, blood transfusion comes with potential risks to the recipient ani-

mals such as development of disseminated intravascular coagulation, acute post-transfusion lung lesion, and many other immune and non-immune reactions (Harrell & Kristensen, 1995; Hardy et al., 2004; Saleh & Walsh, 2013; Thomovsky & Bach, 2014; Radulescu et al., 2021). In particular, this efficient therapeutic method poses threats to the immunity. Complications associated with blood transfusion emerge despite following the protocols of donation, preservation, and transfusion of blood. Risk of such complications increases in cases of non-compliance to the blood-transfusion protocols. For example, a study found that practicing veterinarians in Australia conducted assessments of compatibility of donor blood and blood of recipient animals prior to allogeneic transfusion for only about a half of dogs and cats, leading to development of various post-transfusion reactions (Poh et al., 2021).

Despite the fact that blood transfusion to patients is a life-saving procedure, it nonetheless can lead to threatening complications. Some of them are subclinical, while others manifest in the form of acute clinical impairments that increase morbidity and mortality of the patients. Complications during blood transfusion are usually classified as immune and non-immune-related, and also depending on whether they are acute or delayed in nature. Type and severity of the clinical signs can vary depending on a particular reaction. Many reactions can be prevented using the standard special transfusiology procedures. Those methods include careful collection and storage of blood products, adequate screening, and grouping of blood of donor animals, cross-matching of blood of donor and recipient, use of component therapy, and correct introduction of blood products and use of pre-transfusion prophylaxis if necessary. Since many reactions depend on

dose, it is hard to overestimate the careful monitoring of transfusions. Timely detection of reaction to blood transfusion and immediate cessation of the transfusion, and also required support therapy are essential for a successful treatment of reactions to blood transfusion. If used correctly, transfusion of blood components can be a very beneficial form of low-risk therapy (Harrell & Kristensen, 1995; Brand, 2002; Sreethu et al., 2022).

One of possible causes of post-transfusion complications in the recipients is accumulation of inflammation mediators in containers with blood during storage. Results of the studies by Kristiansson et al. (1996) indicated presence of certain cytokines, in particular, tumor necrosis factor (TNF- α), IL-1, IL-6, and IL-8 in the containers with erythrocytes, although there was a high variance of their concentration during the period of storage and between samples from the containers. In some samples of erythrocytes, the content of cytokines reached levels at which they may be expected to promote the development of systemic inflammation and symptoms of acute post-transfusion reactions (Kristiansson et al., 1996). Similar studies identifying the content of cytokines in samples of stored donor blood were carried out by Shanwell et al. (1997). The researchers found that eliminating leukocytes from whole blood using filtration prior to its storage decreased the contents of IL-1, IL-6, IL-8, and tumor necrosis factor alpha (Shanwell et al., 1997). Then, an experiment by Izbicki et al. (2004) demonstrated that transfusion of leukocyte-unfiltered blood to the recipients can cause acute and transitory leukocytosis in severe non-septic patient. A certain role in this phenomenon is played by interleukin-8, accumulated in packed red blood cells due to white blood cells (Izbicki et al., 2004). Also, a notable, depending on storage time, accumulation of IL-8 in concentrates of canine erythrocytes was noted by Corsi et al. (2014). The study demonstrated that removal of leukocytes from erythrocyte concentrates reduced the accumulation of this cytokine in them. The fact that leukoreduction prevents accumulation of inflammation biomarkers during storage of preserved blood of dogs was confirmed by other researchers (McMichael et al., 2010; Callan et al., 2013; Purcell et al., 2015). Therefore, analysis of the literature data suggests that leukoreduction is a beneficial technical method to minimize complications associated with blood transfusion (Ibojie et al., 2002; Wang et al., 2012).

Strategies oriented at preventing development of immune reactions in animals, caused by transfusion of blood and its components, or alleviation of their manifestation, include optimization of both the methods of preparation of blood and processes of using it. The general objective of such an optimization is reducing the risk of emergence of post-transfusion complications and improving the treatment outcomes.

