SPECIFIC CHANGES OF CYTOKINE STATUS TROUGHOUT THE PROCESSING OF ACUTE NECROTIC PANCREATITIS IN EXPERIMENT

Vitaliy Maksymyuk – PhD, ass. prof.
Igor Polianskiy, MD, PhD, Professor – Chief of Department of Surgery
Volodimir Tarabanchuk – PhD, ass. prof.
Fedir Hrynchuk – MD, PhD, Professor
Igor Davydenko – MD, PhD, Professor
Department of Surgery, Bukovinian State Medical University, Chernivtsi, Ukraine
Email: maxsurg@mail.ru

Summary. Specific changes of cytokine status troughout the processing of acute necrotic pancreatitis in experiment

Using white rats with acute necrotizing pancreatitis and our own experimental model, we studied the changes in TNF-α, IL-2, IL-6 and IL-10 in blood plasma. This proves, that progressing acute necrotizing pancreatitis is characterized by the development of a heavy imbalance of cytokine status with pronounced tendency of anti-inflammatory mediators to be dominate in inflammation. This contributes to profound immune depression, generalized systemic infectious factors and the development of purulent-necrotizing complications; together, they contribute to death in late stages of the septic process. This justifies the necessity to develop adequate methods of anti-inflammatory therapy at various stages of acute necrotic pancreatitis.

Key words: acute pancreatitis, cytokines, TNF-α, IL-2, IL-6, IL-10

Rezumat. Modificările specifice a statutului citokinelor în evoluția necrozei pancreatice în condiții experimentale

În condiții experimentale pe şobolani albi au fost analizate modificările concentrației plasmatice a TNF-α, IL-2, IL-6 and IL-10. În cadrul studiului a fost demonstrat că evoluția necrozei pancreatice acute este caracterizată de imbalanța severă a statutului citokinelor cu tendință marcată de predominare a mediatorilor anti-inflamatorii. Acest fenomen contribuie la o „depresie" imună, generalizarea factorilor infecțioşi sistemici şi dezvoltarea complicațiilor purulent-necrotice; iar în complex acestea induc tanatogeneza în fazele tardive ale procesului septic. Cele menționate argumentează necesitatea de identificare a metodelor adecvate de tratament anti-inflamator la diferite faze evolutive ale pancreonecrozei acute.

Cuvinte-cheie: pancreatita acută, citokine, TNF-α, IL-2, IL-6, IL-10.

Резюме. Особенности изменений цитокинового статуса в процессе развития острого некротического панкреатита в эксперименте

Используя собственную экспериментальную модель острого некротического панкреатита на белых крысах изучены изменения TNF-α, IL-2, IL-6 и IL-10 в плазме крови. Установлено, что прогрессирование острого некротического панкреатита характеризуется развитием тяжелого дисбаланса цитокинового статуса с выраженной тенденцией к доминированию противовоспалительных медиаторов воспаления. Это способствует возникновению глубокой иммунологической депрессии, системной генерализации инфекционных факторов и развития гнойно-некротических осложнений, что в совокупности является основной причиной смерти на поздних стадиях септического процесса. Изложенное обосновывает необходимость разработки адекватных методов антимедиаторной терапии на разных стадиях развития острого некротического панкреатита.

Ключевые слова: острый панкреатит, цитокины, TNF-α, IL-2, IL-6, IL-10

Introduction

An important role in the pathogenesis of acute destructive pancreatitis is the initiation of the cytokine cascade. The most significant cytokines in this cascade are IL-1, IL-2, IL-6, IL-8, IL-10, TNF-α [1-8]; after the activation of pancreatic enzymes and mediators of the kallikrein-kinin system, which include aggressive factors of the third order which play an important role in the pathogenesis of local and systemic inflammatory reactions [2, 4, 7, 8]. However, despite the large number of studies, many questions exist about the interactions of cytokines and their affect on different stages of acute pancreatitis. Therefore, further thorough investigation is warranted.

Material and methods

Experimental research was conducted on 50 white rats. The experimental model of pancreatitis was created by the method of ligation of the hepato-pancreatic duct with injection of medical bile and trypsin into pancreatic tissue where by inducing pancreatitis (Patent № 66667 UA). Blood was collected before the model was induced and then was collected on the 1st, 3rd, 5th, and 7th days thereafter.

We determined the level of TNF-α, IL-2, IL-6 and IL-10 in blood plasma by enzymatic immunoassay analyzer "АИФР-01 «Униплан» (Russia)" using chemicals from the company "Biosuorsce" (Belgium).

