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Original Research

Characteristics of cytokine status in intra-abdominal infection with underlying diabetes mellitus: An experimental study

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Abstract

Background and aims: Diabetes mellitus (DM) is known to have an increased susceptibility to infections and poor treatment outcomes, intraabdominal infections (IAI), in particular. Changes in the functioning of mechanisms for regulating inflammation under the DM influence are the main reason. It is crucial to study the state of the cytokine network. Since cytokines (Ctk) play a major role in the immune response and inflammation regulation. Our study aims to compare changes in cytokine status in IAI with underlying DM in the experiment. **Material and method:** The object of the studies is 100 mature albino nonlinear rats. Their weight is 180–200 g. To create the IAI model, animals have undergone a transesophageal gastric perforation. DM has been induced by subcutaneous administration of a 1.6% alloxan solution in distilled water at a dose of 16 mg per 100 g of body weight. The animals have been divided into the following groups: intact animals, animals with DM models, intact animals with peritonitis models (group 1), animals with peritonitis models with underlying DM (group 2). IAI has been modeled 3 months following the DM simulation. The blood has been taken from the cervical vein both before the IAI simulation and in 6, 12, 24, 48 hours of its simulation. The content of tumor necrosis factor α (TNFa) and interleukins (IL) 2, 6, and 10 in blood plasma have been studied. **Results:** The content of proinflammatory Ctk in animals with DM models has been significantly higher than in intact ones. The IL 10 content has been lower. After 6 hours of IAI modeling, the plasma content of all Ctk, most notably TNFa, has significantly increased in group 1. The amount of TNFa has slightly changed in group 2. The amount of IL 2, IL 6 (table 4) has significantly increased. The amount of IL 10 has increased more than 50 times. The amount of TNFa, IL 6, and 10 have been significantly decreased. The amount of IL has remained at the same level. The number of all Ctk has immediately decreased in group 2. The TNFa number has been the lowest. After 24 hours, the number of TNFa has slightly increased in group 1 since the IAI simulation. The number of other Ctk is unlikely to have been decreased. IL 2 is the most pronounced to have been decreased. The number of TNFa has remained quite the same in group 2. The number of remaining Ctk has continued to decrease rapidly. After 48 hours, the number of TNFa and IL 10 have decreased in group 1 since the IAI simulation. Moreover, the number of IL 10 has become less than the initial indicators. The number of IL 6 has slightly changed, and the number of IL 2 has increased significantly. The number of IL 6 has increased significantly in group 2. The number of IL 6 has increased slightly, while the number of TNFa and IL 10 has remained almost unchanged. **Conclusions:** DM modeling causes a disparity in cytokine status. The reason is a higher number of pro-inflammatory Ctk in the background of a less number of IL 10. Intact animals with IAI models have shown signs of cyclical changes in the activity of various mechanisms of inflammatory regulation within 48 hours. At the same time, in 48 hours, there have been signs of impaired function of the regulation mechanisms of the immune response. There have been differences in the functioning of various mechanisms of inflammatory regulation in 6 hours in animals with IAI models with underlying DM. There have been signs of immunosuppression in 12 hours. These changes have been progressing later on and the signs of immune insufficiency have been shown in 48 hours.

Keywords: diabetes mellitus, intra-abdominal infections, cytokines.

Background and aims

Diabetes mellitus (DM) is known to be associated with increased susceptibility to infections and poor treatment outcomes [1–3]. This fully applies to sepsis [4, 5], in particular intra-abdominal infections (IAI) [6, 7], which is reflected in the guidelines for their treatment [8–10]. Changes in the functioning of mechanisms for regulating inflammation under the DM influence are the main reason [11]. Some of the changes are described in our studies as well [12–14]. It is crucial to study the state of the cytokine network. Since cytokines (Ctk) play a major role in the immune response and inflammation regulation [11, 15]. Our study aims to compare changes in cytokine status in IAI with underlying DM in the experiment.

Material and method

Study design

The object of the studies is 100 mature albino nonlinear rats. Their weight is 180–200 g. To create the IAI model, animals have undergone a transesophageal gastric perforation. DM has been induced by subcutaneous administration of a 1.6% alloxan solution in distilled water at a dose of 16 mg per 100 g of body weight [16].

The animals have been divided into the following groups: intact animals, animals with DM models, intact animals with peritonitis models (group 1), animals with peritonitis models with underlying DM (group 2).

The main criterion of DM has been blood glucose, indicating 5.39±0.25 mmol/l (in intact animals, 3.21±0.53 mmol/l, $p<0.01$). IAI has been modeled 3 months following the DM simulation. The blood has been taken from the cervical vein both before the IAI simulation and in 6, 12, 24, 48 hours of its simulation.

While performing the research, the basic requirements concerning biomedical experiments of the Vancouver Convention were observed (1979, 1994). The animals were taken out from the experiment by overdosing on an anesthetic. All manipulations have been performed under sevoranov anesthesia.

