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Der Pharmacia Lettre, 2016, 8 (5):184-188
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Peculiarities of pathogenesis of complications with account of immuno-physiological disorders in acute response to trauma

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ABSTRACT

*M*echanisms *associated with the* development *of systemic inflammation response syndrome (SIRS) are of great importance in the progression of multiple organ dysfunction syndrome, traumatic disease and progression of pyoinflammatory complications. This study has been shown that period of acute response to trauma corresponds to the period of shock and early postshock period (first two days), which is characterized by hypovolemia and perfusion deficiency. To restore homeostasis allelically-determined powerful energetic mechanisms of urgent adaptation (hypermetabolism), changing the character of carbohydrate, protein and fat metabolism, are involved. Above-noted mechanisms become exhausted that results in decompensation (fatal case) or at adequate treatment functional dominance switches on the mechanisms of long-term adaptation, ensuring restoration of sustainable living. The character of the* early and late complications *is predetermined by the degree of* immunogenic *and metabolic d*isturbances.

Key words: traumatic disease, systemic inflammation response syndrome, multiple organ dysfunction syndrome, pathogenesis.

INTRODUCTION

Nowadays traumatic disease is an important problem which is accompanied by high-incidence disabilities and mortality among the patients. So percent of traumatic diseases in the structure of complications of general traumatism ranges from 10.1 to 20.0%, but percent of mortality causes is 21.3% [1-4].

According to the data of national and foreign authors [3,4], at the present time incidence of traumatic disease is rising from 13.8% in 2005 year to 20.6% in 2011 year. Traumatic disease is accompanied by a multiple organ dysfunction syndrome (MODS).

Scientific researches conducted by the leading hospitals all over the world let us to come to conclusion that a trigger of MODS progression of any nature is a systemic inflammation response syndrome [5, 6].

In the present paper we attempted to estimate place of the abovementioned syndrome at the traumatic disease and its role under the realization of MODS.

MATERIALS AND METHODS

Studying of pathogenesis of trauma complications it is necessary to analyze in depth peculiarities of cellular and extracellular metabolic disorders.

Peculiarities of cellular and extracellular metabolic disorders in 26 patients of both sexes in the age range of 18 to 73 have been studied. According to the research tasks they were divided on two groups: 1group consists of 12 patients with multisystem thoracic injuries and 2 group - 14 patients with multisystem injuries of chest, organs of abdominal cavity and retroperitoneal.

Groups were strictly randomized (χ^2=0.901, p=1.000) on main clinical criteria, severity of condition and foreseeable mortality for group.

To compare dynamics of disorders occurring as a response on acute trauma (it corresponds to a period of wound shock and early postshock period (first two days) and is characterized by hypovolemia and perfusion deficiency) we pointed out two subgroups: 1 – APACHE II score up to 20 points (foreseeable mortality is up to 22%), 2 – 20 points and more (foreseeable mortality is up to 82%).

Factors of specific resistance have been studied: T-cell subset distribution, expression of CD11a receptors, interconnected with the functional state of granulocytic neutrophils and expression of CD16 receptors, mediated phagocytosis, was studied. Also we have studied expression of CD95 receptors, characterized potential ability to cause apoptosis, and CD162 receptors which mediate leukocyte movement across activated endothelium on the immunocompetent cells. Determination of T-cell subset distribution has been performed by indirect immunofluorescence staining technique using MAb monoclonal antibodies and FITC conjugated goat anti-mouse IgG antibodies. Counting of positive cells has been done using fluorescent microscope (set of specific monoclonal antibodies, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine). Nonspecific resistance was estimated by the changing in phagocytic parameters. Activity of neutrophils was determined by the absorbance and elimination of microbial bodies by the polymorphonucleocytes and monocytes of peripheral blood, able to bind on its surface, absorb and digest microbial test-culture. Yeast culture was used. Romanowsky-Giemsa staining has been carried out. Preparations were viewed under the microscope at 1000 × magnification in immersion system. 200 cells were counted and phagocytic indexes were determined.

Content of immunoglobulins class A, G and M in the blood serum has been determined by the spectrophotometric method using IFA -1.

Level of circulating immune complexes (CIC) was assayed by spectrophotometrically after incubation the samples in borate buffer and polyethyleneglycol at room temperature.

