Alterations in innate antibacterial response after immunomodulatory nutrition

Zmiany wrodzonej odpowiedzi przeciwbakteryjnej po żywieniu immunomodulacyjnym

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Słowa kluczowe: wrodzona odporność, żywienie immunomodulacyjne, receptory Toll-podobne.

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Abstract

Septic infections in malnourished surgical patients show the highest morbidity and mortality rate. The attempt to correct postoperative immune and nutritional disorders by introducing immune-enhancing nutrition (immunonutrition) is a promising way of improving outcome, but as yet little is known about the mechanisms of correcting an extensive postoperative inflammatory response (systemic inflammatory response syndrome [SIRS]) to a massive infection using this type of nutrition. A significant role in the innate antibacterial and inflammatory response is played by Toll-like receptors (TLRs) that recognize pathogen-associated molecular patterns (PAMPs). The regulatory impact of immunonutrition on TLR expression in surgical septic patients seems to be a new research direction. In this paper special emphasis was put on clinical trials and the research results for the TLR-dependent immune response and anti-bacterial/anti-inflammatory response applying immunomodulatory nutrition with increased concentrations of glutamine and unsaturated fatty acids.

Streszczenie

Zakażenia septyczne w grupie niedożywionych pacjentów chirurgicznych obarczone są najwyższym wskaźnikiem chorobowości i śmiertelności. Korekcja pooperacyjnych zaburzeń odporności i niedożywienia poprzez żywienie immunomodulujące jest obiecującą metodą uzyskania lepszych wyników leczenia, ale nadal niewiele wiadomo o mechanizmach regulacji zwiększonej pooperacyjnej odpowiedzi zapalnej (*systemic inflammatory response syndrome* – SIRS) na masywne zakażenie za pomocą tego typu terapii. Istotne znaczenie w regulacji wrodzonej odpowiedzi przeciwbakteryjnej i przeciwzapalnej mają receptory TLRs (*Toll-like receptors*), rozpoznające związane z patogenami wzory molekularne (*pathogen-associated molecular patterns* – PAMPs). Ocena regulacyjnego wpływu żywienia immunomodulacyjnego na ekspresję receptorów TLR u septycznych pacjentów chirurgicznych jest nowym kierunkiem badań naukowych. W przedstawionej pracy szczególną uwagę zwrócono na wyniki badań klinicznych i eksperymentalnych dotyczące regulacji zależnej od TLRs odpowiedzi przeciwbakteryjnej/przeciwzapalnej za pomocą żywienia immunomodulacyjnego, zawierającego zwiększone stężenia glutaminy i nienasyconych kwasów tłuszczowych.

Introduction

Despite advances in treatment methods, there is still no therapy available to efficiently reduce the excessive inflammatory response, which can increase the risk of multiple organ failure in patients treated in intensive care units (ICUs). One of the ways to discover new, more efficient treatment methods involves regulating the mechanisms of inflammatory response to a massive infection by using immune-enhancing nutrition (immunonutrition). The results of a meta-analysis of randomized studies have shown that in a group of seriously ill patients (systemic inflammatory response syndrome [SIRS], acute respiratory distress syndrome [ARDS]) immunomodulatory treatment with glutamine and fatty acids improves outcomes, which has not been con-