Laboratory, anthropometric, and clinical data collection

The content of tumor necrosis factor α (TNFa) and interleukins (IL) 2, 6, and 10 in blood plasma have been studied. The study has been performed on an immunoenzyme analyzer AIFR-01 "Uniplan" with reagents from the "Biosuorce" company.

Statistical analysis

The statistical calculations of the research results have been conducted with the help of Microsoft® Office Excel spreadsheets (build 11.5612.5703). The Shapiro – Wilk criterion has checked the law of samples distribution for normality. To test the hypothesis of average equality the student criterion has been used. To check normally distributed samples the Fisher criterion has been used. The Wilcoxon and Wilcoxon–Mann–Whitney criteria have been applied for samples whose distribution was different from normal.

Results

The content of pro-inflammatory Ctk in animals with DM models has been significantly higher than in intact ones. The IL 10 content has been lower.

In 6 hours since IAI modeling, the plasma content of all Ctk, most notably TNFa, has significantly increased in group 1 (Table 2). The amount of TNFa has slightly changed in group 2. The amount of IL 2 (table 3), IL 6 (Table 4) have significantly increased. The amount of IL 10 (Table 5) has increased more than 50 times.

The amount of TNFa, IL 6, and 10 have been significantly decreased. The amount of IL has remained at the same level. The number of all Ctk have immediately decreased in group 2. The TNFa number have been the lowest.

After 24 hours, the number of TNFa has slightly increased in group 1 since the IAI simulation. The number of other Ctk is unlikely to have

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Table 1: The initial amount of cytokines (pcg/ml) in the blood plasma of experimental animals (M±m).

Indicators	Intact animals	Animals with DM models
TNFα	34.256±14.444	203.770±65.602, p<0.01
IL 2	146.002±42.045	299.772±39.224, p<0.01
IL 6	33.461±2.667	55.643±9.527, p<0.05
IL 10	26.166±6.920	8.333±2.092, p<0.01

been decreased. IL 2 is the most pronounced to have been decreased. The number of TNFa has remained quite the same in group 2. The number of remaining Ctk has continued to decrease rapidly.

After 48 hours, the number of TNFa and IL 10 have decreased in group 1 since IAI simulation. Moreover, the number of IL 10 has become less than initial indicators. The number of IL 6 has slightly changed, and the number of IL 2 has increased significantly. The number of IL 6 has increased significantly in group 2. The number of

Table 2: Changes in the amount of TNFa (pcg/ml) during the experiment (M±m).

No	Observation time	Group 1	Group 2
1	6 hours	480.511±180.213	297.717±192.852, p<0.01
2	12 hours	44.531±16.615, p1-2<0.01	22.776±7.230, p1-2<0.01
3	24 hours	59.964±16.983	33.040±25.521
4	48 hours	37.292±18.413, p1-2<0.01	33.237±23.071

Table 3: Changes in the amount of IL2 (pcg/ml) during the experiment (M±m).

No	Observation time	Group 1	Group 2
1	6 hours	361.082±39.312	711.334±82.409, p<0.01
2	12 hours	358.414±42.022	290.201±73.543, p1-2<0.01
3	24 hours	265.112±34.602	171.815±40.734, p<0.05
4	48 hours	379.741±36.404 p3-4<0.01	183.775±22.062, p<0.01, p3-4<0.05

Table 4: Changes in the amount of IL 6 (pcg/ml) during the experiment (M±m).

No	Observation time	Group 1	Group 2
1	6 hours	153.195±59.788	813.288±86.566, p<0.01
2	12 hours	41.455±2.164, p1-2<0.01	149.859±47.533, p<0.01, p1-2<0.01
3	24 hours	34.944±2.250	24.417±4.356, p<0.05, p2-3<0.01
4	48 hours	42.866±4.390	43.589±4.038, p3-4<0.05

Table 5: Changes in the amount of IL 10 (pcg/ml) during the experiment (M±m).

No	Observation time	Group 1	Group 2
1	6 hours	177.834±17.265	449.719±221.575, p<0.05
2	12 hours	68.833±16.441, p1-2<0.01	140.772±50.555, p<0.01, p1-2<0.01
3	24 hours	39.500±11.337	23.833±4.689, p<0.05, p2-3<0.05
4	48 hours	24.833±6.874	24.166±6.154

IL 6 has increased slightly, while the number of TNFa and IL 10 has remained almost unchanged.

Discussion

A significantly higher number of proinflammatory Ctk in the background of a less number of IL10 in animals with DM models is the reason for cytokine status disparity. These changes cause numerous consequences listed below. The development of systemic insulin resistance, chronic stress, hypercoagulation syndrome, impaired immune response, etc. [11, 17–21].

