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Percentage of CD4+, CD8+, and CD25+ T lymphocytes in peripheral blood of pigs in the course of experimental burns and necrectomy

Roman Aleksiewicz[1], Krzysztof Kostro[2], Marek Kostrzewski[3], Beata Lisiecka[3], Marcin Bojarski[3], Paweł Adam Mucha[3]

[1]University Centre of Veterinary Medicine, University of Agriculture in Krakow, 31-120 Krakow, Poland;
[2]Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences, 20-612 Lublin, Poland;
[3]Association of Silesian Veterinary Polyclinics, 41-500 Chorzow, Poland
provet1@poczta.onet.pl

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Abstract

The aim of the study was the evaluation of changes in the percentage profile of CD4+, CD8+, and CD25+ T lymphocytes, and their predictive value with respect to the course of experimental skin burns and early necrectomy in pigs. Thirty Large White Landrace pigs of both genders, weighing 50 kg (±2 kg), were used. Burns to their skin were performed with the use of a computer-controlled heating plate, applied to the animal's body and heated to 2000ºC, using 2.5 kg pressure for 10 s. It produced a burn of 30% (±2%) of body surface with a range of damage between II bº and IIIº. In animals of each experimental group fascial necrectomy was performed, according to the testing module. Blood from experimental and non-treated control animals was collected from the external jugular vein before the beginning of the experiment (hour 0) and at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 h of the experiment. An immune response profile was evaluated using flow cytometry analysis of the level and expression dynamics of CD4+, CD8+, and CD25+ particles on the surface of T lymphocytes. The study demonstrated that experimentally-induced burns in pigs caused cell-mediated immune response reflected in the changes in the percentage of CD4+, CD8+, and CD25+ T lymphocytes, and that early necrectomy in burnt pigs acted in a protective manner for the organism, based on the immunological index values. The study also proved that the dynamics of cell-mediated immunological response intensification determined on the basis of the percentage of CD4+, CD8+, and CD25+ T lymphocytes is conditioned by the size of the burnt surface and the time of necrectomy procedure.

Keywords: pigs, burn wound, necrectomy, T lymphocytes, hypovolaemia.

Introduction

Burns disturb skin integrity and the skin immune system (SIS), protective against the activity of external pathogens. Disturbances in organism's SIS activity create favourable conditions for local infections, while systemic immunity dysfunctions encourage bacteraemia and sepsis (3, 27). The first phase of organism response is pro-inflammatory, and in the case of severe burns may lead to the development of systemic inflammatory response syndrome (SIRS). Such responses are related to the dominance of Th1-type lymphocytes responsible for cell-mediated immune response (4). The second phase is the anti-inflammatory response CARS (compensatory anti-inflammatory response syndrome) which inhibits cell-mediated response resulting in a shift in balance toward humoral immune response with dominance of Th2-type lymphocytes and production of anti-inflammatory cytokines (3, 14). A significant role in CARS formation is also played by CD8+ T lymphocytes (TCD8+). TCD8+ cells enhance CARS development due to selection of anti-inflammatory cytokines, the composition of which is similar to the cytokine pattern of Th2 cells (21, 26, 27).

In severe burns immunosuppression development is observed, leading to reduced activity of CD4+ T (supporting) and CD8+ T (suppressor/cytotoxic) lymphocytes; moreover, the rate of CD4+ is lowered in comparison to CD8+ cells. Immature T cells are noted,

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and the number of natural killer (NK) cells decreases, which results in anergy of both inborn and adaptive immunity (11, 13, 25). CD4+ and CD25+ regulatory T lymphocytes (Treg) play a significant role in inflammatory reaction modulation. As a result of direct interactions, these lymphocytes are able to inhibit proliferation of CD4+ and CD8+ T lymphocytes, and suppress production and release of cytokines (1, 7, 16, 23).

The presence of necrotic tissues in deep and wide burns prolongs intoxication and is the main factor responsible for delaying the healing process (15, 17, 22). Early necrectomy may partially counter immunosuppression by facilitating cytoprotection (5, 15). An early necrectomy of a burn significantly contributes to mitigating immunosuppression (15). Some immunological factors, essential for the initiation of inflammatory reaction, the control of its course, and the healing process of burn wounds, are useful markers which enable burn course monitoring (6, 21, 25, 27).

The study aimed to evaluate the changes in percentage profile of CD4+, CD8+, and CD25+ T lymphocytes, and their predictive value with respect to the course of experimental skin burns and early necrectomy in pigs.