When doing research we followed the generally accepted norms of international and domestic guidelines for biology and medicine, namely: Universal Helsinki declaration of human rights, Vancouver convention of biomedical research (1979, 1994) and other legislation which are used on the Ukrainian territory. Statistical dependence between variable, were assessed by criteria of Student.

Results

Our research conducted that after 24 hours since initialing of acute pancreatitis it was noted parallel growth in levels of TNF-α by 3,7 times ($p<0,05$), IL-6 – by 17,0 time ($p<0,001$), IL-10 – by 1,9 time ($p<0,01$). However the level of IL-2 had no substantial change (**Fig. 1-4**).

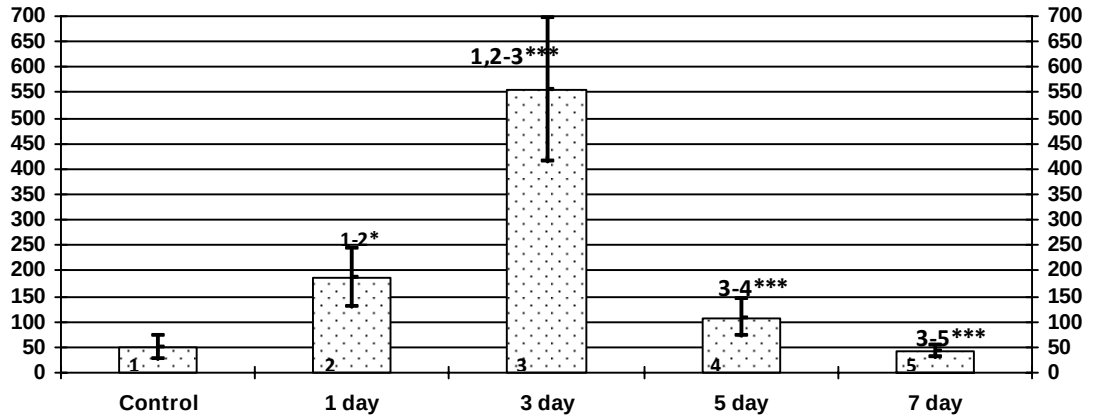

Fig. 1. *Dynamics the level of TNF-α (pg/ml) in blood plasma of experimental animals in the development of acute destructive pancreatitis*

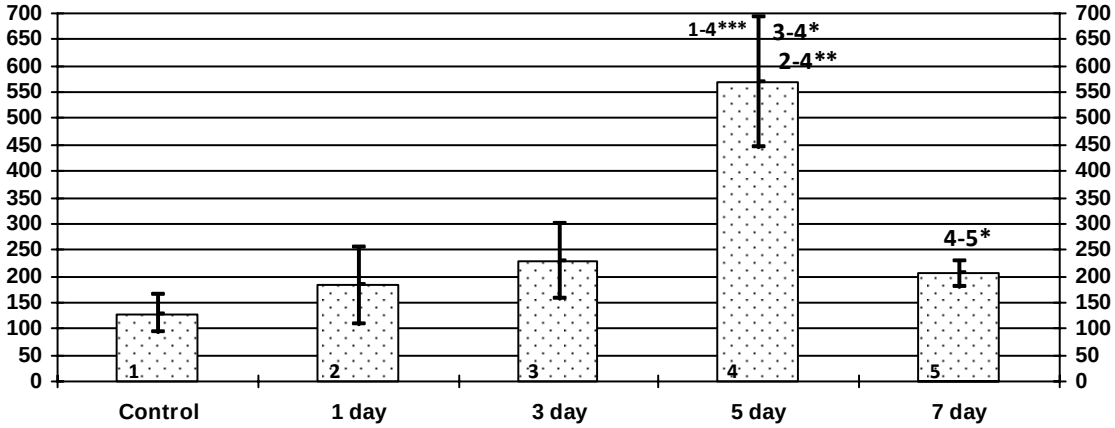

Fig. 2. *Dynamics the level of IL – 2 (pg/ml) in blood plasma of experimental animals in the development of acute destructive pancreatitis*

Note: *- probability ratio P between indicated observation period < 0,05; **- < 0,01; ***- < 0,001.

From the 1st to the 3rd day the development of acute necrotic pancreatitis the experimental level TNF-α continued to increase, exceeding the parameters by 11,1 times ($p<0,001$). Initially in the first 3 days the concentration of IL-6 substantially decreased however after the 3rd day levels rose by a factor of 6,3 times ($p<0,05$). Levels of IL-2 and IL-10 did not change in the first 3 days but IL-10 rose by 2,7 times after the 3rd day ($p<0,05$) (**Fig. 1-4**).