Complement was determined by the way of determination of tissue antibodies in the blood serum according to N.I. Kondrashova tecnique (1973).

Evaluation of plasma levels of IL- 2, 4, 6 and 8 has been done by the immunofluorescence assay (creator LLP «Protein contour», St. Petersburg, Russia).

Response to massive blood loss was estimated before the surgery: according to the plasma endothelin-l levels using IFA and standard commercial set (No. EIAH – 6901, PENINSULA LABORATORIES, INC., USA).

Antibodies to Lipopolysaccharide (LPS) *E. coli* of the group K30 classes G, M, A (anti-LPS-lg A,-lgM, -lgG) were defined. Commercial obtained LPS preparation made from biomass of gram-negative *Escherichia coli* of the group K30 (Serva) was used during the investigation. Blood plasma was obtained by a common method and stored at the temperature +4 ^{0}C. Antibodies to Lipopolysaccharide *E. Coli* of the group K30 classes G, M, A were determined by IFA. Absorbance of end product in enzymatic reaction was measured at 492 nm using the immunoassay analyzers AKI–C01. Dynamics of anti-LPS-lg A,-lgM, -lgG among the patients of main group was estimated before the surgery, in 24, 48 hours and in 10-14 days after surgery. Control indexes of anti-LPS-lg A,-lgM, -lgG antibodies content in the blood plasma of healthy donors (25) were taken during the examination in laboratory of immunology in «V.T. Zaytsev Institute of General and Emergency Surgery of NAMS of Ukraine».

Time frame for study: I period of traumatic disease is a period of acute response to trauma (up to 2 days).

RESULTS AND DISCUSSION

All functional disorders can influence on the strategy of human body's compensatory adaptive reactions. Sustainable disturbances of immunopathological reactions and changes in the indexes of nonspecific resistance can

develop in patients with traumatic shock on the background of blood loss and multiple organ dysfunction syndrome (MODS).

Clinical heterogeneity of disorders at traumatic shock depends on the different combinations of metabolic disturbances. It is associated with initial state of general resistance and with the character of organ failure, presence of virus and bacterial infection and type of immuno-physiological reaction (table. 1).

Conducted researches have been shown that reliable reduction in CD3 expression on average of 46.7% was observed in patients from subgroup 2. Reduction in CD4 expression was 41.8%. Along with this expression of CD8 suppressor (Ts) cells increased in subgroup 2 on average of 19.6%, however it were lower than reference values (p=0.088).

Table 1. Dynamics of changes in indexes of specific resistance in patients

Index	APACHE II score	Average value at the stages of study	p
CD3+, %, (61.4±4.3)	Up to 20 points	50.28±2.13*	0.053
	20 points and more	26.81±2.32*	
CD4+, %, (39.12±6.4)	Up to 20 points	26.84±3.47*	0.035
	20 points and more	15.63±4.34*	
CD8+, %, (18.1±5.4)	Up to 20 points	13.5±0.42*	0.404
	20 points and more	16.15±2.33	
CD4/CD8, (1.91±0.15)	Up to 20 points	1.75±0.11*	0.000
	20 points and more	0.89±0.11*	
CD11a+, %, (65.2±11.6)	Up to 20 points	51.9±2.05*	0.014
	20 points and more	42.6±1.8*	
CD11b+, %, (62.2±11.8)	Up to 20 points	52.5±3.76*	0.043
	20 points and more	48.15±7.14*	
CD19+, %, (12.4±5.1)	Up to 20 points	9.43±0.62*	0.014
	20 points and more	6.72±0.54*	
CD54+ (ICAM1), %, (19.23±6.22)	Up to 20 points	15.28±0.86*	0.031
	20 points and more	6.71±0.54*	
CD95+, %, (15.6±3.4)	Up to 20 points	9.93±2.41	0.017
	20 points and more	6.79±2.86*	
CD 162, %, (65.2±11.8)	Up to 20 points	54.95±2.48*	0.013
	20 points and more	37.8±1.98*	
	20 points and more	7.75±0.63*	

*Note: * - reliable with control, p<0.05*

Immunoregulatory index in second subgroup of patients was an average of 49.1% that is lower than in a subgroup 1. Besides, studied index was reliably lower than reference values in both subgroups (an average of 8.4% and 53.4%, respectively).