Material and Methods

The study involved 30 pigs of Large White Landrace breed, of both genders, weighing 50 kg (±2 kg). The pigs were healthy and their blood indices were within the reference values for pigs (25). During the 7 d acclimation period the pigs were kept in a day/night lighting system and given complete mixture and water *ad libitum.* A starvation diet was introduced 24 h before the start of the experiment. The animals were divided into six equal groups: group K – burnt animals without necrectomy, group A – burnt animals on which necrectomy was performed 12 h after burning, group B – burnt animals with necrectomy performed 24 h after burning, group C – burnt animals with necrectomy performed 72 h after burning, group D – burnt animals with necrectomy performed 120 h after burning, and control animals without injury. The time of experiment needed was established as 180 h. Before anaesthesia, the animals were weighed. For preemptive analgesia, 0.4 mg/kg b.w. of meloxicam i.m. (Melovem, Dopharma B.V., the Netherlands) was administered. Atropine s.c. at the dose of 0.04 mg/kg b.w. (W.Z.F. Polfa, Poland) and 0.02 mg/kg of butorphanol s.c. (Torbugesic, Zoetis, USA) were used for premedication, and 3 mg/kg b.w of azaperone i.m. (Stresnil, Janssen Pharmaceutica NV, Belgium) and 10 mg/kg b.w. of ketamine i.m. (Bioketan, Vetoquinol, Poland) were administered after 30 min. For pentobarbital anaesthesia, 6 mg/kg b.w. of Vetbutal (Biowet Pulawy, Poland) was infused intravenously. Cannulation of the external jugular vein was performed in each pig. The burning procedure required to obtain 30% body surface area (BSA) burn in each pig was precisely established using the following mathematical formula: BSA (cm^2) = $734BW^{0,656}_{kg}$ (26). The burns were obtained using a touch/burn (T/B) heating plate controlled with a computer software that calculated adjacent area, which in the case of pigs weighing 50 kg was 1% of BSA (3). The burns were produced by placing the burning device on the animal's body, using pressure resulting from the device weight, *i.e.*, 2.5 kg for 10 s. The temperature of 2000ºC was obtained after its application onto skin. The number of required uses was individually established for each pig, so as to obtain the assumed 30% ± 2% BSA of II bº and IIIº burns. The degree of the burn was verified through visual assessment of the burnt skin and histological analysis of burnt sites. Necrectomy of the burn wound was performed by removing the necrosis with a scalpel, up to the fascia. The depth of skin necrosis cut was determined based on the observation of bleeding from capillaries. The loss formed was entirely covered with dermo-epidermal grid graft, collected with a Brown dermatome with a grid add-on device. Blood (2 mL) from experimental animals and from the control group was collected to sterile test tubes with EDTA K3 from the external jugular vein before the beginning of the experiment (hour 0) and at 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 h of the experiment. After the procedure, the animals were euthanised by 40 mg/kg b.w. of sodium pentobarbital i.v. (Morbital, Biowet Pulawy, Poland) and the carcasses were disposed of.

An evaluation of immune response profile after the induction of burn inflammation and necrectomy was performed based on analysis of the level and expression dynamics of CD4+, CD8+, and CD25+ particles on the surface of T lymphocytes. The analysis was carried out using a flow cytometry method (Epics XL Beckman – Coulter flow cytometer, Comesa Polska, Poland) using single-cell labelling. The quality control and self-standardisation of cytometry were performed prior to the analysis using Coulter Flow-Check, Coulter Flow-Set, and compensation reagent preparations (Comesa Polska, Poland). The half-peak coefficient of variation (HPCV) with expected value was verified in order to check the stability of optic and flow systems. Light dispersion and fluorescence were standardised using Flow-Set preparation, adjusting voltage and amplification to the specified mean position on the basis of the control application. In addition, the balancing reagents were used to establish the colours of the system. Using these control and experimental protocols, the cytometry analysis was performed.

Mouse monoclonal antibodies directed toward surface particles were used for this purpose. They were antibodies to CD4+ and CD8+, which are mainly present on Th and Tc lymphocytes in pigs (mouse anti-pig CD4:FITC/CD8:RPE; Serotec Immunological

Excellence, UK), and to CD25+ (mouse anti-pig CD25:FITC; Serotec Immunological Excellence, UK). The examinations were performed according to the procedure enclosed with the antibody sets. Solutions containing 10 µL of mouse monoclonal antibodies directed toward surface particles CD4+, CD8+, and CD25+ were added to 100 µL of pig whole blood. These samples were incubated for 45 h in darkness. After incubation with antibodies, 2 mL of lytic agent was added in order to remove erythrocytes. After 20 min of cell incubation with lytic agent at room temperature in darkness, the labelled cells were measured by flow cytometer.

Excel 2010 for Windows software and the Statistica 10.0 statistical packet (Statsoft, USA) were used for database preparation and for statistical calculations. The results were presented as arithmetical means and standard deviations, and Student's *t*-test was used for significance of differences calculation. Statistical significance was determined for $P < 0.05$. The relationship between the variables was evaluated using correlation coefficients (*r*) according to Pearson with assumed value $P < 0.05$.

Results

Changes in the total white blood cells (WBC) count. Mean WBC count determined in whole blood is presented in Fig. 1. The mean WBC count of control group was 21 000/mm^3. In group K (without necrectomy), WBC count increased and at 12 h reached 130% of WBC value in the control group. Further WBC increase was observed between 12 and 60 h, up to the value of 210% ($P < 0.001$). Between 60 and 180 h, a decrease in WBC value down to the level of 145% of WBC for the control group ($P < 0.05$) was noted. In group A (necrectomy after 12 h), the WBC value increased and at 12 h it equalled 181% of control value ($P < 0.001$). After necrectomy, between 12 and 36 h, the WBC value decreased and reached 73% of WBC value for the control group ($P < 0.05$). Between 36 and 48 h, WBC value increased rapidly by 28%. Between 84 and 180 h of the experiment, a further increase in WBC was noted up to 106% compared to the control group. In group B (necrectomy after 24 h), WBC value increased, and at 12 h it reached 204% of control value ($P < 0.001$). Between 12 and 24 h, the WBC value decreased by 65% and reached 139% of the value of the control group ($P < 0.05$). After necrectomy, between 24 and 60 h, WBC decreased down to the value of 60% compared to the control group ($P < 0.05$). From 60 to 180 h, WBC increased reaching 112% of WBC value for the control group. In group C (necrectomy after 72 h), WBC value was subject to an intense growth up to 72 h, when it reached 230% compared to WBC value in the control group ($P < 0.001$). After necrectomy performed between 72 and 96 h, there was a distinct decrease in WBC value down to 59% of WBC in the control group ($P < 0.05$). Between 96 and 120 h, WBC count increased, by 25.3% on average. From 120 to 180 h, WBC value decreased reaching 86% of WBC value in the control group at 180 h. In group D (necrectomy after 120 h), WBC value increased, and at 12 h it equalled 174% of WBC value in the control group ($P < 0.05$). Between 12 and 108 h of the experiment, WBC count decreased reaching a value of 101%. Between 108 and 156 h, an increase in WBC value was noted, reaching 163% of control group WBC value ($P < 0.05$). From 156 to 180 h, WBC count decreased. At hour 180 it amounted to 125% compared to the control value. (Fig. 1)