Thus, donor blood can be transfused to a recipient as fresh whole blood or in the form of prepared whole blood after storage at the temperature of 4 °C. Also, whole blood can be separated into components, including packed red blood cells, plasma, and thrombocyte concentrate. Those components are stored separately in different storage conditions and are given to the recipient animals with various pathologies (packed red blood cells in cases of anemia, blood plasma in cases of hypoproteinemia, and thrombocyte concentrate to treat thrombocytopenia). Transfusion of whole blood to the recipient animal is also associated with high risk that alien antigens can affect the immune system, therefore entailing a post-transfusion immune response to them. This is another reason why transfusion doctors recommend a component therapy instead of transfusion of whole blood (Kristensen & Feldman, 1995).

Component blood transfusion is used due to several reasons. First of all, a patient receives only the necessary blood component, which increases the potential of one unit of whole blood for treatment of several critically ill recipient animals. This maximizes the benefits of each unit of whole blood and improves economic efficiency. Secondly, administration of only part of whole-blood unit is less risky because a lower volume is considered safer for patients with risk of overloading with the fluid, including patients with cardiovascular diseases and kidney failure. Thirdly, by administering only the necessary component of blood, the recipient receives a smaller amount of cytokines and antigens, which reduces the risk of immunologic reactions (Chin-Yee et al., 1997; Wadhwa et al., 2000; Lanevski & Wardrop, 2001; Chiamonte, 2004; Herring et al., 2013; Cummings et al., 2016).

Reactions of an animal body, related to transfusion of donor blood and its components to it, could be divided into the following categories:

fever non-hemolytic reactions, respiratory reactions, allergic reactions, hemolytic reactions, delayed serologic reactions, post-transfusion infections, hypocalcemia/citric toxicity, hyperammonemia, hypotensive reactions, post-transfusion purpura, and reactions of transfused blood against a recipient (Davidow et al., 2021).

Given that the majority of post-transfusion reactions are immune-related, it is relevant to conduct studies on the immune status of recipient animals following the transfusion of whole blood or its components.

Therefore, the objective of our study was determining absolute and relative contents of white blood cells and their subpopulations in the blood of the recipient rabbits after allogeneic transfusion of packed red blood cells (pRBCs).

Materials and methods

The experiments were performed according to the requirements of the General Ethic Principles of Performing Experiments on Animals, adopted by the 1st National Congress of Bioethics and the Positions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, and the Law of Ukraine On Protection of Animals From Abuse. Permission to use the animals in the experiments, according to the developed scheme, was approved by the Commission of Bioethics of the National University of Life and Environmental Sciences of Ukraine (as of 10/27 2020, Protocol No. 31-1).

The studies were conducted in 2022–2023 at the Bank of Animal Blood of the Scientific-Research Laboratory of Academician I. O. Povazhenko Department of Surgery and Pathophysiology of the National University of Life and Environmental Sciences of Ukraine in the conditions of the Vetmedservis Research-Production Center.

For the experiments, we used clinically healthy non-pedigree rabbits. The diet of the experimental animals corresponded to their needs of nutrients and biologically active compounds. The animals had free access to water and food. During the studies, we controlled the main clinical parameters in the experiment animals (body temperature, frequency of cardiac contractions, respiratory rate).

The material for the studies were samples of blood serum, collected from 5 rabbits on the 3rd, 7th, and 23rd day after pRBC transfusion. The blood of the donor rabbits was withdrawn from the jugular vein using a partially enclosed method. In the region of blood collection, hair was shaved and the skin was treated with a solution of ethyl alcohol. The samples of donor blood were collected into polymer containers with CPDA anticoagulant.

Separation of whole blood into components was performed using a Rotanta 460R centrifuge (HETTICH GmbH, Germany). Centrifugation lasted for 20 min at the rate of 2,500 rpm. Centrifugation time did not include the braking time. The temperature during centrifugation was 5 °C. After centrifugation, the bag was carefully taken out of the centrifuge and transferred to a plasma extractor to separate the plasma from the mass of erythrocytes (Kenichiro & Holowaychuk, 2016).

Allogeneic transfusion of pRBCs without leukoreduction in calculation of 5.5 mL/kg of body mass to the recipient rabbits was performed once (Kenichiro & Holowaychuk, 2016). So as to prevent the complications related to the procedure, we determined compatibility of blood of the donor and recipient rabbits prior to pRBC transfusion using a large cross test. This cross test was carried out *in vitro* at the temperature of 37 °C on a Micromed BV-4 water bath (Ukraine). At the same time, the serum of the recipient animals and blood of the donor animals was mixed in 1:5 and 1:10 proportions on microscope slides. After a 5-min exposure, we performed microscopic studies. In cases of no agglutination, we conducted biological (*in vivo*) tests for individual compatibility.

