

# The Effects of Immunoglobulin Treatment Upon Th Polarization in Rats with Experimentally Induced Sepsis

## DeneySEL Sepsis Modeli Oluşturulan Sıçanlarda İmmünoglobulin Tedavisinin Th Polarizasyonu Üzerine Etkisi

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**ABSTRACT Objective:** The aim of this study was to investigate the effects of intraperitoneal (i.p.) immunoglobulin (Ig) on T helper (Th) polarization in rats exposed to an experimentally induced sepsis model. **Material and Methods:** Wistar Albino rats were randomly allocated to 4 groups. Group C was identified as the Control Group, groups P, F and S were given i.p. *Escherichia coli* lipopolisaccharide (LPS) to induce sepsis. Group P received i.p. IgM-IgA enriched Ig for three consecutive days starting on the first day of the experiment. Group F received IgG according to the same time schedule with Group P. Blood lymphocyte subgroups were analysed by Flow Cytometry. Interferon (IFN)- $\gamma$  and interleukin (IL)-4 cytokine levels were measured at baseline and at 24 and 72 hours after intervention. Survival on days 7-14 was determined. **Results:** CD4<sup>+</sup>+26<sup>+</sup> level in Group F at 72 hours increased significantly in comparison to the levels in Groups S and P. While significant decreases were observed in the IFN- $\gamma$  levels of the study groups with respect to the Control Group, IL-4 and CD4<sup>+</sup>+30<sup>+</sup> levels seemed to increase. **Conclusion:** In rats with experimentally induced sepsis, i.p. Ig administration reduced the Th2 response and increased survival time particularly in the group which received Ig enriched with IgM-IgA.

**Key Words:** Sepsis; immunoglobulins, intravenous; T-Lymphocytes, helper-inducer

**ÖZET Amaç:** Çalışmamız, deneysel sepsis modeli oluşturulan sıçanlarda intraperitoneal (i.p.) immünoglobulin (Ig) uygulamasının yardımcı T hücrelerinin [T helper (Th)] polarizasyonu üzerindeki etkilerini araştırmak amacıyla planlanmıştır. **Gereç ve Yöntemler:** Wistar-Albino cinsi 30 adet sıçan rastgele 4 gruba ayrıldı. Grup C, kontrol grubu olarak ayrıldıktan sonra, Grup S, P ve F'ye *Escherichia coli* lipopolisakkaridi (LPS) i.p. olarak uygulanmak suretiyle sepsis modeli oluşturuldu. Grup P'ye IgM ve IgA ile zenginleştirilmiş, Grup F'ye ise IgG içeren Ig ilk gün LPS'den 6 saat sonra ve sonraki iki gün i.p. olarak uygulandı. Tüm gruplardaki deneklerden başlangıçta ve girişimden 24 ve 72 saat sonra kan alınarak, Flow sitometri yöntemi ile periferik kan lenfosit alt grupları, IFN- $\gamma$  ve IL-4 sitokin düzeyleri ölçüldü. Deneklerin 7-14 günlük yaşam süreleri değerlendirildi. **Bulgular:** CD4<sup>+</sup>+26<sup>+</sup> düzeylerinin 72. saatte Grup F'de, Grup S ve P'ye göre anlamlı ölçüde artmış olduğu gözlemlendi. Kontrol grubuna göre, çalışma gruplarında IFN- $\gamma$  düzeylerinde anlamlı azalmalar, IL-4 ve CD4<sup>+</sup>+30<sup>+</sup> düzeylerinde ise artış gözlemlendi. **Sonuç:** Deneysel sepsis modeli oluşturulan sıçanlarda i.p. Ig uygulanmasının Th2 yanıtını azalttığı ve özellikle IgM-IgA ile zenginleştirilmiş Ig alan grupta daha belirgin olmak üzere yaşam süresini uzattığı saptandı.