firmed after the enteral administration of arginine [1]. Another meta-analysis covering the perioperative results of immunonutrition aimed at enhancing immunity in patients operated on for tumors of the digestive tract has shown a decrease in the number of postoperative complications, reducing the length of hospital stay and improving selected immune parameters such as an increase in the total number of lymphocytes, subpopulation of CD4 lymphocytes, and concentration of IgG, and a decrease in the concentration of IL-6 [2]. Compared with the standard immunonutrition, the enteral administration of glutamine, arginine and/or fatty acids (n-3 PUFAs: n-3 polyunsaturated fatty acids) in patients with acute pancreatitis shows no significant impact on decrease in the number of complications, the length of hospital stay or mortality rate [3]. Considering another review of study results the advantageous impact of enteral and parenteral administration of immunonutrition in patients with acute pancreatitis can be enhanced by administering inflammatory and immune response modulators [4]. In the group of patients after extensive surgical trauma, adding glutamine has always resulted in a decrease of post-surgical complications, reduction in the length of hospital stay and even a reduction of the mortality rate in seriously ill patients [5]. Multi-center studies have shown that the administration of high doses of glutamine associated with antioxidants in seriously ill patients hospitalized in ICUs results in a significant increase in morbidity and mortality [6]. The majority of studies that have been carried out to date show that n-3 PUFAs have a significant regulative impact on the immune response and outcomes in patients after surgery with serious infections including ARDS [7, 8]. The enteral administration of unsaturated fatty acids in septic patients is still controversial due to the lack of prospective randomized studies. We still know very little about the mechanism of action of n-3 PUFAs which is among other things supposed to reduce the negative impact of n-6 PUFAs on the immune system (inflammatory response reduction). Considering the summary of the recent meta-analysis covering the outcomes of 3013 patients treated mainly in ICUs, immunomodulating nutrition containing fish oils significantly improved the outcomes for those patients [9]. The authors of the meta-analysis emphasize that arginine-enriched diets with no glutamine or fish oil added do not improve outcomes as compared with standard immunonutrition.

The promising results of experimental studies on treating severe infections with lipopolysaccharide (LPS) inhibitors, tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), platelet activating factor (PAF), nitric oxide (NO), arachidonic acid metabolites, complement component inhibitors or free radicals did not considerably reduce the highest mortality rate in septic patients [10]. Other interesting strategies for the treatment of sepsis based on attempts to block LPS-binding receptors and on blocking signaling pathway proteins for the antibacterial response, e.g. blockade of Toll-like receptor (TLR)4, caspases, FasL-Fas or NF-κB activity, and blocking the high mobility group box 1 (HMGB1) pathway as well as on attempting to regulate neutrophil and lymphocyte apoptosis (e.g. by overexpression of anti-apoptotic proteins such as Bcl-2) are still under experimental research [10-14]. It is well known that neutrophils and monocytes/macrophages taking part in the innate immune response to trauma and infection play a significant role in the elimination of microorganisms as well as in local and systemic inflammatory response regulation (SIRS: systemic inflammatory response syndrome) that increases the risk of multiple organ failure [15]. It is suggested that the modulation of TLR expression found in the cells of intestinal mucous membrane, neutrophils, monocytes and dendritic cells (DCs) binding bacterial antigens and the modulation of expression of signaling pathway proteins of those cells by early administration of appropriate immunonutrition can help efficiently eliminate microorganisms and reduce the inflammatory response (production of cytokines, chemokines). These issues make us ask the basic and still open question: Can the re-programming of signal transduction pathways in intestinal mucosa and innate immunity cells of septic patients after administering immunonutrition contribute to attenuation of the local and systemic hyperinflammatory response in massive bacterial load?