Absolute content of white blood cells and their individual subpopulations in blood of the experimental rabbits was measured using a Mindray BC-2800 Vet veterinary hematologic analyzer (China). The relative amount of T lymphocytes was identified using the Jondal Method. The method of measuring the amount of T lymphocytes is based on the interaction between the membrane receptors of lymphocytes and ram erythrocytes. The number of erythrocytes adsorbed by one lymphocyte indicates the level of activity of T cells, because the phenomenon of rosette formations is conditioned by density of the receptors on their surface (Mazurkevych

et al., 2014). The relative amount of B lymphocytes was identified using the Bianco Method. The principle of the method is based on the fact that B lymphocytes bear receptors to C₃-component complement on their surface, but have no receptors to ram erythrocytes, and therefore direct interaction of such erythrocytes with B lymphocytes is impossible. Attachment of erythrocytes to B lymphocytes requires mediation between complement and anti-erythrocyte antibodies. For this purpose, erythrocytes are treated with hemolytic serum that contains anti-erythrocyte antibodies. Thus, there forms an antigen-antibody complex, with which complement binds. Since all B lymphocytes have receptors to C₃-component complement, the number of such lymphocytes that had formed rosettes (attached three and more erythrocyte-antibody-complement complexes) reflects the number of B lymphocytes in a certain volume of animal blood (Mazurkevych et al., 2014).

The relative amount of 0 lymphocytes was estimated according to the residual principle after determining the relative amount of T and B lymphocytes by the method of rosette formation using the formula: 0 lymphocytes = 100% – (T lymphocytes, % + B lymphocytes, %) (Duda & Prus, 2019). The cells were examined microscopically using a Sigeta Biogenic LED microscope (China) with an installed Sigeta MDS-560 CCD camera (China).

The results were statistically analyzed using the Tukey Test with the Bonferroni Correction. The data are presented as mean value ± standard deviation (x ± SD).

Results

Absolute content of leukocytes in the blood of the recipient rabbits exceeded such in the initial state throughout the studies (Table 1), indicating that pRBC transfusion caused leukocytosis. Therefore, as compared with the initial state, the absolute content of white blood cells in blood of the experimental animals (7.04 10⁹/L) increased 27.0% on the 3rd day, measuring 8.94 10⁹/L; 40.1% on the 7th day, measuring 9.86 10⁹/L; and 34.1% on the 23rd day, measuring 9.44 10⁹/L.

Table 1

Dynamics of absolute content of leukocytes and their individual subpopulation in blood of the experimental animals after allogeneic pRBC transfusion (x ± SD, n = 5)

Immunocompetent cells	Initial state	Day 3	Day 7	Day 23
Leukocytes, 10 ⁹ /L	7.04 ± 0.43 ^a	8.94 ± 1.32 ^{ab}	9.86 ± 1.15 ^b	9.44 ± 0.89 ^b
Granulocytes, 10 ⁹ /L	2.72 ± 0.43 ^a	4.54 ± 0.50 ^b	6.02 ± 0.53 ^c	5.08 ± 0.64 ^{bc}
Lymphocytes, 10 ⁹ /L	3.98 ± 1.30 ^a	4.08 ± 0.88 ^a	3.54 ± 1.30 ^a	4.10 ± 1.14 ^a
Monocytes, 10 ⁹ /L	0.341 ± 0.089 ^a	0.324 ± 0.084 ^a	0.304 ± 0.187 ^a	0.262 ± 0.055 ^a

Note: different letters indicate significant difference (P < 0.05) within one line according to the Tukey Test with the Bonferroni Correction.