**Anahtar Kelimeler:** Sepsis; immünoglobulinler, intravenöz; T-lenfositler, yardımcı-uyarıcı

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Defined as the systemic inflammatory response to infection, sepsis is one of the most common death causes in intensive care units.<sup>1</sup> Although sepsis pathophysiology has been well understood and mortality rates have been decreased by means of new treatment methods and

better intensive care support in the recent years, sepsis is still the leading cause of death in the world.<sup>2</sup> T helper (Th) lymphocytes exposed to an antigenic stimulus induce the release of inflammatory cytokines by changing into Th1 lymphocytes, and/or release of antiinflammatory cytokines by changing into Th2 lymphocytes. While Th1 lymphocytes produce cellular immune response, Th2 lymphocytes produce humoral (antibody) immune response. Regarding the immunosuppression mechanisms, which develop in sepsis, conversion of Th1 response to Th2 response is one of the most important factors. Enhancement of Th2 response in sepsis has negative effects on mortality. Various studies have suggested that the administration of antiinflammatory agents (IL-10, IL-12, etc.) decreases mortality by converting Th2 responses to Th1 responses.<sup>3,4</sup>

Researchers suggest that using intravenous immunoglobulines (IVIG) in order to increase Ig levels, which are depressed during sepsis, is a beneficial approach in the treatment of children and adults with sepsis. In studies on sepsis, antibiotic treatment is shown to preserve its popularity. Moreover, Ig administration (especially Ig enriched with IgM and IgA) is reported to be useful for immune support treatment.<sup>5,6</sup>

Fow-cytometry analysis is a diagnostic method used to identify surface antigens and to detect lymphocyte cell subgroups at the single cell level. This method ensures detection of even a very small number of cells in a large cell population; thus, it plays a crucial role in clinical diagnosis.<sup>7</sup>

In essence, Flow Cytometry facilitates the investigation of biochemical and physical properties at the level of single cell depending on the size and granular structure of cells. This system is used for various purposes including identification of surface antigens of suspended cells, detection of B cells and T cell subgroups, determination of leukemia and lenfoma types, DNA analysis, phagocytosis and autoantibody detection and chromosome analysis. Regarding its clinical use, Flow Cytometry has important properties such as counting a large number of cells rapidly, detecting a very small number of neoplastic cells among a large cell population, sort-

ing out sub-groups of cells and heterogeneous cell populations.

In this study, we examined the effects of intraperitoneal (i.p.) administration of two different Ig preparations (Ig enriched with IgM and IgA and Ig with IgG alone) for 3 consecutive days on cytokine levels, lymphocyte subgroups, T helper polarization and survival rates in rats with experimentally induced sepsis.

## MATERIAL AND METHODS

Following the approval of the Faculty Ethic Committee, 30 Wistar-Albino rats weighing between 200-250 grams were included in the study. Ethical provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" were followed and care was taken to use the minimum number of subjects in the study. The subjects were kept in an air-conditioned room with lighting (22-24°C 70-75% humidity). They were fed on standard rat pellet and tap water. The subjects were marked and were randomly divided into 4 groups using the "closed envelope" method. Anesthesia was induced in all subjects using intramuscular Ketamin HCl (Ketalar®, Eczacıbaşı, Istanbul 90 mg/kg) and intramuscular Xylazine (Romphun® Bayer Istanbul 10 mg/kg).

Group C (n=6) subjects were given i.p. 1 ml 0.9% NaCl. The remaining subjects received *Escherichia coli* lipopolysaccharide (LPS) in 1 ml 0.9% NaCl (2.5 mg/kg 0.111:B4; Sigma Chemical, St. Louis, MO) to induce experimental sepsis.<sup>8</sup>

After the LPS injection, Group P (n=8) subjects were administered Ig enriched with IgM and IgA (i.p. 4 ml/kg Pentaglobin, Biotest Pharma GmbH, Dreieich, Germany) at 6, 24 and 48 hours.<sup>9</sup> Group F (n=8) subjects were given i.p. Ig containing IgG alone (500 mg/kg Flebogamma 5% Grifols USA) at same time intervals.<sup>10</sup> The subjects in Group S (n=8) were not subject to any intervention after the i.p. LPS injection.

About 6-8 hours after LPS injection, clinical symptoms reported by Wichterman were observed (the subjects snuggled together to sustain their

body temperatures; their motions slowed down; piloerection developed; crusting and bleeding developed in their eyes).<sup>11</sup> Rectal body temperatures of the subjects were measured using a digital thermometer. Subjects without sepsis symptoms within 12 hours were excluded from the study. They were replaced with new ones to have a total number of 30 subjects.