TLR signaling and immunonutrition

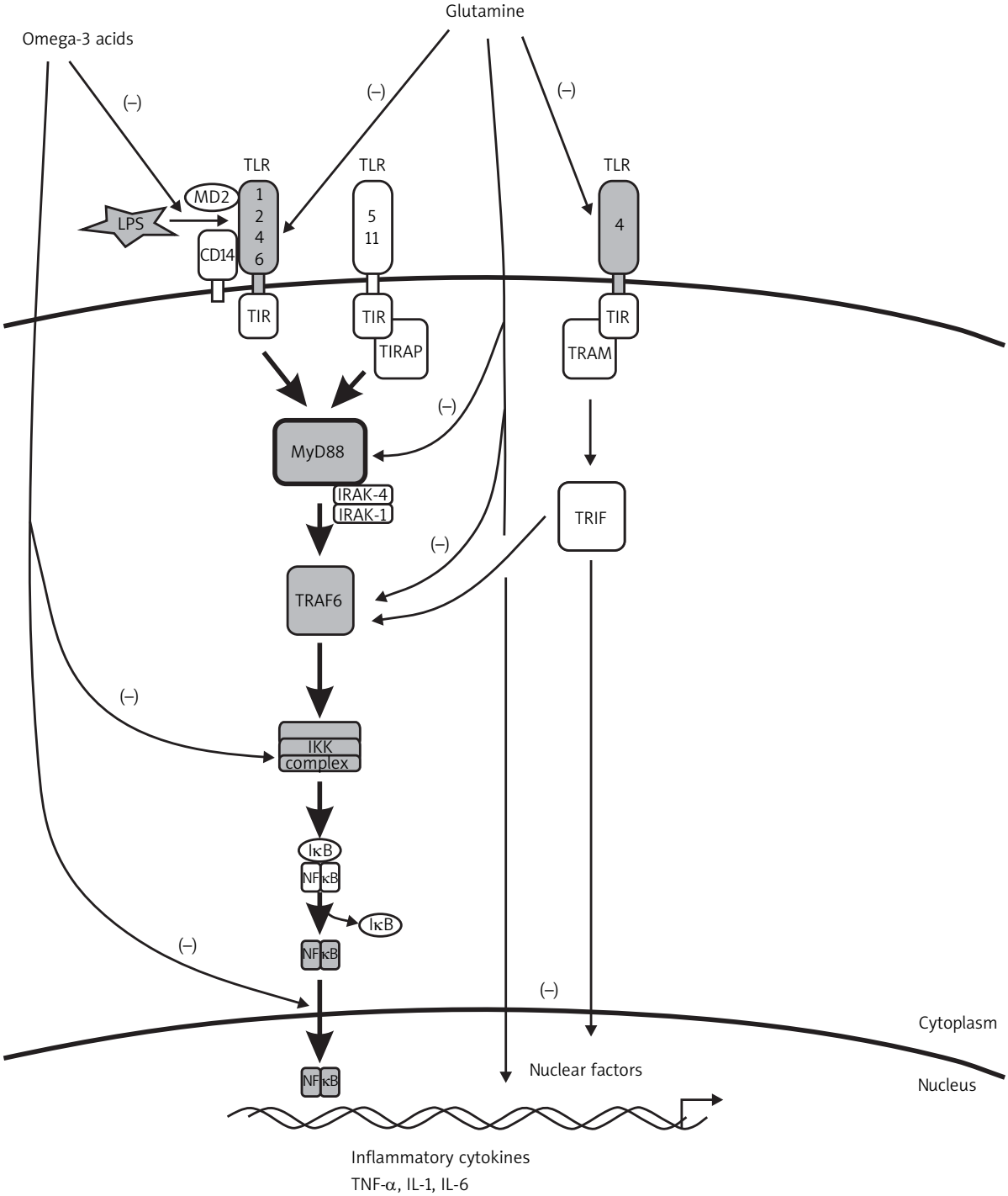
TLR-dependent anti-bacterial response

TLRs recognize a few highly conservative structures existing in prevailing microorganisms. Thirteen TLRs have been identified (including 11 in humans). Additionally, it was indicated that they showed mainly affinity to bacteria (TLR1, 2, 4, 5, 6). Some of them (TLR3, 7, 8, 9) also show some affinity to viral RNA and DNA. The prevailing TLRs show expression on the surface of cells (TLR1, 2, 4, 5, 6), but some of them can also be active inside cells and occur on the surface of endosomes (TLR3, 7, 8, 9) [16, 17]. However, it is still unknown how ligands for those receptors (e.g. exogenous ligands: LPS, peptidoglycan, bacterial DNA) penetrate into the cells. The endogenous ligands for TLRs include mainly some heat shock proteins (HSP60, HSP70) released from injured cells.