Absolute content of granulocytes in the blood of the experimental animals after transfusion of pRBCs was also higher than initially throughout the studies. Compared with the initial condition (2.72 10⁹/L), the absolute content of granulocytes increased 66.9% on the 3rd day after transfusion, equaling 4.54 10⁹/L; 2.21-fold on the 7th day, measuring 6.02 10⁹/L; and 1.87-fold on the 23rd, equaling 5.08 10⁹/L (Table 1). Percentages of the absolute granulocyte content in blood of the experimental animals suggest their higher relative content throughout the experiment compared with the initial state (38.5%), surging 50.7%, 60.2%, and 53.8% on days 3, 7, and 23 of the experiment, respectively (Table 2). The number of lymphocytes and monocytes did not change significantly throughout the experiment, although a downward tendency was observed for the content of monocytes in blood (Table 1). Percentage of monocytes in blood of the experimental animals also had a tendency towards decline throughout the experiment, from 4.87% to 2.79% on the 23rd day (Table 2).

Therefore, the parameters of absolute and relative content of white blood cells and their individual subpopulations in blood of the experimental animals after allogeneic pRBC transfusion indicate onset of leukocytosis, occurring due to granulocytes.

Relative content of T lymphocytes in blood of the recipient rabbits was lower than initially throughout the studies (Table 3). Compared with the initial state (73.0%), the relative content of T lymphocytes in the blood

of the experimental rabbits decreased by 20.6% on day 3, equaling 58.0%; by 30.1% on day 7, equaling 51.0%; and 25.8% on day 23, measuring 54.2%.

Table 2

Percentage of subpopulations of white blood cells (%) in blood of the experimental animals after allogeneic transfusion of packed red blood cells (x ± SD, n = 5)

Immunocompetent cells	Initial state	Day 3	Day 7	Day 23
Granulocytes	38.5 ± 7.2 ^a	50.7 ± 6.2 ^{ab}	60.2 ± 10.4 ^b	53.8 ± 4.9 ^b
Lymphocytes	56.7 ± 18.1 ^a	45.8 ± 8.9 ^{ab}	36.6 ± 11.1 ^b	43.5 ± 11.4 ^{ab}
Monocytes	4.9 ± 1.4 ^a	3.6 ± 0.6 ^{ab}	3.3 ± 2.5 ^{ab}	2.8 ± 0.7 ^b

Note: see Table 1.

It has to be noted that the relative content of B lymphocytes in the blood of the recipient rabbits after allogeneic pRBC transfusion was higher throughout the studies compared with the initial state (11.4%). It increased by 94.7% on the 3rd day, equaling 22.2%; 2.23-fold on the 7th day, measuring 25.4%; and 2.51-fold on the 23rd day, equaling 28.6%.

Table 3

Dynamics of relative content of lymphocytes (%) in blood of the experimental animals after pRBC allogeneic transfusion (x ± SD, n = 5)

Types of lymphocytes	Initial state	Day 3	Day 7	Day 23
T lymphocytes	73.0 ± 4.3 ^a	58.0 ± 3.2 ^b	51.0 ± 4.7 ^b	54.2 ± 2.2 ^b
B lymphocytes	11.4 ± 2.4 ^a	22.2 ± 3.0 ^b	25.4 ± 2.1 ^{bc}	28.6 ± 1.1 ^c
0 lymphocytes	15.6 ± 2.1 ^a	19.8 ± 1.6 ^b	23.6 ± 3.2 ^b	17.2 ± 1.9 ^{ab}

Note: see Table 1.

The relative content of 0 lymphocytes in the blood of the recipient rabbits after allogeneic pRBC transfusion was also higher than before throughout the studies. Therefore, on the 3rd, 7th, and 23rd days of the experiment, it was higher by 26.9%, 51.3%, and 10.3%, measuring 19.8%, 23.6%, and 17.2%, respectively.

Therefore, after allogeneic transfusion of pRBCs to the rabbits, the ratio between various kinds of lymphocytes in their blood changed, in particular, there was seen decrease in the relative content of T lymphocytes and increase in B lymphocytes.

Discussion

Transfusion of blood or pRBCs to the recipient animals increases their ability to carry oxygen, although can cause implications that are potentially harmful for severely ill patients, in particular transmission of causative agents of infectious and parasitic diseases, and immune modulation. Furthermore, stored donor blood, as well as pRBCs, can exert pro-inflammatory effects after transfusion due to containing white blood cells and biologically active compounds they synthesized during storage (Saleh & Walsh, 2013). After transfusion of allogeneic blood, the recipients can experience non-septic leukocytosis (Fenwick et al., 1994).