Changes in the percentage of CD 4+ T lymphocytes. Mean percentage of CD4+ T lymphocytes determined in whole blood is presented in Fig. 2. The average percentage of CD4+ subpopulation determined in the pigs from the control group was 25.6. In group K (without necrectomy), the percentage of CD4+ increased and at hour 12 it was 103 compared to the control group. Between 12 and 36 h of the experiment, the percentage of CD4+ lymphocytes decreased rapidly, reaching a value of 66 of the control group ($P < 0.001$). Between 84 and 132 h, the percentage of the lymphocytes decreased rapidly up to the value of 41 ($P < 0.001$). Some changes concerning the percentage of CD4+ lymphocytes were noted between 132 and 180 h, and at hour 180 it was 45 compared to the control group ($P < 0.001$). In group A (necrectomy at 12 h), the percentage of CD4+ lymphocytes decreased and at 12 h it was 86 compared to the control group. Between 12 and 48 h of the experiment, the percentage of CD4+ lymphocytes was still decreasing reaching 50 of the control group value ($P < 0.001$). A distinct increase in the percentage of the lymphocytes was observed between 48 and 72 h, and it reached a value of 70 compared to the control group ($P < 0.05$). A decrease in the percentage of CD4+ lymphocytes was noted up to 144 h, and it equalled 40 related to the control group, while further increase in the percentage of the lymphocytes was noted from 144 to 168 h of the experiment up to the value of 52 ($P < 0.001$); however, between 168 and 180 h there was a rapid decrease in the percentage of CD4+ lymphocytes up to 38 compared to the control group ($P < 0.001$). In group B (necrectomy at 24 h), the percentage of CD4+ lymphocytes decreased and it was 82 at 12 h compared to the control group. Further decrease was noted between 12 and 48 h, up to 40% compared to the control group, and the mean decrease between the periods was 10.2% ($P < 0.001$). From 48 to 72 h of the experiment, the percentage of CD4+ lymphocytes increased, reaching a value of 75, and the mean increase from that period was 11.6%. A constant decrease in the percentage of CD4+ lymphocytes was observed from 72 to 180 h, up to the value of 32 ($P < 0.001$) compared to the control group ($P < 0.001$). In group C (necrectomy at 72 h), the percentage of CD4+ lymphocytes decreased and at 72 h it was 86

compared to the control group (P < 0.001). Between 72 and 96 h of the experiment, there was a further decrease in the percentage of CD4+ lymphocytes up to 50%, and the mean decrease between the periods was 12% (P < 0.001). Between 96 and 168 h, there was an increase up to the value of 62%; however, between 168 and 180 h of the experiment there was a rapid decrease in the percentage of CD4+ lymphocytes down to 47 compared to the percentage in the control group (P < 0.001). In group D (necrectomy at 120 h), the percentage of CD4+ lymphocytes increased and it reached 106% at 12 h compared to the control group (P < 0.001). Between 12 and 144 h, there was a decrease in the percentage of CD4+ lymphocytes down to the value of 59 (P < 0.001). In turn, an increase in the percentage of CD4+ lymphocytes was noted between144 and 180 h, up to 72% compared to the control group (P < 0.001) (Fig. 2).

Changes in the percentage of CD 8+ T lymphocytes. The mean percentage of CD 8+ T lymphocytes determined in whole blood is presented in Fig. 3. The mean percentage of the lymphocytes, determined in control group pigs, was 11.5%. In group K (without necrectomy) the percentage decreased and at 36 h it equalled 50% as compared to the control group (P < 0.001). Between 36 and 84 h of the experiment, there were some fluctuations in the percentage of CD8+ lymphocytes with a small upward tendency (mean between the periods = 1%). Between 84 and 132 h, the percentage of CD8+ lymphocytes decreased significantly down to 36 (P < 0.001). A distinct increase of up to 51 in the percentage of the lymphocytes, as compared to the control group, was noted between 132 and 156 h (P < 0.001). A decrease in the percentage of CD8+ lymphocytes was noted from 156 to 180 h compared to the percentage of the lymphocytes in the control group (P < 0.001). In group A (necrectomy at 12 h), the percentage of CD8+ lymphocytes decreased and it reached 50 at 50 h compared to the control group. Between 24 and 84 h, the percentage of CD8+ lymphocytes increased considerably up to 100 (P < 0.05). A constant decline in the percentage of CD8+ lymphocytes was observed at other hours, and at hour 180 it reached 47 compared to the control group (P < 0.05). In group B (necrectomy at 24 h), the percentage of CD8+ lymphocytes increased and at 12 h it equalled 149 compared to the control group. Between 12 and 48 h, the percentage of CD8+ lymphocytes decreased rapidly reaching 48 of the value in the control group (P < 0.001). Between 48 and 72 h, the percentage of CD8+ lymphocytes increased considerably up to 106 (P < 0.05). From hours 72 to 180, a decrease in the percentage of CD8+ lymphocytes down to 37 compared to the control group was observed (P < 0.001). In group C (necrectomy at 72 h), the percentage of the lymphocytes increased and at 72 h it equalled 118 compared to the control group (P < 0.05). Between 72 and 96 h of the experiment, the percentage of CD8+ lymphocytes decreased rapidly reaching a value of 69 (P < 0.001). An increase in the percentage of the lymphocytes was noted between 96 and 180 h, and it reached 93 compared to the percentage of CD8+ lymphocytes in the control group (P < 0.001). In group D (necrectomy at 120 h), the percentage of CD8+ lymphocytes declined up to 108 h of the experiment and it was 49% with respect to the control group. Between 108 and 144 h, the percentage of CD8+ lymphocytes increased up to 68 compared to the control group. Some fluctuations in the percentage of CD8+ lymphocytes were observed between 144 and 180 h, and their value was 75 at 180 h compared to that in the control group (Fig. 3).