The production of the LPS receptor complex (CD14, TLR4, MD-2) induces dimerization of TLR4 and initiates a signaling cascade which results in activation of phos-

phorylation kinases of NF-κB (IκB) transcription factor inhibitor. This process induces transcription of genes whose products take part in the inflammatory response (e.g. cytokines TNF-α, IL-1, IL-6, IL-12). Apart from the role of extracellular TLRs in the signaling cascade of the antibacterial response, there are also such "participants" as a cytoplasmic part referred to as the TIR domain (Toll-IL-1 receptor), adaptor protein MyD88 (myeloid differentiation factor 88), TIRAP (TIR-domain-containing adaptor protein) and TRAM (TRIF-related adaptor molecule), the protein TRIF (Toll/IL-1-receptor domain-containing adaptor inducing interferon), TRAF6 (TNF-α receptor-associated factor 6), kinases (IRAK1, IRAK4 – IL-1 receptor-associated kinases; TAK – transforming growth factor β (TGF-β)-activated kinases) and a kinase complex (IKK– inhibitory κB kinase complex) [18-20]. The MyD88, TRAF6, TRIF and TRAM proteins are key proteins of signaling pathways initiated by TLRs, which can be of significant importance in treating sepsis [21, 22]. This process results in the activation of NF-κB and cytokine promoter genes. The production of inflammatory cytokines begins as soon as 60 min after the activation of macrophages by TLR4-binding LPS. In the case of MyD88-dependent transduction of the signal from TLR4 and TLR2, the TRAF6 protein plays a significant role, while in the case of the MyD88-independent pathway the main participants include TRAM and TRIF proteins (Figure 1). Attempts to block the signaling cascade in patients with severe infections attract the interest of many scientists. Especially interesting may be the use of negative regulation of signaling pathways associated with TLRs (e.g. RP 105/CD180 protein: TLR homolog, single immunoglobulin IL-1-receptor-related protein, signaling TIR family ligand, Toll-interacting protein; or Smad6), whose mechanism consists in suppressing the location of the bond between the ligand (LPS) and the receptor, proteolysis of TLR, blocking intracellular receptors binding antibacterial antigens, blocking the activity of kinases (suppression of phosphorylation process) and suppressing the activation of NF-κB [23-28]. Numerous experimental studies indicate the possibility of conduction of signals from TLRs to the inside of cells after connecting with LPS, which may prevent the activation of NF-κB. Mice deprived of signaling pathway proteins associated with TLR4 (TIRAP, MyD88, TRIF) do not respond to LPS and are resistant to septic shock [29-32]. On the other hand, mice deprived of TLR2 and the MyD88 adaptor protein are more susceptible to *Staphylococcus aureus* infections [33]. This indicates a protective action of some TLRs and adaptor proteins. In mice deprived of MyD88 protein, the systemic inflammatory response to LPS was significantly reduced but still present, which suggests cooperation of other routes of signal conduction in the antibacterial response [34]. The above-mentioned fact at least partly shows that the effects of treatment are insufficient due to the blockage of signal conduction routes.

TLRs expressed in mucosal cells and in the cells that take part in the innate response to infections play a significant role in antibacterial response modulation in patients with severe infections. The TLRs recognize the structural components of microorganisms and occur both inside and outside cells (intra- and extracellular TLRs). Some studies show that trauma reduces, whereas a severe infection increases the expression of TLRs recognizing bacterial antigens (e.g. LPS, peptidoglycan) [35-38]. Compared with healthy people, the expression of TLR4 in the monocytes of trauma patients was reduced [36]. Therefore, it is tempting to postulate that increased expression of TLRs would have a beneficial effect in trauma patients. Polymorphism of TLRs was one of the factors that increased the susceptibility to infections caused by particular bacteria, but in patients with mixed severe infections did not show any significant effect on their course and outcome [39]. In some experimental studies a lack of TLRs increased the susceptibility to infections in mice [40] and caused disorders in inflammatory mediator secretion as well as disorders in phagocytosis and antigen presentation [41-43]. Intraperitoneal administration of living *Escherichia coli* bacteria or LPS TLR4 had a significant impact on the phagocytic activity of peritoneal macrophages. Lack of TLR2 and TLR4 in macrophages caused disorders in bacterial phagocytosis (*E. coli*, *Staphylococcus aureus*). The experimental findings suggest that TLR4 plays a key role in regulating the expression of inflammatory cytokines in the lung during endotoxic shock [44]. Six hours of LPS administration induced a significant increase in pulmonary TNF-α, IL-1β and IL-6 mRNA in control ($TLR4^+$) mice compared to TLR4-deficient mice [44]. Recent studies showed that apoptotic macrophages can have a protective action in septic shock caused by LPS administration in mice [45]. This study for the first time demonstrated that the administration of apoptotic cells could protect mice against LPS-induced death, even when the apoptotic cells were administered 24 h after the LPS challenge. The beneficial effects of administration of apoptotic cells included the reduced circulation of pro-inflammatory cytokines (TNF-α), enhancement of IL-10 expression by LPS-activated macrophages, suppression of neutrophil infiltration in target organs, and decreased serum LPS levels. The apoptotic cells were an LPS carrier, which facilitated its phagocytosis and LPS elimination. This study showed that not TLR4, but CD14 is one of the receptors for LPS-coated apoptotic cell