The absolute content of white blood cells, i.e. overall concentration of leukocytes present in a unit of blood volume, is one of the commonest identified blood parameters, because it is assessed in detailed immunogram. Increase in total white blood cells (leukocytosis) in most cases is characterized by increase in neutrophils and lymphocytes, much rarer – eosinophils and monocytes. The criterion of leukocyte content becomes indicative only when considered in conjunction with the formula of blood cells, as it provides a more comprehensive assessment of the patient's condition. At the same time, the application of an individual parameter of leukocyte content in blood is often completely justifiable because it provides an opportunity to quickly and approximately assess the patient's condition.

Our studies revealed that allogeneic transfusion of stored pRBCs to the recipient rabbits caused development of non-septic leukocytosis, which remained throughout the studies (Table 1). We should note that our earlier studies demonstrated that allogeneic transfusion of whole blood to recipient rabbits also caused leukocytosis throughout the studies due to increases in lymphocytes and granulocytes (Maliuk et al., 2023). The results of our studies of allogeneic pRBC transfusion are consistent with the

studies by other authors. Lozano et al. (2019) compared the levels of inflammation markers after transfusion of pRBCs to dogs with and without leukocytes, finding that the general amount of leukocytes in blood of the dogs that had received leukocytes-containing pRBCs was much greater 24 h after transfusion than in the group of dogs that had received leukocytes-removed pRBCs (Lozano et al., 2019). Another study of pRBC transfusion showed leukocytosis in the patients' blood, its level correlating with the concentration of IL-8 in transfused stored pRBCs (Izbicki et al., 2004). Increase in the absolute concentration of white blood cells 8–24 h after blood transfusion was also observed in newborns (Wright & Skinner, 2001). At the same time, an experiment revealed no differences in concentrations of leukocytes in blood of dogs after receiving erythrocyte transfusion both with and without leukocytes. The authors assumed that absence of detected differences in changes in the numbers of leukocytes between the groups of animals was due to the sampling size and its heterogeneity (Claus et al., 2022). In the studies by Frey (2002), which dealt with preterm babies, the general amount of leukocytes and neutrophils 48 h after blood transfusion also did not change considerably. The numbers of lymphocytes, eosinophils, basophils, and plasmatic cells underwent no changes, although the content of monocytes increased (Frey, 2002).

The results of our studies indicate that transfusion of pRBCs entailed leukocytosis in the recipient animals, resulting from heightened concentration of granulocytes in their blood (Table 1). Although we did not study the mechanism through which leukocytosis develops, the literature data suggest that the cause of leukocytosis after pRBC transfusion without prior leukoreduction is accumulation of cytokine erythrocytes in stored samples, synthesized by donor leukocytes, and also leukocytes proper and other blood antigens (Kristensen & Feldman, 1995; Ibojie et al., 2002; McMichael et al., 2010; Wang et al., 2012; Callan et al., 2013; Corsi et al., 2014; Purcell et al., 2015; Remy et al., 2018).

The study by McMichael et al. (2010) on blood of the dogs that had received pRBCs without leukoreduction also revealed significant increase in the absolute content of leukocytes and segmentonuclear neutrophils compared with the initial level. Similar results were obtained by Balasundaram et al. (2023) while studying changes in the profile of white blood cells in blood of preterm newborns with low body mass after pRBC transfusions. The results of this study suggest that during a 72-h period after transfusion of pRBCs, the blood of the children underwent increase in the absolute amount of white blood cells, with simultaneous increases in neutrophils, eosinophils, and monocytes (Balasundaram et al., 2023).

It has to be noted that detection of non-septic leukocytosis after transfusion of red blood cells is crucial, as it helps to avoid unnecessary investigation and therapy after erroneous suspicion of sepsis.