Percentage of CD 25+ T lymphocytes. The mean percentage of CD 25+ T lymphocytes determined in whole blood is presented in Fig. 4. The mean percentage of subpopulation CD25+ determined in pigs from control group was 48.5. In group K (without necrectomy), the percentage of CD25+ lymphocytes decreased and at 12 h it reached 78% compared to the percentage of CD25+ lymphocytes in the control group. Between 36 and 180 h of the experiment, the percentage of CD25+ lymphocytes increased significantly up to 144 compared to the control group (P < 0.001). In group A (necrectomy at 12 h), the percentage of CD25+ lymphocytes decreased, and at hour 24 it reached 82 in relation to that in the control group (P < 0.001). An increase in the percentage of CD25+ lymphocytes was noted from 24 to 72 h, up to the value of 128 (P < 0.001). Between 72 and 96 h, there was another decrease in the content of CD25+ lymphocytes down to 117% compared to the control group (P < 0.05). Both fluctuations and an increase in the percentage of CD25+ lymphocytes were noted between 96 and 180 h of the experiment, up to 140 compared to the percentage of CD25+ lymphocytes in the control group (P < 0.001). In group B (necrectomy at 24 h), the percentage of CD25+ lymphocytes decreased and at hour 24 it reached 70 compared to that in the control group (P < 0.001). A significant increase in the percentage of CD25+ lymphocytes was noted between 24 and 48 h of the experiment, up to the value of 105 (P < 0.001). Then, between 48 and 72 h of the experiment, a decrease in the percentage of CD25+ lymphocytes was noted down to the value of 80, followed by an increase up to 118 compared to the control group, observed between hours 72 and 180 (P < 0.001). In group C (necrectomy at 72 h), the percentage of CD25+ lymphocytes decreased and at hour 12 it equalled 30 compared to the percentage of CD25+ lymphocytes in the control group. An increase in the percentage of the lymphocytes up to the value of 118 was noted between hours 12 and 72 (P < 0.001) (with the mean increase of 6.3% between the periods). Between 72 and 96 h of the experiment, a further increase in the percentage of CD25+ lymphocytes was observed, up to the value of 135 (P < 0.001) (with the mean increase of 5.6% between the periods). A decrease in the percentage of the lymphocytes down

to the value 104 was noted between 96 and 120 h ($P < 0.001$), and a constant increase in the percentage up to 117 was noted from hours 120 to 180, compared to the percentage of CD25+ lymphocytes in the control group. In group D (necrectomy at 120 h), the percentage of CD25+ lymphocytes decreased, and at 84 h it reached 96 compared to that in the control group. Between 84 and 120 h, a significant increase in the percentage of CD25+ lymphocytes was noted, up to the value of 145, and an average increase between the periods amounting to 12.25% ($P < 0.001$). From 120 to 180 h, an insignificant decrease was noted in the percentage of CD25+ lymphocytes, reaching 140 compared to the percentage of the lymphocytes in the control group (Fig. 4).

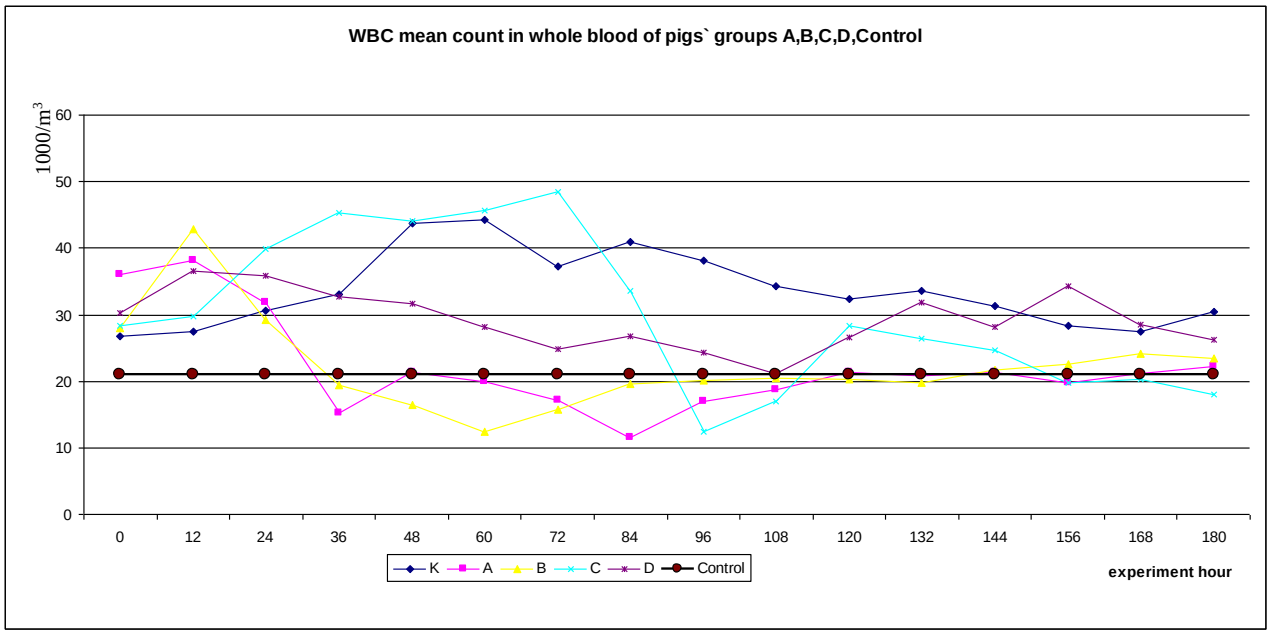


Fig. 1. Changes in leukocyte counts in whole blood of pigs from groups K, A, B, C, D, and control