### IMMUNOLOGICAL ANALYSIS

Blood samples were collected from the tail veins of the rats for immunological analysis at baseline and at 24 and 72 hours after the intervention. To determine the lymphocyte subgroups, the samples were analysed with Flow Cytometry (Epics XL; MCL; Beckman Coulter USA) using rat monoclonal antibodies [CD45<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup> 26<sup>+</sup> (Cedarlane Laboratories, Canada), CD14<sup>+</sup>, CD4<sup>+</sup> 30<sup>+</sup> (BD Biosciences, Canada)]. Serum Interferon (INF)- $\gamma$  and interleukin (IL)-4 levels were measured with ELISA (Triturus Grifols, España) using rat cytokine kits (Biosource, USA). Sensitivity values of the kits used for analyses were recorded as <13 pg/mL and <2.0 pg/mL for INF- $\gamma$  and IL-4 respectively. The survival rates from 7-14 days were recorded.

### STATISTICAL ANALYSIS

Statistical Package for Social Sciences 12.0 (SPSS) Programme was used for statistical evaluation. Data obtained were expressed as mean $\pm$ standard deviation and median (min-max). For inter-group comparisons, Kruskal Wallis and Mann-Whitney U tests were employed. Wilcoxon analysis was used to analyze repeated measurements. Chi-square (Fisher's Exact) and Kaplan-Meier analysis were used for the analysis of survival rates.  $p < 0.05$  was considered significant.

## RESULTS

The body weights of the rats included in the study did not show a significant difference between the groups ( $p > 0.05$ ). The body temperature in Group S reached its maximum value at 72 hours, whereas temperature changes in the remaining groups remained within very small limits.

### FLOW-CYTOMETRIC ANALYSIS

CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> (Total T lymphocyte, T helper lymphocyte, T cytotoxic lymphocyte) lymphocyte subgroups: Significant differences were not observed in the CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> levels of Group C and Group S in comparison with the baseline values. However, in the Ig administered groups (Group P, Group F), CD3<sup>+</sup> lymphocyte levels decreased with respect to the baseline values ( $p = 0.03$ ,  $p = 0.008$ ) at 24 hours. At 72 hours, the levels increased; however, the increase was significant only in Group F ( $p = 0.008$ ). CD4<sup>+</sup> lymphocyte levels of Group P and Group F decreased in the period following intervention before which a significant increase was detected. On the other hand, CD8<sup>+</sup> lymphocyte levels of the Ig administered groups initially decreased at 24 hours and then increased at 72 hours with respect to baseline values. These changes were significant in Group F ( $p < 0.05$ ). The CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> lymphocyte levels did not show a significant difference between the groups for the same time intervals.

CD4<sup>+</sup> 26<sup>+</sup> (Th1) Results: CD4<sup>+</sup> 26<sup>+</sup> lymphocyte levels of all groups demonstrated a decrease at 24 hours, which was not significant with respect to the baseline values. However, at 72 hours, there was an increase which was significant for Group F only ( $p = 0.01$ ). The increase in levels was significantly high in Group F when compared with Groups S and P ( $p < 0.05$ ) (Table 1, 2, Figure 1).

CD4<sup>+</sup> 30<sup>+</sup> (Th2) Results: While there was no notifiable change in the Control Group, Group P showed a significant increase at 24 hours with respect to the baseline values. On the other hand, Groups F and S demonstrated similar changes at 24 and 72 hours ( $p < 0.05$ ). These changes observed in all groups were significant in comparison to the Control Group (Table 1, 2, Figure 2).

INF- $\gamma$ : While there was no significant difference in the Control Group with respect to baseline values, INF- $\gamma$  levels displayed significant decreases in the remaining 3 groups ( $p < 0.05$ ). INF- $\gamma$  levels significantly decreased at 24 hours in Groups S and F and at 72 hours in all groups compared to the Control Group ( $p < 0.05$ ). This decrease was smaller