TLR – Toll-like receptor, TIR – Toll-IL-1 receptor, TIRAP – TIR-domain-containing adapter protein, TRAM – TRIF-related adapter molecule, TRIF – Toll/IL-1-receptor domain-containing adapter inducing IFN, MyD88 – myeloid differentiation factor 88, TRAF6 – TNF-α receptor-associated factor 6, IKK – inhibitory κB kinases complex, NF-κB – nuclear factour κB, TNF-α – tumor necrosis factor-α, IL-1 – interleukin 1, IL-6 – interleukin 6, LPS – lipopolysaccharide

Fig. 1. Schematic diagram of TLR4, MyD88 and TRAF6 down-regulation in rat intestinal mucosa following glutamine administration and LPS-induced endotoxemia. N-3 PUFA inhibition of TLR signaling pathway at the extracellular (DHA interference with TLR4 receptor) and intracellular level: inhibition of the phosphorylation and degradation of IκB, inhibition of NF-κB activation and inflammatory cytokine production in LPS-stimulated human leukocytes and macrophages

Ryc. 1. *Schemat hamowania kaskady sygnałowej TLR4, MyD88 i TRAF6 w błonie śluzowej jelita po podaniu glutaminy u szczurów z zakażeniem wywołanym przez LPS. Zależne od kwasu tłuszczowego (n-3 PUFA) zewnątrzkomórkowe hamowanie kaskady sygnałowej TLR (przez wpływ kwasu DHA na receptor TLR4) i wewnątrzkomórkowe (przez hamowanie fosforylacji i degradacji czynnika I B), hamowanie aktywacji czynnika NF-κB i produkcji cytokin zapalnych w ludzkich leukocytach i makrofagach po stymulacji LPS*

uptake. The experimental study suggests that the LPS-induced decrease of neutrophil apoptosis takes place mainly through TLR2, not through TLR4, but apoptosis-associated factors such as Bcl-XL or Bak do not play a major role in this process [46]. The regulation of this signaling pathway is crucial for decreased neutrophil apoptosis during sepsis, which may amplify and prolong any systemic inflammatory response.

After partially successful attempts to block LPS-binding receptors in septic patients and blocking the signaling pathway proteins associated with the antibacterial immune response (e.g. suppression of HMGB1), the attempts to modulate the response by immunonutrition seem to be a new and promising direction of studies. To date, several randomized clinical trials have evaluated the efficacy of arginine, glutamine, ω-3 fatty acids, nucleotides and trace elements with antioxidant properties in critically ill patients with trauma and/or infections, but the basic molecular mechanisms that can attenuate the overwhelming inflammatory response in sepsis are still unclear. In malnourished surgical patients with infections, the direct factor that intensifies the failure of local "first line" antibacterial defense may be disorders of PAMPs (e.g. LPS, peptidoglycan, teichoic acids, bacterial DNA) recognition by innate immunity cells. The increased activation of receptors taking part in PAMP recognition can lead to development of severe infections. The hypothesis that one of the main reasons for false recognition of bacterial antigens by immune system cells (mainly by phagocytic cells) is malnutrition is highly probable. The deficiency of immunoactive nourishing substances (e.g. glutamine, fatty acids or trace elements such as selenium and zinc) can intensify the disorders of expression of bacterial antigen binding extracellular receptors and intracellular proteins/receptors. The excessive accumulation of bacterial wall fragments and the microorganisms proliferating in tissues intensify the inflammatory response and increase the release of cytokines into the blood.