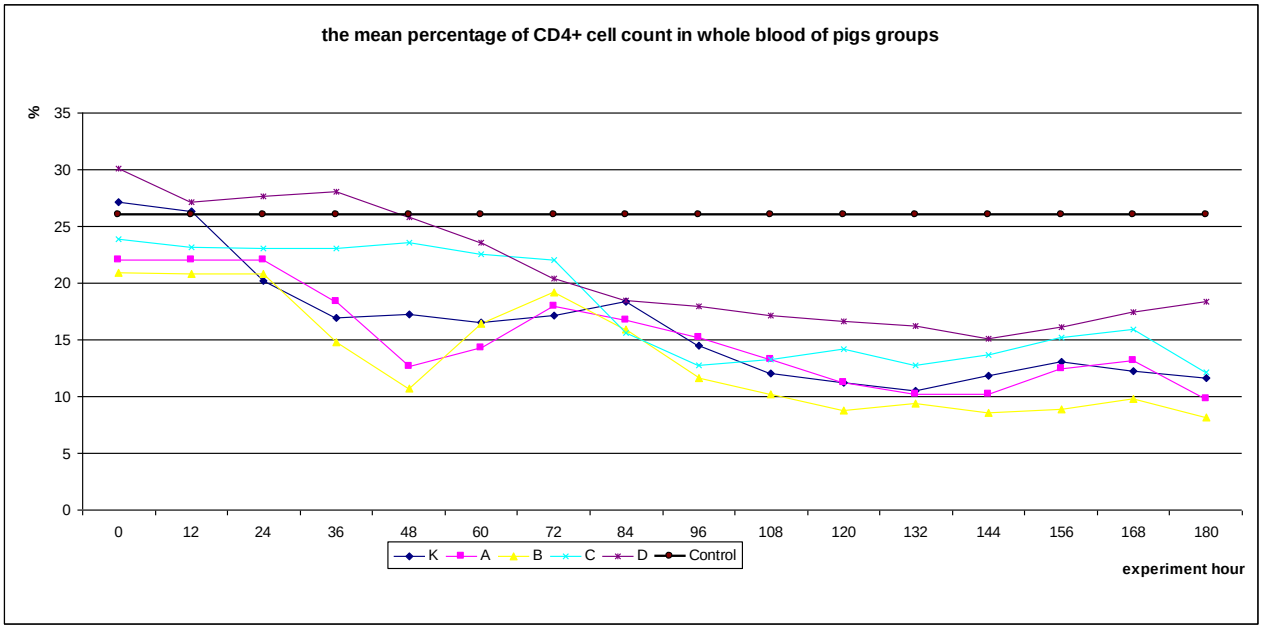


Fig. 2. Changes in the percentages of CD4+ lymphocytes in whole blood of pigs from groups K, A, B, C, D, and control

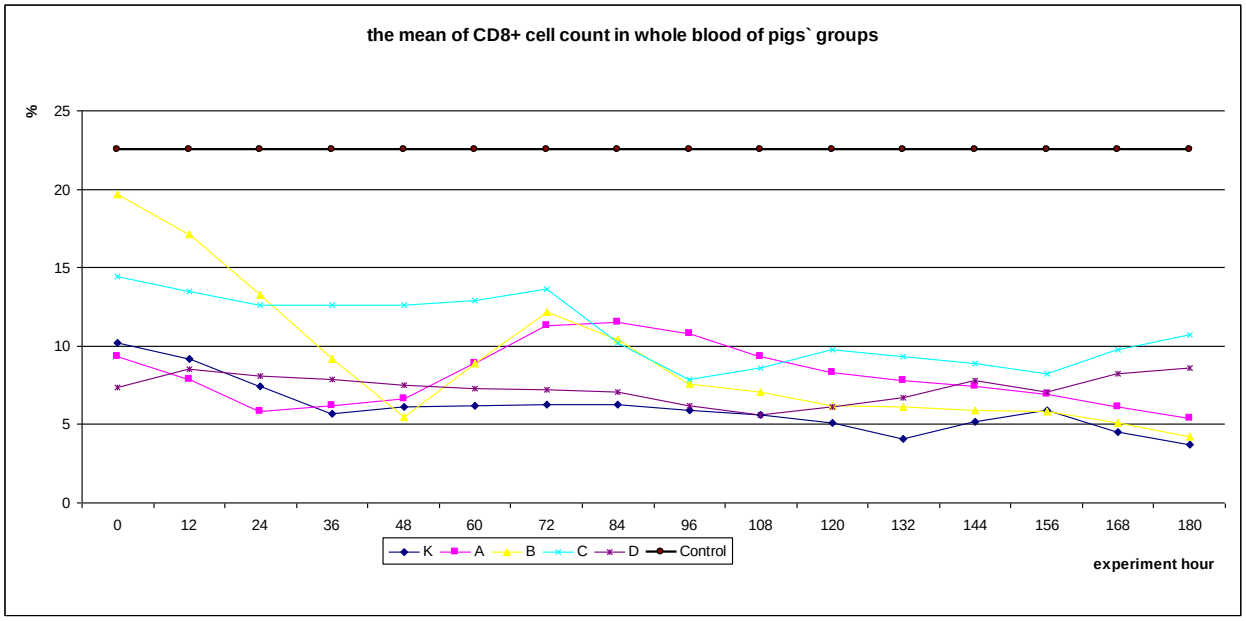


Fig. 3. Changes in the percentages of CD8+ lymphocytes in whole blood of pigs from groups K, A, B, C, D, and control