**TABLE 1:** Comparison of data according to baseline values [Median (Min.-Max)].

Variable	Baseline	24 hours	72 hours	P value		
				Baseline-24	Baseline-72	
Group C	CD24+ 26+	42.25(37.20-48.10)	40.06 (29.49-48.30)	43.07 (35.08-49.08)	0.17	0.60
	CD24+ 26+	3.00 (2.30-3.58)	2.97 (2.41-3.58)	3.18 (2.39-3.49)	0.34	0.08
	IFN-γ	45.20 (43.90-58.60)	46.60 (45.20-58.00)	42.80 (39.80-52.60)	0.14	0.50
	IL-4	28.75 (22.70-34.10)	29.25 (22.70-33.00)	27.20 (23.90-32.40)	0.29	0.07
Group S	CD24+ 26+	38.25 (36.60-48.80)	37.92 (34.40-41.71)	36.96 (35.63-47.63)	0.11	0.60
	CD24+ 26+	3.06 (2.41-3.49)	2.89 (2.39-3.74)	3.67 (2.92-3.97)	0.02	0.02
	IFN-γ	44.50 (42.70-58.60)	40.20 (37.60-43.90)	38.45 (35.10-41.50)	0.02	0.02
	IL-4	29.75 (24.10-30.40)	30.50 (23.40-34.90)	31.85 (22.90-35.30)	0.02	0.02
Group P	CD24+ 26+	38.60 (29.40-40.70)	31.98 (29.58-43.83)	39.71 (27.27-46.44)	0.13	0.76
	CD24+ 26+	2.91 (2.14-3.21)	2.90 (2.19-3.84)	3.25 (2.59-3.70)	0.008	0.11
	IFN-γ	46.70 (41.70-50.10)	45.20 (42.30-51.90)	39.80 (35.10-49.20)	0.08	0.01
	IL-4	26.50 (20.70-35.80)	27.50 (19.50-36.30)	29.60 (24.10-39.00)	0.008	0.008
Group F	CD24+ 26+	38.80 (35.30-49.80)	35.64 (28.36-42.64)	47.32 (37.20- 48.10)	0.06	0.01
	CD24+ 26+	2.93 (2.46-3.78)	2.88 (2.42-3.89)	3.26 (2.58-3.77)	0.008	0.008
	IFN-γ	49.60 (41.20-54.70)	46.60 (41.50-53.90)	40.60 (36.80-52.60)	0.01	0.008
	IL-4	26.90 (20.10-31.20)	27.50 (23.60-32.40)	25.70 (21.60-31.60)	0.008	0.18

CD4+ 26+ : T lymphocyte cluster of differentiation (T helper 1), CD4+ 30+ : T lymphocyte cluster of differentiation (T helper 2), IFN - γ: Interferon- gamma, IL-4: Interleukin 4.

**TABLE 2:** Comparison of percentage change compared to baseline values [Median (Min.-Max)].

Variable	Group-C	Group-S	Group-P	Group-F	Statistical analysis (p)	
CD4+ 26+	0-24 <sup>th</sup>	0.97 (0.79-1.03)	0.95 (0.76-1.03)	0.90 (0.76-1.11)	0.97 (0.79-1.03)	C-S 0.025
	0-72 <sup>nd</sup>	0.99 (0.89-1.14)	0.97 (0.90-1.01)	1.02 (0.84-1.16)	1.10 (0.95-1.27)	C-P 0.007 C-F 0.013 C-S 0.423 C-P 0.157 C-F 0.238
CD4+ 30+	0-24 <sup>th</sup>	0.94 (0.78-1.55)	0.89 (0.80-1.55)	1.08 (0.70-1.89)	1.02 (0.92-1.26)	C-S 0.004 C-P 0.001 C-F 0.001
	0-72 <sup>nd</sup>	0.99 (0.78-1.51)	1.12 (0.84-1.64)	1.14 (0.89-1.40)	1.12 (0.71-1.42)	C-S 0.004 C-P 0.018 C-F 0.001
IFN-γ	0-24 <sup>th</sup>	1.02 (0.99-1.04)	0.87(0.74-0.97)	0.98 (0.87-1.03)	0.98 (0.81-1.05)	C-S 0.004 C-P 0.087 C-F 0.002
	0-72 <sup>nd</sup>	0.94 (0.89-1.01)	0.86(0.70-0.91)	0.88 (0.71-1.14)	0.99 (0.89-0.98)	C-S 0.004 C-P 0.008 C-F 0.001
IL-4	0-24 <sup>th</sup>	1.01 (0.96-1.03)	1.03(0.97-1.17)	1.04 (0.85-1.25)	1.03 (1.01-1.30)	C-S 0.016 C-P 0.077 C-F 0.059
	0-72 <sup>nd</sup>	0.96 (0.88-1.05)	1.07(0.75-1.35)	1.12 (0.92-1.47)	1.03 (0.75-1.19)	C-S 0.006 C-P 0.860 C-F 0.018

CD4+ 26+ : T lymphocyte cluster of differentiation (T helper 1), CD4+ 30+ : T lymphocyte cluster of differentiation (T helper 2), IFN - γ: Interferon- gamma, IL-4: Interleukin 4.

for the Ig administered groups at 24 and 72 hours but significant in comparison with the Sepsis Group (Table 1, 2, Figure 3).