Expression of CD19 receptors (B-lymphocytes) reduced significantly as in relation to the reference values (an average of 24% and 45.8% in subgroup 1 and 2, respectively) and in the subgroup 2 studied index was an average of 28.7% lower than the same index in subgroup 1.

CD95 proapoptotic receptor expression was an average of 31.6% lower than indexes in subgroup 1, but studied index was also significantly lower than reference values (an average of 36.3% and 54.5%, respectively).

Expression level of adhesion molecule (CD54) was an average of 20.5% in subgroup 1 and on 65.1% lower than reference values. Differences between the groups of patients were significantly: in the subgroup 2 adhesion molecule (CD54) expression was an average of 56% lower than in the subgroup 1.

CD11a integrin expression on the neutrophils in the subgroup 1 was 51.9±2.05%, and 42.6±1.8% in subgroup 2, that differ significantly from indexes in control group (65.2±11.6%). Expression of CB11b reduced to 52.5±3.76% that is statistically lower than reference values (62.2±11.8%) an average of 15.6%.

Moreover, patients of subgroup 2 had a more significant reduction of studied index compared to control (an average of 22.6%) and subgroup 1 (an average of 8.3%). Decreased reception of integrins can imply about the reducing of neutrophil phagocytic activity in patients. In addition, it also indicates the decreasing of adhesive properties of endothelial cells that can be a result of endothelial dysfunction as well as endothelial death.

Thus, during the study of neutrophil phagocytic activity the following abnormalities were detected: phagocytic index in patients from subgroup 2 was on average 12.1% less than in patients from subgroup 1; phagocytic number was on average

30% lower than in patients from subgroup 1; index of phagocytosis completeness was on average 29.4% lower than reference values.

It is known that complement system takes part in the progression of acute inflammatory response; fragments of complement interact with polymorphonuclear neutrophils raising the expression of adhesion molecules ICAM-1. Research of this index has also shown that complement activity in patients from subgroup 2 was on average 12.1% less than in a subgroup 1.

That way, conducted researches have shown that already in the first period of traumatic disease patients have combined structure–functional cell and humoral immune deficiency. This can be confirmed by lymphopenia, decreasing in the number of CD3 T- lymphocytes and CD 19 B–lymphocytes and also by the abnormality of neutrophil phagocytic activity.

Decreased expression levels of CD95, CD54 show reduction of functional activity of lymphocytes. All this in conjunction with the lymphopenia can be evidence of intensifying proapoptotic effects on the cells of immunocompetent system and its death maybe through apoptosis.

We think that this problem should be considered within a theory of general adaptation syndrome. From this perspective severe injury is a sign of initial disadaptation or breakdown of compensatory-adaptive mechanisms. To avoid excessive signs of systemic inflammation next to the SIRS negative control mechanisms mediated by the production of anti-inflammatory cytokines and soluble inhibitors of proinflammatory cytokines are involved.

In the treatment of patients with severe injury it is essential to take into account pathophysyiological changes, particularly, abnormality of immune regulation key role of which is a secretion of cytokines. The table 2 shows dynamics of changes in the indexes of some cytokines in patients depending on the severity of the state on APACHE II score.

Table 2. Dynamics of changes in the indexes of some cytokines in patients depending on the severity of the state on APACHE II score

Index	APACHE II score	Average value at the stages of study	p
IL- 6, pg/ml, (2.13±0.73)	Up to 20 points	50.4±10.47*	0.006
	20 points and more	86.62±10.91*	
IL- 8, pg/ml, (52.9±12.35)	Up to 20 points	119.6±7.14*	0.037
	20 points and more	160.4±3.8*	
IL- 2, pg/ml, (16.4±2.6)	Up to 20 points	133.4±9.83*	0.007
	20 points and more	273.1±7.57*	
IL- 4, pg/ml, (24.8±9.7)	Up to 20 points	132±5.2*	0.031
	20 points and more	20.3±2.55*	

*Note: * - reliable with control, p<0.05*

As it can be seen from the table 2 middle level of IL-6 in patients with a number of points up to 20 is on average of 26.2% higher than in patients from control group while for patients with a 20 points and more this level is on average of 39.7% higher than in a control group.