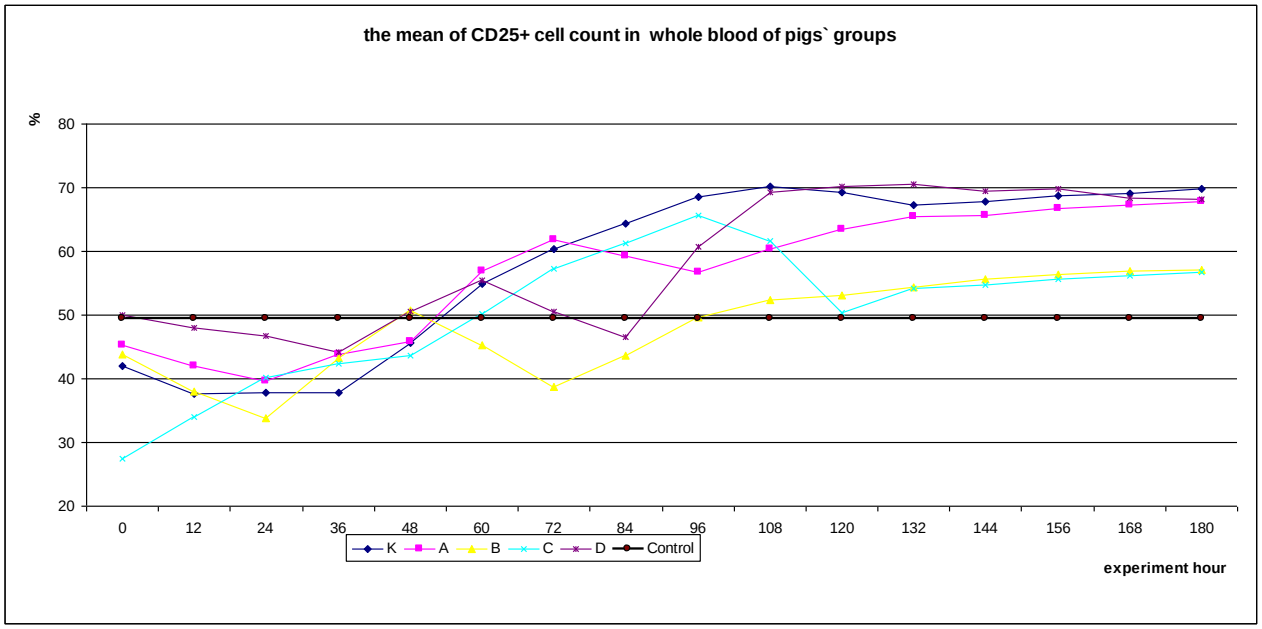


Fig. 4. Changes in the percentages of CD25+ lymphocytes in whole blood of pig's from groups K, A, B, C, D, and control

Discussion

Burn and burn necrosis are an abundant source of numerous toxins, which cause systemic reactions of varying intensity dependent on burn surface and depth of tissues hyperthermia (13, 24). Burns of II bº and IIIº of an area of 30% BSA were obtained in the study, leading to systemic inflammatory reaction observed in groups K, A, B, C, and D. Leukocytes play a fundamental role in inflammatory, protective, and regeneration reactions of the organism. They are also an index of inflammatory reaction intensity and development direction. Leukocytosis was noted in all groups of pigs subjected to burns, and it was visible as early as 12 h from injury. In group K, leukocytosis was visible throughout the whole experiment, and its highest values were noted at 60 h after burning. The number of leukocytes in groups A, B, and C dropped at 12 h, and the highest decrease was observed in group C. This was presumably a result of secondary bacterial infection that usually develops in the case of delayed necrectomy (9, 13, 18, 24, 27). This could be confirmed by the results obtained in groups A, B, and C, where necrectomy was performed at 12, 24, and 72 h after

burning. The significant decrease in leukocyte count observed in these groups may be considered a good predictive factor pointing to a decreased risk of sepsis. According to some authors, the dynamics of leukocyte count change is not an objective factor for evaluation of the intensity of inflammatory processes developing during the course of burn injury, since a positive correlation between leukogram results and inflammatory process intensity is sometimes lacking (2, 19). Numerous authors indicate the importance of the healing-promoting effect of burn necrosis excision, *i.e.* a burn wound being brought up to the condition of a clean surgical wound (5, 9, 10, 13). Early necrectomy may help to maintain cell ultrastructure, stabilise blood biochemical parameters, limit immunosuppression, and modify inflammatory mediator synthesis (5, 15).

Lymphocytes are a strategic element of immune response after burning. They are also a significant predictive index of inflammatory reaction development. These cells are essential in inflammatory reaction initiation, its course control, as well as in the control of the healing process in burn wounds (6, 21, 25, 27). For that reason, they find an application in an evaluation of cell-mediated immune response in the course of burns and necrectomy. The study involved an analysis of the dynamics of cell-mediated immune response based on the percentage of lymphocytes containing surface particles TCD4+, TCD8+, and TCD25+. A significant decrease in the percentage of the CD4+ T lymphocyte subpopulation was noted on the first day after burning. Significant differences were observed in particular experimental groups during further period of observation. Constant, distinct decrease in the percentage of CD4+ T lymphocytes was observed in group K, while the decreasing trend in groups A and B was less clear. In groups C and D, after necrectomy procedure, the percentage of TCD4+ was subject to an insignificant increase. A significant decrease in the percentage of lymphocytes of TCD8+ phenotype was observed in all experimental groups during the first hours after injury, but a constant decreasing tendency was only noted in group K. In turn, in groups A, B, C, and D, in which burn necrosis was removed, a gradual increase in TCD8+ lymphocyte percentage was noted after 48 h. The changes in the percentages of CD4+ T lymphocytes demonstrated high positive correlation with the changes in the percentage of CD8+ T lymphocytes. The values of correlation coefficient r amounted to + 0.92926 in group K, + 0.92273 in group B, and + 0.89778 in group C (as shown in Figs 5, 6, and 7).