Glutamine

Glutamine, the most abundant amino acid in the human body, performs multiple roles in the intestine and may even serve as a signaling molecule [47]. Glutamine is an important energy source for lymphatic tissue and glutamine-enriched enteral nutrition has been found to reduce the incidence of sepsis in trauma patients, due to maintaining the integrity of intestinal mucosa [5, 48, 49]. Animal studies indicate that glutamine-supplemented total parenteral nutrition decreases bacterial translocation [50]. The experimental studies showed that adding glutamine to a culture of neutrophils taken from patients after surgical trauma increases their antibacterial activity [51]. Low plasma glutamine concentrations (< 0.42 mM) at admission to ICUs were associated with higher severity of illness and higher mortality rates [52]. Glutamine deprivation can upregulate an important pro-inflammatory mediator (LPS-induced IL-8 production) via decrease of the inhibitor κB in both the immature and mature human intestine, showing the greatest response with the immature intestine [53]. The production of IL-8 by LPS-stimulated human blood monocytes *in vitro* was increased along with increasing glutamine concentration [54]. The results of recent studies show the regulative impact of glutamine on the inflammatory response in severe infections and indicate that it is necessary to administer high doses (e.g. in parenteral administration 0.35 g/kg × day) to obtain a better therapeutic effect [49, 55, 56].

Some of the most recent experimental studies show that the enteral administration of glutamine reduces the increased expression of TLR4, signal adaptor protein MyD88 and TRAF6 mRNA in intestinal mucosa as a response to LPS-induced endotoxemia in rats (Figure 1) [14]. In addition, the above-mentioned studies found a decreased level of injury to the mucous membrane of the small intestine. It is well known that TLR4 mRNA is present in mouse and human intestinal epithelial cell TLR4 lines [57] and the upregulation of expression of TLR4 and MyD88 after LPS leading to the induction of inflammatory cytokines such as TNF-α, IL-1 and IL-6 can explain the development of intestinal mucosal injury.

Another study [58] suggested that downregulation of TRL4 expression may be a mechanism used by intestinal epithelial cells to protect against dysregulated immune signaling in response to Gram-negative bacteria. In the case of critical care patients a recent study showed that parenteral nutrition supplemented with glutamine does not increase the expression of TLR2 or TLR4 in peripheral blood monocytes [59]. In trauma patients in the ICU, total parenteral nutrition (TPN) supplemented with glutamine does not improve the expression or the functionality of TLRs in peripheral blood monocytes [60]. The mechanisms whereby glutamine prevents the occurrence of infection are still unclear, but it is well known that glutamine decreases the production of pro-inflammatory cytokines [61, 62] and improves the bactericidal function of neutrophils in the case of surgical or burn patients [63]. In cultured colonic biopsies from patients with active Crohn's disease, glutamine was found to decrease TNF-α and the main pro-inflammatory cytokines released by NF-κB [64]. The impact of glutamine on TLR4 may suggest modulation of expression of other signaling proteins through omitting the main pathway with the MyD88 protein (e.g. by

regulating the expression of TRIF protein). Our last study suggested that in malnourished pancreatic cancer patients glutamine may be a modulator for the innate immune system (unpublished data). In patients with pancreatic cancer a statistically significant increase of TLR4 ($p = 0.0001$) and NOD1 ($p = 0.004$) gene expression (measured by real-time RT-PCR) was observed in peripheral blood leukocytes as compared with the control group of healthy volunteers. Treatment with glutamine (pre-operative enteral administration for 5 days, 20 g of glutamine per day) was associated with significantly decreased TLR4 gene expression (Figure 2). We suggested that one of the mechanisms by which pre-operative enteral immunonutrition with glutamine decreases the incidence of post-operative septic complications in malnourished pancreatic cancer patients is probably associated with down-regulation of TLR4 expression.