IL-4: IL-4 levels increased significantly with respect to the baseline values in all groups at 24 hours (p<0.05). At 72 hours however, the levels increased

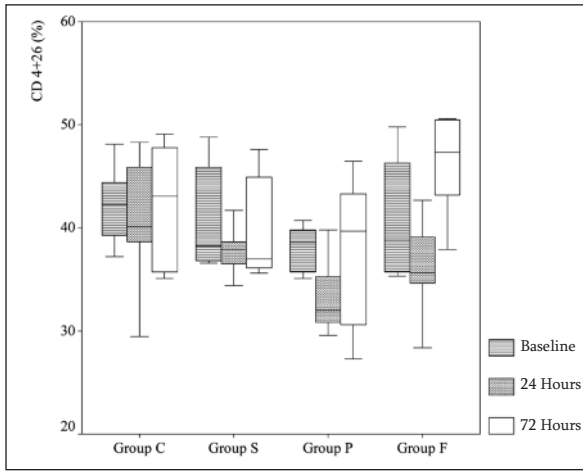


FIGURE 1: CD4+ 26+ lymphocyte levels of the subjects.

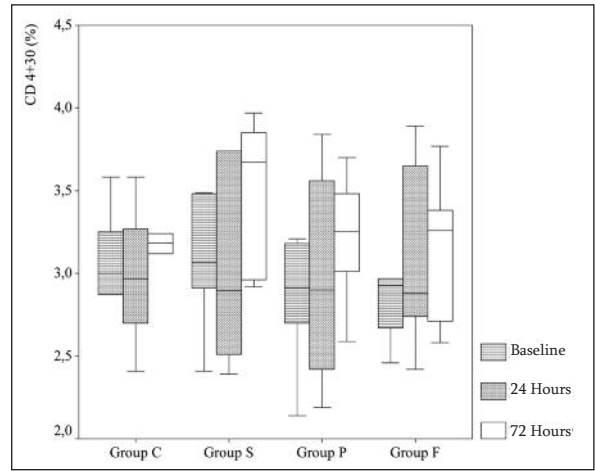


FIGURE 2: CD4+ 30+ levels of the subjects.

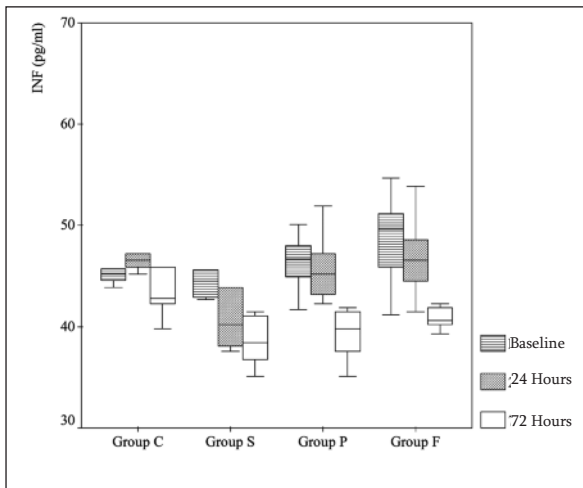


FIGURE 3: Serum IFN- $\gamma$  levels of the subjects.

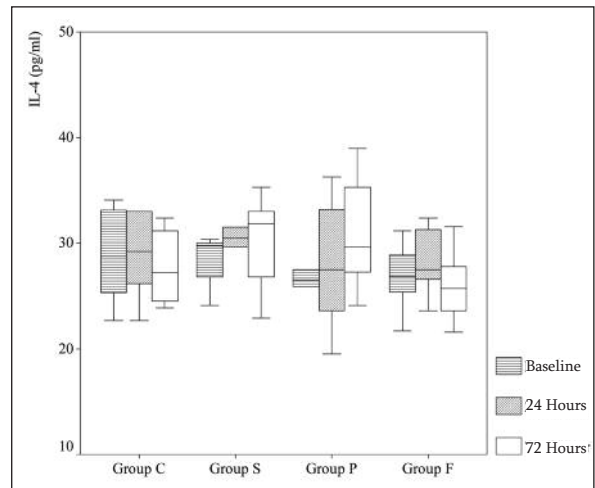


FIGURE 4: Serum IL-4 levels of the subjects.

only in Groups S and F. On the other hand, IL-4 levels in Group P decreased to approach baseline values. At 24 hours IL-4 levels seemed to increase significantly in Group S compared to Groups C, P and F, ( $p < 0.05$ ). At 72 hours, the comparison of Groups S and F with Groups C and P showed significant increases in these levels (Table 1, 2, Figure 4).