Similar results in the case of human patients were obtained by Fayazov *et al.* (65) and Schäffer and Barbul (22), who observed both a decrease in the percentage of CD4+ and CD8+ T lymphocyte subpopulations during the course of burns and a decrease in the TCD4+:TCD8+ ratio. The dynamics of changes in the ratio of CD4+ to CD 8+ T lymphocytes is equivalent to strong anergy of cell-mediated immune response. Numerous authors emphasise the significance of immunosuppressive factors, proinflammatory cytokines (IL-1, IL-6, and TNF-α), stress hormones, nitrogen oxide, and prostaglandin PGE_2 (6, 25). These mediators directly effect a decrease in lymphocyte count and a depression of T-dependent immunity. According to Fayazov *et al.* (6), a decrease in the percentage of T lymphocytes in the burn course may be additionally caused by apoptosis. It was demonstrated in the study conducted that the percentage of CD4+ lymphocytes did not reach initial values in any of the experimental groups, which reflects the state of immunosuppression caused by burns. Only small increases in the count of CD4+ T lymphocytes were noted in groups C and D after necrectomy of burns, which may prove that this procedure contributes to immunosuppression reduction, and thus faster healing. A similar kind of immunosuppression after burning was described by Hansbrough *et al.* (10). The authors implanted the burnt skin to healthy mice, which caused immunosuppression and inhibited proliferation of T lymphocytes and phagocytic activity of macrophages. The decrease in the percentage of CD25+ T lymphocytes was noted in all the groups as early as on the first day after burning. In turn, a constant increase in the percentage of CD25+ T lymphocytes was observed starting from 48 h in group K. In groups A, B, and C after the necrectomy procedure, the percentage of CD25+ T lymphocytes was subject to fluctuations, with the tendency to a rise in percentage. Only the necrectomy performed on group D did not affect the percentage of CD25+ T lymphocytes. According to some authors, an increase in the expression of the TCD25+ particle, which is a receptor for IL-2 on activated T lymphocytes, may prove the activation of Th2 cell-mediated response, which stimulates lymphocytes to secrete anti-inflammatory cytokines and to intensify observed post-burn immunosuppression (14, 20, 25, 27). CD4+ T lymphocytes demonstrated high negative correlation with TCD25+ lymphocytes. Values of correlation coefficient r amounted to – 0.6372 in group K– 0.6710 in group A, and – 0.6844 in group C, as shown in Figs 8, 9, and 10. It is worth noticing that sometimes the correlation is only due to necrectomy.

Based on extensive investigations it can be concluded that the dynamics of the cellular immune response in pigs burnt determined by the evolution of the percentage of lymphocytes TCD4 + TCD8 + and TCD25 +, depends on the size of the surface scalded and the time of necrectomy.

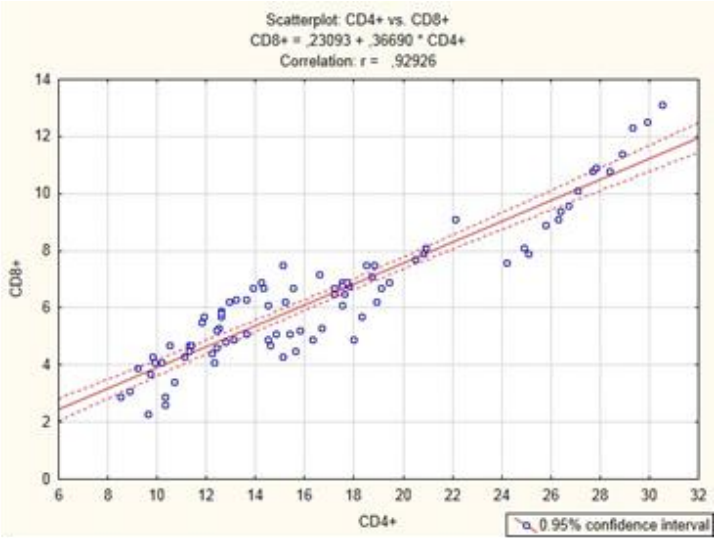


Fig. 5. Correlation between the percentage of CD4+ T lymphocytes and the percentage of CD8+ T lymphocytes in group K

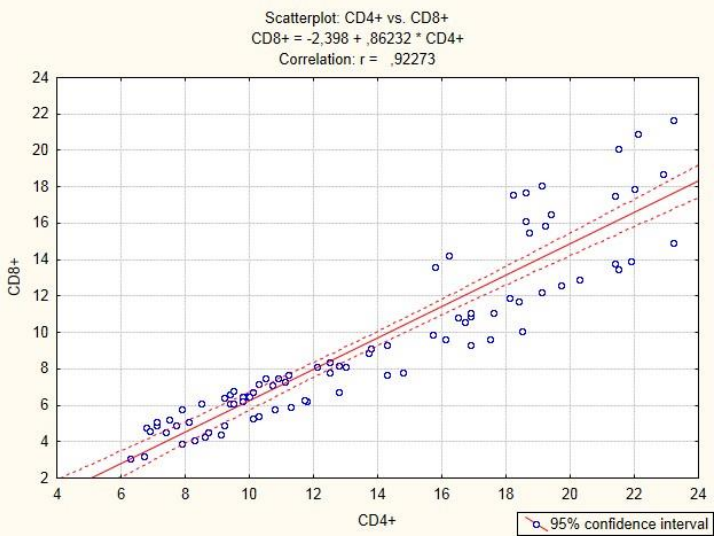


Fig. 6. Correlation between the percentage of CD4+ T lymphocytes and the percentage of CD8+ T lymphocytes in group B