Lymphocytes are one of the largest populations of immune-competent cells. However, in the traditional analysis of blood, their clinical value can be limited compared with neutrophils, eosinophils, and monocytes. This is because total lymphocytes include a number of morphologically monotypical cells, identifying which can help alleviate impacts of physiological and pathological changes in cells of individual subpopulations in the body. T lymphocytes are one of the informative parameters of immunogram. This is related to the fact that T cells in blood are a very labile indicator, sensitive to changes in the immune system. The number of T lymphocytes in the blood is of great practical significance, especially to assess the level of antigen loading on the body. Using this parameter in many cases has an individual significance, outside the entire immunogram (contrast to most other parameters). T lymphocytes are the fastest among all the immunocompetent cells to react to an antigen stimulus – their level decreases (Lebedev, 1996). In our study, the relative content of T lymphocytes in blood of the recipient rabbits was lower throughout the period compared with the initial state (Table 3). Molina-Aguilar et al. (2020) observed changes in the content of T lymphocytes in the blood of people alloimmunized using donor red blood cells. Moreover, decrease in the content of Treg-lymphocytes and distortion of the CD4/CD8 ratio was also accompanied by increase in B-lymphocytes in blood of the recipients. Thus, decrease in the relative content of T-lymphocytes in the blood of the rabbits after pRBC transfusion is a reaction of the immune system to an alien material invading the body and characterizes the start of countering it. For example, decline in the level of T lymphocytes was observed in animals after vaccination (Zhipeng et al., 2023). Decline in the level of

T lymphocytes suggests a normal reaction of the immune system to an alien object, while absence of such a decrease when subject to an antigen stimulus or inflammatory processes indicates absence of reactions of the immune system.

B lymphocytes that are found in peripheral blood are a mixture of mature cells that enter blood from the bone marrow and gradually settle in the lymph nodes and other secondary formations of the lymphoid tissue, and from recirculating B cells with immunological memory for various antigens, formed in the secondary lymphoid tissue, and migrate from there to other lymphoid formations. Release of cells of both types into the blood flow increases during their active proliferation in response to antigen stimuli (Lebedev, 1996). In our study, the relative content of B lymphocytes in blood of the recipient rabbits was higher than initially throughout the study (Table 3). Transfusion of red blood cells can augment concentration of B lymphocytes in people as well (Remy et al., 2018; Molina-Aguilar et al., 2020). Active proliferation of B lymphocytes in the secondary organs of immunogenesis in response to the antigen stimulus, which is a donor pRBCs, is a precondition of development of hemolytic post-transfusion reactions (Harrell & Kristensen, 1995; Hendrick, 2020; Davidow, 2021).

Naive lymphocytes are cells with reduced physiological activity: young, immature, or aging, defective lymphocytes, or cells that are temporarily devoid of receptors or with blocked T- and B-lymphocyte receptors. Naive lymphocytes include cells that have a killer activity. In most cases, change in the level of naive cells is determined by T lymphocytes, because their number in peripheral blood is several-fold higher than such of B lymphocytes. Therefore, increase in the number of naive cells oftentimes occurs simultaneously with decline in the number of T lymphocytes, therefore confirming the observed shift in T cells (Lebedev, 1996). This phenomenon was confirmed completely by the results of our studies (Table 3).

Conclusions

Allogeneic transfusion of pRBCs to the recipient rabbits caused development of leukocytosis in their blood: as compared with the initial condition, the absolute content of white blood cells in blood of the experimental animals increased 27.0% on the 3rd day after transfusion, equaling $8.94 \cdot 10^9/L$; 40.1% on the 7th day, measuring $9.86 \cdot 10^9/L$; and 34.1% on the 23rd day, measuring $9.44 \cdot 10^9/L$. Against the initial condition, development of leukocytosis resulted from increase in granulocytes in the blood: 66.9% on the 3rd day after transfusion the absolute content of granulocytes, equaling $4.54 \cdot 10^9/L$; 2.21-fold on the 7th day, measuring $6.02 \cdot 10^9/L$; and 1.87-fold on the 23rd day, measuring $5.08 \cdot 10^9/L$.

Allogeneic transfusion of pRBCs to the recipient rabbits caused decline in the content of T lymphocytes in their blood and increase in B lymphocytes. Compared with the initial condition, the relative content of T lymphocytes in the blood of the experimental animals decreased 20.6% on the 3rd day of the study, equaling 58.0%; 30.1% on the 7th day, measuring 51.0%; and 25.8% on the 23rd day, measuring 54.2%. Relative to the initial state, the relative content of B lymphocytes increased 94.7% on the 3rd day of the study, equaling 22.2%; 2.23-fold on the 7th day, equaling 25.4%, and 2.51-fold on the 23rd day, measuring 28.6%.

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