From the 3rd to the 5th day there was a sharp decrease in the level of TNF-α by 5,2 times ($p<0,001$) and IL-6 – by 3,6 times ($p<0,05$). Against a background of significant growth of IL-2 – 4,4 times ($p<0,001$) and IL-10 by 9,3 times ($p<0,001$) accordingly (**Fig. 1-4**). With this we can observe a balance correlation between the anti-inflammatory and inflammatory cytokines (**Fig. 5**).

From the 5th to the 7th day the level of IL-2 decreases by 2,8 times ($p<0,05$), but the concentration of TNF-α and IL-6 decreased till initial levels (**Fig. 1-3**). However, the level of IL-10 substantially grew reaching to a level by 15,7 times greater ($p<0,001$) (**Fig. 4**). Therefore on the 7th day the balance correlation of cytokines shifted back to dominance of anti-inflammatory mediators in inflammation (**Fig. 5**).

Discussion

The obtained results show that in the process of development of acute necrotic pancreatitis balance correlation of cytokines shifted to the dominance side of anti-inflammatory mediator inflammation. This indicates that in prolonged development of systemic inflammatory process there is an excessive secretion of cytokines. Therefore the following conditions call for a decrease in the functional activity of immune-competent cells. Namely in this situation not only is the uncontrolled hyperproduction of anti-inflammatory cytokines, but absolute disregulation of systemic inflammatory reaction. This facts are corresponding with a dates of international literature [2, 3, 5] and

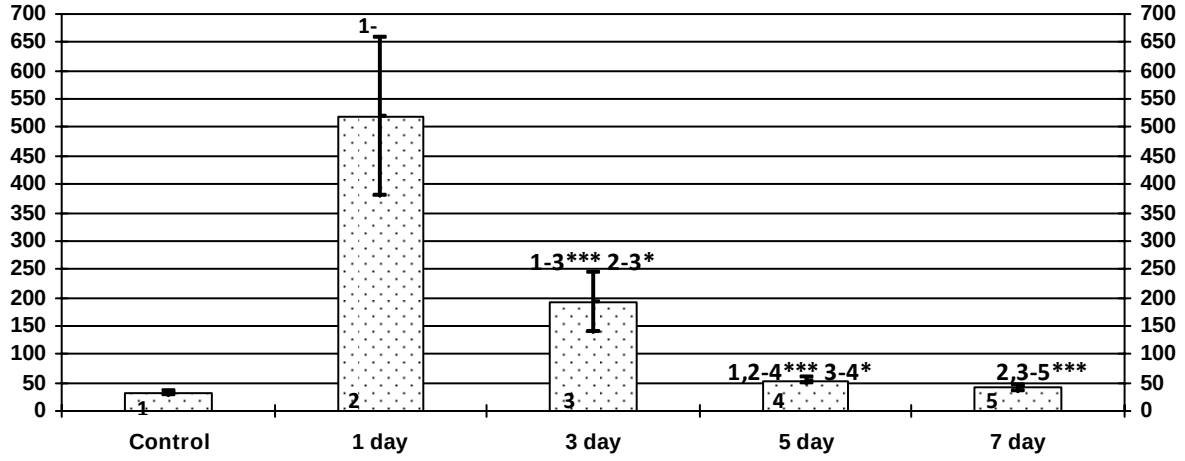

Fig. 3. *Dynamics the level of IL–6 (pg/ml) in blood plasma of experimental animals in the development of acute destructive pancreatitis*

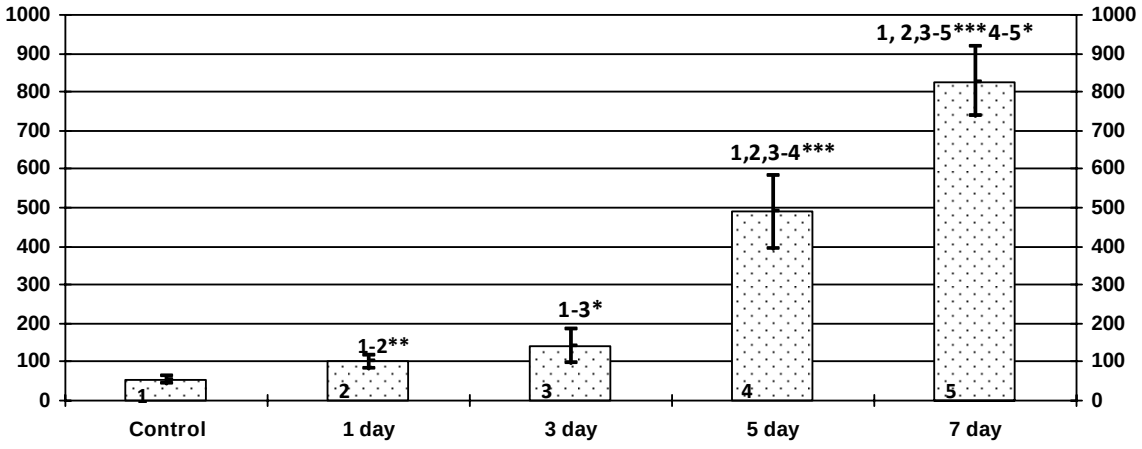

Fig. 4. *Dynamics the level of IL–10 (pg/ml) in blood plasma of experimental animals in the development of acute destructive pancreatitis*