Omega-3 fatty acids

The anti-inflammatory action of unsaturated fatty acids (including mainly n-3 PUFAs) and their use in treating early sepsis (in the first phase of sepsis syndrome) still seem to be very interesting. In traumatized and surgical patients an enteral diet containing n-3 fatty acids significantly reduced infectious complications and septic events [65-67]. Enhanced survival and reduced lung failure after enteral or parenteral administration of n-3 PUFAs was observed in experimental models of sepsis [68-70]. Interestingly, by incorporation into various membrane (phospho)-lipid pools, n-3 PUFAs may affect lipid-signaling events in different cell types [71, 72]. The ω-3 fatty acids also have an ability to selectively suppress the signaling cascade associated with the innate antibacterial response (mainly leukocytes and macrophages), independently at successive stages: 1) endotoxin interaction with TRL4, 2) activation of inhibitor phosphorylation kinases of the NF-κB (IκB) transcription factor and 3) translocation to the nucleus and connecting NF-κB to an appropriate DNA sequence (suppressing the transcription of inflammatory response mediator genes; Figure 1) [73-80]. The concept of free n-3 PUFAs as a TLR4 antagonist has not been demonstrated *in vivo*. Docosahexaenoic acid (DHA) can block the conduction of signals at the TRL4 level [11], which indicates the key role of the receptor in regulating the response to increased concentrations of fatty acids in peripheral blood during sepsis. The interaction of those fatty acids with TRL4 intensifies the inflammatory response, and increases insulin resistance and tissue injury [81]. We can prevent it by administering appropriate nutrition mixtures. It has been demonstrated that the enteral administration of a diet enriched in unsaturated fatty acids (eicosapentaenoic acid – EPA) and glutamine in septic patients treated in ICUs reduced the inflammatory response and mortality rate caused by acute lung injury (ALI) [82, 83]. The enteral administration of n-3 acids in septic patients modulated the functions of neutrophils, and changed the disadvantageous proportion of n-6 acids to n-3 in the direction of higher concentration of n-3 acids, which was associated with lower concentrations of pro-inflammatory cytokines [84, 85]. In mice, EPA- and DHA-enriched diets modulate the balance between pro- and anti-inflammatory cytokines, alter the early response of the host to *Pseudomonas aeruginosa* infection, and affect the early outcome of infection [86]. Recent findings for the first time indicate that LPS administration causes the downregulation of TLR4 at the mRNA and protein level in pig adipose tissue and that dietary n-3 PUFA blocks this response [87]. In spleens from mice fed a fish oil diet, an increased proportion of macrophages secreting TNF-α and IL-10 and expressing the LPS receptor complex molecules CD14 and TLR4/MD-2 was observed [88]. The expression of TLR2 and TLR4 on peritoneal macrophages and DCs from mice treated with a combined diet (soybean isoflavones and green tea) was significantly decreased [89].

The investigation of Lee and Hwang revealed that saturated and unsaturated fatty acids circulating in the blood can modulate TLR4 signaling by acting as agonists or antagonists [90] For instance, lauric acid (C12:0, a component of *E. coli* lipid A) was a potent NF-κB activator. The activation of NF-κB and COX-2 expression

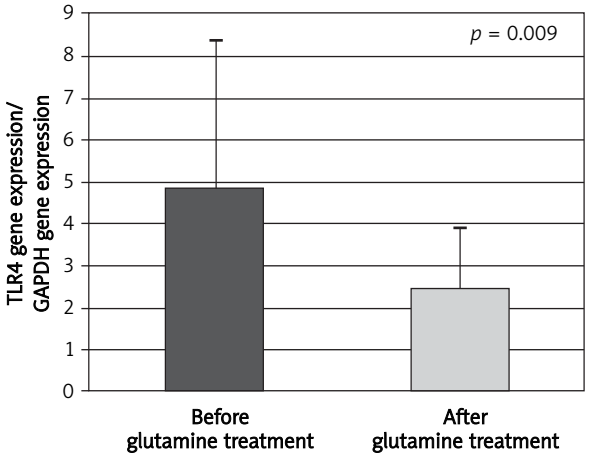


Fig. 2. Significantly decreased TLR4 gene expression in peripheral blood leukocytes of malnourished patients with pancreatic cancer after preoperative enteral glutamine administration