**Survival Rates:** Mortality rates decreased significantly in the Ig administered groups with respect to the Sepsis Group at 7 days after the intervention ( $p = 0.001$ ). The Ig administered groups did not show a significant difference in mortality rates (28.7% in Group P, 13.9% in Group F) ( $p = 0.319$ ). The mean survival time during the 14-day follow-up was  $92.33 \pm 4.85$  hours for Group

S (95% confidence interval 82.33-101.84),  $238.00 \pm 24.97$  hours for Group P (95% confidence interval 189.05-286.95) and  $148.70 \pm 10.79$  hours for Group F (95% confidence interval 127.55-169.85). Survival rates for the 14-day follow-up were significantly longer for Group P rats than for Group F rats ( $p = 0.003$ ) (Figure 5).

## DISCUSSION

A number of studies have investigated the effects of antimicrobial and/or antiinflammatory agents against pathogens or the inflammatory response in experimentally induced sepsis and peritonitis models. These studies have shown that a series of immuno-inflammatory reactions are induced due to

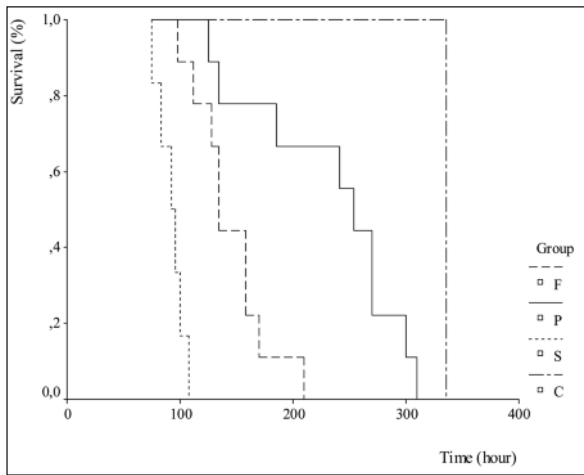


FIGURE 5: Survival rates of the subjects.

the release of pro-inflammatory cytokines, which lead to the suppression or activation of the immune system.<sup>12</sup>

As pro-inflammatory cytokines have a short half-life in the peripheral circulation and since their natural inhibitors exist (such as interleukin-1 receptor antagonist, tumor necrosis factor- $\alpha$  antagonists), it can be misleading to measure the serum levels alone to explain the inhibition or activation properties of these cytokines.<sup>13</sup> Examining the peripheral blood lymphocyte subgroups (Th1, Th2) or intracellular cytokine levels in the lymphocytes are among the most effective ways for identifying the polarization between Th1 and Th2 type lymphocytes in sepsis.<sup>14</sup>

The fact that treatment with normal immunoglobulins obtained from healthy donors becomes effective through neutralization of endotoxins or other bacterial products forms the basis of immunotherapy. Chitkara et al. have shown that in the early period after major surgery, serum IgG and IgM levels decrease, and Ig concentration has a correlation with postoperative infection risk.<sup>15</sup> Other important factors responsible for the success of immunological treatment are the antibody spectrum of the agents used and the concentration of existing antibodies. In previous studies, the existence of antibodies against *Staphylococcus*, *Escherichia*, *Streptococcus* and *Pneumococcus* in IVIG preparations was shown with

ELISA.<sup>16-20</sup> The activation of the complement system is known to play an important role in sepsis pathogenesis. In sepsis, while the alternative way is activated by bacterial lipopolysaccharides, classical complement activation cascade starts with the attachment of C-reactive protein to the cell membrane. Immunoglobulins play an important role in sepsis treatment both by neutralizing the bacteria based toxins and by inhibiting the complement activation.