Along with this level of studied index in patients from subgroup 2 was on average of 71.9% higher than average values of IL-6 in subgroup 1 (p=0.006). It has been established that IL-6 has many effects, including promotion of growth and differentiation of T- and B-lymphocytes. It stimulates the production of acute-phase proteins by hepatocytes and is an endogenous pyrogen.

The study of changes in IL-8 in the patients' serum has shown that its increase also depends on the severity of the state: in the subgroup 2 (20 points and more on APACHE II score) its average values were on 34.1% higher than indexes in subgroup 1 (up to 20 points on APACHE II score).

Multiple elevation of average values of IL-8 in all patients gives evidence of possible disorder in hepatic cytokine clearance, namely IL-8, increasing of which occurs after the increasing of type-1 cytokine (TNFα, IL 1 etc.) production.

Multiple elevation of serum IL-2 levels gives evidence of formation of T-cell tolerance to specific mediator exposures. Wherein average level of IL-4 in patients from subgroup 2 (20 points and more on APACHE II score) was on average of 84.6% less than indexes in subgroup 1 (up to 20 points on APACHE II score).

Most died patients had IL-4 level near 0 pkg/ml.

As is shown in conducted researches average level of anti-LPS-Ig of all classes was greater than control indexes in acute period of traumatic disease. It depended on the severity of the patients' state (table 3). And along with this an average total immunoglobulines IgA, –M и –G indexes in patients in all subgroups were decreased, that indicated on abnormalities of patients' nonspecific resistance in acute period of traumatic disease.

Decreasing concentration of anti-LPS-Ig of all classes was observed in the postoperative period in patients with fatal case with every successive study before death occurs: anti–LPS–IgA: r_{Sp} was 0.905, p=0.004; anti–LPS–IgM: r_{Sp} was 0.893, p=0.007; anti–LPS–IgG: r_{Sp} was 1.000, p=0.000.

This is due to the intensive translocation of microorganisms and massive endotoxin entering in blood flow which bound with existing in blood antiendotoxic antibodies.

Table 3. Dynamics of changes of average concentrations of anti–LPS–IgA, –IgM and –IgG in patients in acute period of traumatic disease

Index	APACHE II score	Average value at the stages of study	p
anti–LPS–Ig A, 0.042± 0.001	Up to 20 points	0.129±0.01*	0.031
	20 points and more	0.160±0.01*	
anti–LPS–Ig M, 0.008±0.001	Up to 20 points	0.042±0.02*	0.054
	20 points and more	0.073±0.012*	
anti–LPS–Ig G, 0.006±0.001	Up to 20 points	0.105±0.01*	0.023
	20 points and more	0.133±0.01*	
Ig A, 2.23 ± 0.29	Up to 20 points	1.1 ± 0.11*	0.133
	20 points and more	0.94 ± 0.06*	
Ig M, 0.84 ± 0.01	Up to 20 points	0.6 ± 0.04*	0.053
	20 points and more	0.48 ± 0.02*	
Ig G, 14.9 ± 1.8	Up to 20 points	10.2 ± 0.57*	0.045
	20 points and more	7.4± 0.28*	

*Note: * - reliable with control, p<0.05*

CONCLUSION

Thus, conducted researches have shown that period of acute response to trauma corresponds to the period of shock and early postshock period (first two days), which is characterized by hypovolemia and perfusion deficiency. To restore homeostasis allelically-determined powerful energetic mechanisms of urgent adaptation (hypermetabolism), changing the character of carbohydrate, protein and fat metabolism are involved.

Above-noted mechanisms become exhausted that results in decompensation (fatal case) or at adequate treatment functional dominance switches on the mechanisms of long-term adaptation, ensuring restoration of sustainable living. Nature of the early and late complications is predetermined by the degree of disturbances of immunogenesis and metabolism.

Occurring most apparent dyscrasias are accompanied by disorders of vascular tone, disturbances of microcirculation, changes in cellular and humoral immunity, inhibition of macrophage chemotaxis and reducing of their phagocytic activity, expulsion of humoral factors activating SIRS. This period lasts from 12 to 48 hours and it should be considered as a period of perfusion deficiency and induction phase of SIRS with a formation of MODS.

In this regard character of metabolic processes and usage of proteins, fats and carbohydrates for energetic purposes changes significantly with the development of hypermetabolic intoxication.

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