Ryc. 2. *Istotne obniżenie ekspresji genu TLR4 w leukocytach krwi obwodowej u niedożywionych osób z rakiem trzustki po przedoperacyjnym dojelitowym podaniu glutaminy*

was attenuated by DHA, probably in the mechanisms of TLR4/NF-κB pathway blockage at the receptor level [11]. This stimulatory effect is not limited to immune cells. In adipocytes and endothelial cells saturated fatty acid (C18:0, palmitic acid) stimulated IKK and induced IL-6 and TNF-α expression also in a TLR4-dependent pathway [91, 92]. The divergent TLR response to saturated and unsaturated fatty acids suggests some clinical implications of immunonutrition. Parenteral nutrition with fish oil increased free EPA and DHA levels and modulated cytokine response in patients with sepsis [85]. An experimental study revealed that diets enriched with fish oil/DHA might reduce inflammation after tissue damage [93]. Resolvin D1 and protectin D1, DHA-derived lipid mediators, are involved in these mechanisms. In a model of peritonitis in mice, intraperitoneal administration of protectin D1 preceding zymosan A reduced the number of neutrophils and the levels of pro-inflammatory cytokines and chemokines [94]. This means that the administration of n-3 PUFAs modulates the profile of lipid mediators not only by their impact on TLR-dependent signaling. Moreover, leukotrienes, thromboxane, and prostaglandins formed from EPA are considered to have less of a pro-inflammatory impact on the immune response [95]. We believe that parenteral n-3 PUFA administration during a hyperinflammatory reaction may have positive effects on the outcomes of critically ill patients.

Probiotics

Recently there has been evidence that TLRs may mediate immunomodulatory activity of probiotics via ligation with certain probiotic antigens and/or nucleic acids [96, 97]. TLRs' interactions with bacterial ligands support intestinal barrier function and communication to immune cells (e.g. DCs) that play important roles in regulating intestinal homeostasis in the healthy intestine and in the dysregulated response seen in the inflammatory bowel diseases [98, 99]. In experimental colitis (hapten-induced colitis in BALB/c mice, and the IL-10 knockout mice model) the DNA of bacterial origin that binds to TLR9 has been shown to reduce inflammation [100]. The immunomodulatory function of probiotic bacterial DNA has also been demonstrated in a study of peripheral blood mononuclear cells taken from healthy donors where *Bifidobacterium* genomic DNA caused the induction of secretion of the anti-inflammatory IL-10 [101]. In addition, probiotics have been shown to reduce NF-κB activation in intestinal inflammation [102]. A possible mechanism by which probiotics interfere with the NF-κB pathway is based on the fact that a mixture of probiotic bacteria elicits immunosuppressive activity by stabilizing IκB levels and inhibiting both NF-κB activation and IL-8 secretion [103]. These inhibitory effects on the proinflammatory NF-κB pathway may be an important mechanism used by probiotics to regulate intestinal inflammation and response to infection. There are insufficient data to recommend the use of probiotics in critically ill patients during enteral nutrition. In the Besselink study, there was a significantly higher need for surgical intervention and frequency of organ failures and bowel ischemia associated with the use of pre/probiotics in acute pancreatitis [104]. It was noted that their use after multiple trauma might be associated with a reduction in diarrhea in critically ill patients [105]. There is also emerging evidence for the use of probiotics in treating gastrointestinal infections, to prevent postoperative bacterial translocation, irritable bowel syndrome, in ulcerative colitis and Crohn's disease [106].

In conclusion, the above-presented results show that improving outcomes in the group of patients with severe infections requires more attention to be paid to explaining the molecular mechanisms regulating the innate antibacterial response. One of the preconditions necessary to achieve progress in treating the most severely ill patients is to find out more about the impact of nutrition, severe infections and immunonutrition on the expression of selected signaling pathway proteins of innate antibacterial response cells. Attempts to modulate the innate antibacterial immune response by administering immunonutrition are promising and indicate that in the future it may be a valuable way of assisting the basic therapy by blocking selected signaling pathways aimed at reducing the life-threatening effects of massive infection, including the increased inflammatory response.

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