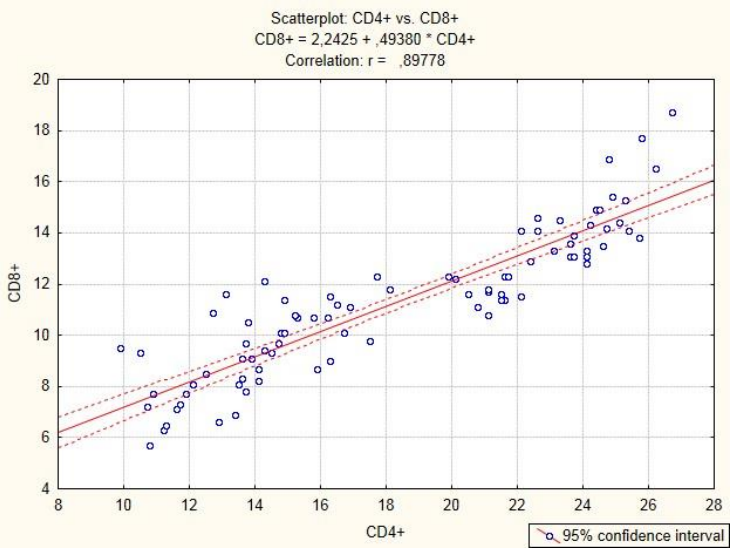


Fig. 7. Correlation between the percentage of CD4+ T lymphocytes and the percentage of CD8+ T lymphocytes in group C

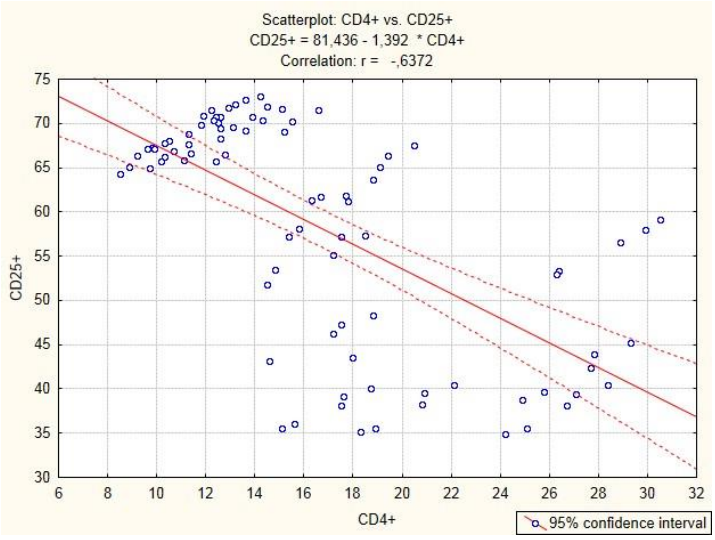


Fig. 8. Correlation between the percentage of CD4+ T lymphocytes and the percentage of CD25+ T lymphocytes in group K

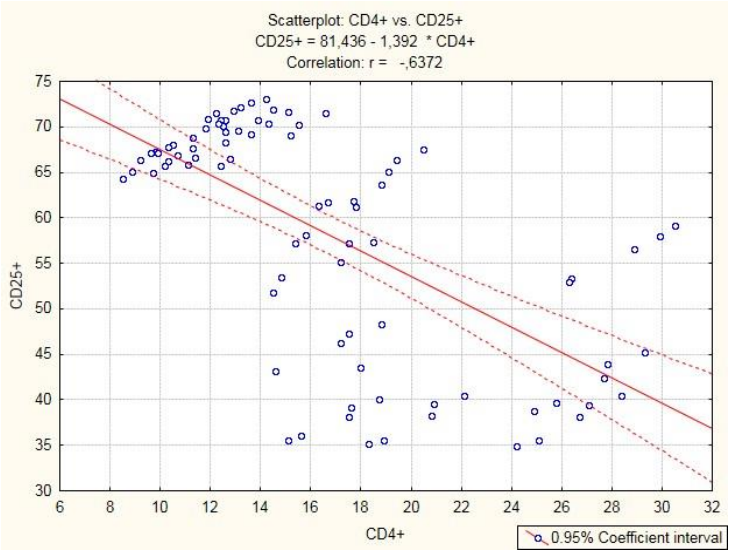


Fig. 9. Correlation between the percentage of CD4+ T lymphocytes and the percentage of CD25+ T lymphocytes in group A

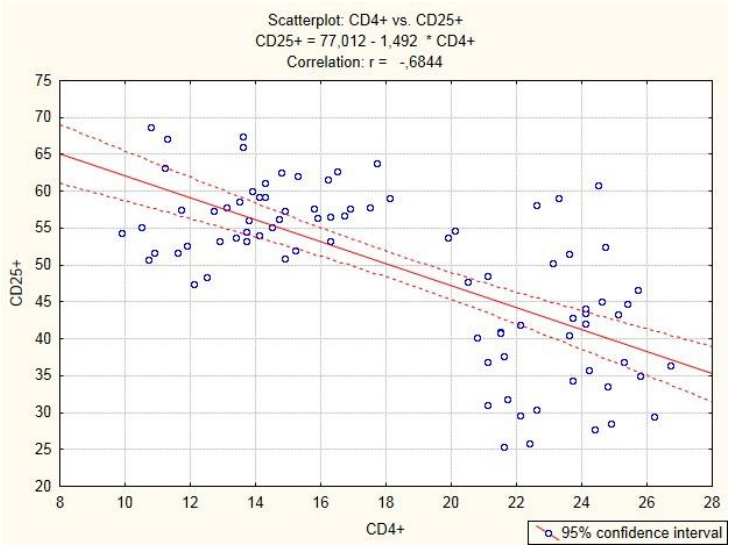


Fig. 10. Correlation between the percentage of CD4+ T lymphocytes and the percentage of CD25+ T lymphocytes in group C

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: The authors declare that the experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regards care and use of laboratory animals.

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