Note: *- probability ratio P between indicated observation period < 0,05; **- < 0,01; ***- < 0,001.

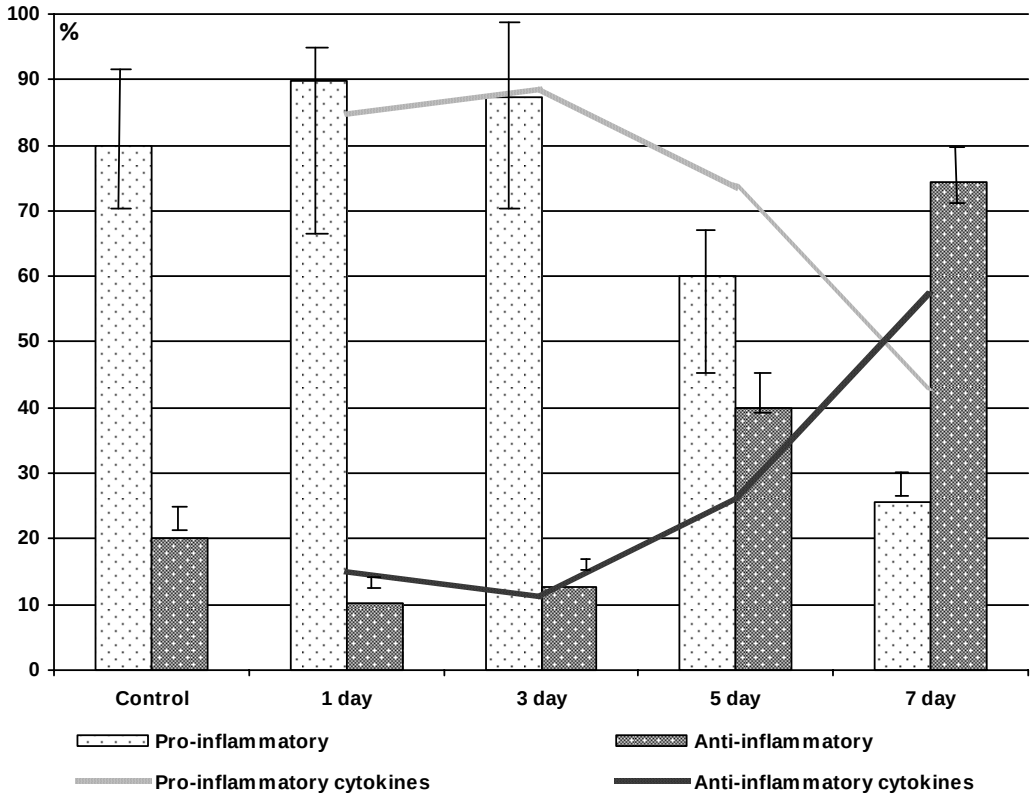

Fig. 5. *Ratio equity levels (%) pro- and anti-inflammatory cytokines in the development of acute destructive pancreatitis*

can characterized as "mediator chaos" [7, 8]. It is obvious that the occurrence of such "immunological paralysis" is accompanied by the development of deep immunosupression and further deepening of the manifestation of systemic inflammation [1, 5, 7]. This in turn facilitates the task of generalizing infections factors the formation of purulent necrotic complications and the development of multiple organ failure, which together determine the fatal outcome in the later stages of sepsis [1, 7, 8].

Conclusions

Summarizing the research result we can conclude that cytokine cascade plays a significant role in the pathogenesis of acute necrotic pancreatitis, which includes inflammatory cytokines from one side and anti-inflammatory mediators from the other. Characteristically such "immunological swings" having combined action with acute destructive pancreatitis create a severe immundepressive state. The latter promotes systemic generalized infections factors and formation of purulent necrotic complications. A set of pathological disorders identified fatal outcomes of the disease at different stages of sepsis. This justifies the need to develop adequate methods of anti-mediator therapy for different stages of acute necrotizing pancreatitis.

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