The increased content of Ctk, which has been found 6 hours after IAI modeling, is a well-known natural phenomenon [8, 9, 22, 23]. This is considered as the activation of mechanisms for regulating inflammation [11, 15]. Although the dynamics of individual indicators in groups of animals have differed.

The number of TNFa has got the top-most in group 1, which is consistent with the literature data [8, 9, 21]. The predominant increase in the amount of IL 2 and 6 among pro-inflammatory Ctk in group 2 indicates a predominant activation of other mechanisms of inflammatory regulation. A relatively smaller increase in the amount of TNFa may have happened due to due to inhibition of its synthesis under the IL 6 influence [15]. It should be noted that IL 6 is one of the main stimulators of stem cell proliferation and granulocytopoiesis [11, 15]. Therefore, the growth of its synthesis has been directed specifically at these mechanisms. A lack of non-specific cellular factors might be the reason.

It is noteworthy that one of the effects of TNFa is the activation of neutrophilic leukocyte deposit [11, 15]. In our opinion, its relatively low content may be one of the reasons for the suppression of the activity of non-specific immune cells as well as some diagnostic difficulties observed in patients with DM and IAI [24].

A significantly higher amount of anti-inflammatory IL 10 in group 2 is also worth paying attention. This may have been due to a simultaneous increase in the production of pro-inflammatory IL 2 and 6. In general, it shows differences in the functioning of immune mechanisms for regulating inflammation under the DM influence.

A significant decrease in the amount of TNFa and IL 6 in 12 hours in group 1 indicates inhibition of the response of non-specific cells. At the same time, an almost constant amount of IL 2, indicates the development of a specific immune response [11, 15]. Since it can stimulate the proliferation and function of different classes of lymphocytes. A number decrease of IL 10 indicates a consistent function of different protection links.

A rapid number reduction of all Ctk in group 2 indicates significant differences in the function of immune mechanisms. A simultaneous decrease in the amount of TNFa and IL 2 indicates a decrease in the stimulation of various parts of the immune system. The relatively high amount of IL 6, which was higher than in group 1, may indicate that the immune system plays a major role in regulating inflammation. It is possible though that this is a consequence of disorders of the immune system. This is confirmed by a disproportionately high amount of IL 10, which indicates activation of immunosuppression mechanisms [11, 15].

A decrease in the amount of IL 2, 6, 10 in group 1 in 24 hours indicates inhibition of the function of the producing cells of these Ctk. A simultaneous increase in the amount of TNFa indicates the activation of its producers.

TNFa is one of the main activators of non-specific cells [11, 15]. Given the permanent changes in microbial inducers of IAI during its development [23, 25], an increase in its number may indicate the activation of non-specific cells. They are assigned a primary role in curbing the spread of the inflammatory process [11, 15].

A decrease in the amount of IL 2, 6, 10 in group 2 against the background of a constant amount of TNFa indicates the functional inhibition of producers of these Ctk, which indicates suppression of the immune response. Let's focus separately on the meaning of low TNFa content. TNFa is a stimulator of procoagulant factor synthesis [11, 15]. It promotes blood clot growth on the endothelial surface and increases the number of adhesive receptors on its cells [11, 15]. Therefore, the inhibition of TNFa synthesis contributes to impaired hemocoagulation and the spread of IAI.

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A significant decrease in the amount of TNFa and IL 6 in 12 hours in group 1 indicates the response inhibition of non-specific cells. An increase in the amount of IL 2 indicates the activation of specific immune mechanisms [11, 15]. A decrease in the amount of IL 10 might have been associated with a decrease in the production of pro-inflammatory Ctk. Though a constant decrease in the amount of IL 10 could rather be regarded as a function violation of the mechanisms of the immune response regulation.

Minor changes in the amount of TNFa, IL 2 and 10 against the background of IAI progression in group 2 indicate the insufficient function of their producers. In our opinion, the insufficiency of peripheral immunocompetent cells was one of the reasons for the increase in the number of IL 6 aimed at activating the proliferation of inflammatory effector cells. According to the literature, such ratios of indicators indicate immune insufficiency due to immune paralysis [11, 15].

Later on, the study was not conducted, since most of the animals from group 2 died after 48 hours. Therefore, it became impossible to conduct a comparative analysis.

Conclusions

DM modeling causes a disparity in cytokine status. The reason is a higher number of pro-inflammatory Ctk in the background of a less number of IL 10.

Intact animals with IAI models have shown signs of cyclical changes in the activity of various mechanisms of inflammatory regulation within 48 hours. At the same time, in 48 hours, there have been signs of impaired function of the regulation mechanisms of the immune response.

There have been differences in the functioning of various mechanisms of inflammatory regulation in 6 hours in animals with IAI models with underlying DM. There have been signs of immunosuppression in 12 hours. These changes have been progressing later on and the signs of immune insufficiency have been shown in 48 hours.

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