In-vitro studies in T cell cultures of sepsis patients have shown that there is a decrease in both Th1 and Th2 (such as IL-2, IFN- $\gamma$ , IL-4 and IL-10) type cytokine levels.<sup>21</sup> These studies have also shown that while Th2 (such as IL-4, IL-5, IL-6, IL-10) type cytokines increase, there is a decrease in Th1 (such as IFN- $\gamma$  and IL-12) type cytokines.<sup>22</sup> A clinical study investigating the relation between intracytoplasmic cytokine levels and mortality by using the flowcytometric method in patients who developed sepsis after burning, has shown that Th2 (such as IL-4, IL-5, IL-6 and IL-10) type cytokine levels become dominant whereas Th1 (such as IFN- $\gamma$  and IL-12) type cytokine levels are suppressed.<sup>23</sup> IL 10 is an anti-inflammatory cytokine, which blocks TNF- $\alpha$ , IL-1 and IL-12 that have a role in cellular immunity. Various studies investigating the experimentally induced sepsis model have suggested that IL-10 administration produces an antipyretic effect and increases Th2 (IL-4) cytokine level.<sup>8,24</sup> In other studies carried on rats with cecal ligation and puncture (CLP) induced peritonitis, i.p. IL-12 administration resulted in increased IFN- $\gamma$  levels.<sup>25</sup> Trautman et al. studied LPS antibody levels in IVIG preparations and found that the highest concentration was achieved by pentaglobulin. They concluded that IVIG treatment could produce maximum efficiency in the neutralization of endotoxin.<sup>26</sup> Among rats with experimentally induced sepsis, those that received different concentrations of IgM and IgA were seen to have higher complement inhibition than the rats that received IgG alone.<sup>10</sup> Greenberg et al. found that in rats with induced sepsis, leukocyte numbers decreased 24 hours after IgG administration.<sup>27</sup>

In our study, we saw that in the IVIG treated groups, Th2 (both as cytokin IL-4 and Th2 subgroup lymphocyte CD4<sup>+</sup> 30<sup>+</sup>) levels decreased but Th1 (both as cytokin IFN- $\gamma$  and Th1 subgroup lymphocyte CD4<sup>+</sup> 26<sup>+</sup>) levels increased in the late period in comparison to the Sepsis Group. We also found that the levels of CD3<sup>+</sup> (Total T) and CD4<sup>+</sup> (T Helper) lymphocytes, which enhance the defence mechanism by improving macrophage phagocytic ability increased in the late period. Thus, we suggest that the cytokine profile of the IVIG treated groups and the change in lymphocyte subgroups are a result of the anti-inflammatory effects of IVIG preparations. Experimental studies have shown that IL-10 and IgG administration increases survival rates, and clinical studies have proven that IgM and IgA enriched Ig administration decreases mortality rates in patients with sepsis induced during the neutropenic, abdominal and neonatal periods.<sup>24,25,28-33</sup> However, these results were not statistically significant.

In our study, we saw that survival times of IVIG treated groups were longer compared to the other groups excluding the Control Group (survival times being further prolonged in the IgM and IgA enriched Ig groups). This may be attributed to antigen binding of IVIG preparations, their endotoxin neutralizing and complement inhibiting effects and the administration of IVIG preparations at the beginning of endotoxemia before organ dysfunction develops.

Considering the antibiotic resistance in microorganisms and the developments in sepsis biopathology and physiopathology, we suggest that the use of adjuvant agents in the treatment of sepsis might be beneficial. Although there is still a need to carry out comprehensive studies to determine the place of immunoglobulins in sepsis treatment, based on our observation that immunoglobulin administration decreased Th2 response and mortality rates (in the long term) in rats with experimentally induced sepsis, we think that the administration of IVIG preparations (especially immunoglobulin preparations enriched with IgM and IgA) will yield successful results in this respect.

In an experimental study IVIG was administered 2 and 20 hours after peritonitis.<sup>27</sup> In another study, it was injected simultaneously with bacterial inoculation.<sup>34</sup> In our study, we used IVIG 6-8 hours after intraperitoneal LPS injection after observing the clinical signs of sepsis and sustained IVIG administration for 3 days as suggested in the literature.<sup>30,31</sup> Clinical and experimental studies reported survival times of 28-30 days and 7-14 days, respectively.<sup>27,30,31,35</sup> Thus, we believe that it is necessary to interpret the results of studies related with IgM and IgA enriched immunoglobulin very carefully, and it requires further investigation with larger groups of subjects and with longer follow-up to identify a more accurate place for Ig in standard sepsis treatment.

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