Immunonutrition and lipopolysaccharide – induced Toll-like receptor signaling

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Abstract

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

Key words: immunonutrition, toll-like receptors, sepsis.

Malnutrition is a major global public health problem and can be defined as a state of nutrition in which a deficiency of energy, protein and other nutrients like arginine, glutamine, fatty acids, vitamins and trace elements causes measurable effects on body and tissue function and clinical outcome. Surgical trauma increases immune system suppression and deepens disease related malnutrition. The immune disorders and malnutrition worsen in the early postoperative period, considerably affecting the process of wound healing, intestinal barrier function and the number of post-operative infections. Infections in malnourished surgical patients increase morbidity and mortality rate. Despite advances in treatment, there is still no therapy available to efficiently reduce the excessive inflammatory response, which can increase the risk of multiple organ failure (MOF) [1]. The aim of this study is to efficiently reduce the excessive inflammatory response, above all reducing the activation of nuclear factor κB (NF-κB), the production of post-inflammatory cytokines (TNF, IL-1, IL-6), chemokines and adhesive molecules.

More efficient therapy consists influences the mechanisms of inflammatory response to a massive infection. After a major surgery complicated by severe infection special attention should be paid to modulation of the expression of signaling pathway proteins in cells that take part in early (innate) immune response to infection by applying immunonutrition. It is well known that neutrophils and monocytes/macrophages that take part in innate immune response to trauma and infection play a significant role in the elimination of microorganisms and in local and systemic inflammatory response regulation (SIRS – systemic inflammatory response syndrome) that increases the risk of MOF [7]. The disorders of phagocytosis and microorganism elimination in the site of bacterial penetration (extensive surgical wound, catheter in a large vein) intensify the pro- and anti-inflammatory response (CARS- compensatory anti-inflammatory response), which and on attempting to regulate the neutrophil and lymphocyte apoptosis (e.g. by over expression of anti-apoptic proteins such as Bcl-2) are still subject of experimental research [2-6]. The aim of this study is to efficiently reduce the excessive inflammatory response, above all reducing the activation of nuclear factor κB (NF-κB), the production of post-inflammatory cytokines (TNF, IL-1, IL-6), chemokines and adhesive molecules.

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in regulating the expression of inflammatory cytokines in the
disorders in phagocytosis and antigen presentation [34-36].
[33] and caused disorders in inflammatory mediator secretion,
trauma patients was reduced [29]. In experimental studies the
antigens (e.g. LPS, peptidoglycan) [29-32]. As compared with
increases the expression of TLRs recognizing bacterial
pathways in intestinal mucosa and innate immunity cells of septic patients
after immunonutrition contribute to the attenuation of local
and systemic hyperinflammatory response in massive bacterial
load.

The immunomodulatory action of unsaturated fatty acids
affects the decrease in activity of neutrophils, monocytes,
lymphocytes, and the production of cytokines [9-12]. Immu-
nostimulatory action of amino acids increases the phagocytic
activity of leukocytes, enhances immunity to infections and
accelerates wound healing [13-19]. In randomized studies it
has been found that enteral immunonutrition improves the
clinical course, decreases the frequency of severe infections,
shortens hospital stays, reduces treatment costs and signi-
ficantly decreases mortality in severe ill patients with MOF
[20-25]. These benefits were found to be most impressive in
surgeon patients. In patients with severe trauma and infection
receiving immunonutrition significant decrease in the duration of
SIRS and in the frequency of MOF have been found [20,
23, 24, 26]. In the clinical setting immunonutrition (with
arginine, nucleic acids and n-3 fatty acids) reduced infections
complications in critically ill patients after trauma and cancer
[22, 23, 25, 27, 28]. The studies have been performed
in various populations patients, which makes it difficult to
compare their results. The most frequently included patients
are treated in intensive care units. In the majority of those studies,
the changes in nutritional and immune status in the course
of immunonutrition and infection have not been monitored.
Despite the advantage of the positive effects of immuno-
nutrition on the treatment of surgical patients, the impact of
this nutrition on the immune system still remains unclear.
A better knowledge of advantages of immunomodulating
nutrition in treating surgical infections requires studying the
changes in the expression of signaling cascade proteins
associated with their stimulation account not only for
pathological inflammatory response to trauma or infection,
but they can also have a protective action (e.g. increasing the
apoptosis of selected cells, stimulation of signaling pathway
inhibitors).

In regulating the mechanisms of local and systemic
inflammatory response to a massive infection in surgical
patients a significant role is played by Toll-like receptors
(TLRs) expressed in gut mucosa cells and the cells that
take part in innate response to infection. Some studies
performed show that trauma reduces, whereas severe infection
increases the expression of TLRs recognizing bacterial
antigens (e.g. LPS, peptidoglycan) [29-32]. As compared with
healthy people, the expression of TLR4 in the monocytes of
trauma patients was reduced [29]. In experimental studies the
lack of TLRs increased the susceptibility to infections in mice
[33] and caused disorders in inflammatory mediator secretion,
disorders in phagocytosis and antigen presentation [34-36].
The experimental findings suggest that TLR4 plays a key role
in regulating the expression of inflammatory cytokines in the
lung during endotoxic shock [37]. 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.

To date, several randomized clinical trials have evaluated
the efficacy of arginine, glutamine, omega-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 can be the disorders of pathogen-
associated molecular pattern (PAMPs) (e.g. LPS, peptido-
glycan, teichoic acids, bacterial DNA) recognition by innate
immunity cells. 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) 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 being proliferated in tissues intensify
the local inflammatory response and increase the release of
cytokines into the blood.

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 [38-40].
Low plasma glutamine concentrations (<0.42 mM) at ad-
mission to intensive care units were associated with higher
severity of illness and higher mortality rates [41]. The
results of recent studies show the regulative glutamine
impact on inflammatory response in severe infections and
indicate that it is necessary to administer high doses (e.g.
in parenteral administration 0.35g/kg·day⁻¹) to obtain
a better therapeutic effect [39, 42, 43]. Some most recent
experimental studies show that the enteral administration
of glutamine reduces the increased TLR4 expression, signal
adaptor protein MyD88 (myeloid differentiation factor 88)
and TRAF6mRNA (TNF-α receptor-associated factor 6)
in intestinal mucosa as a response to LPS induced endoto-
xemia in rats (Fig. 1) [5]. In addition, the above-mentioned
studies found a decreased injury to the mucous membrane
of the small intestine. The effect of glutamine on intestinal
TLR4 expression may be considered as a mechanism via
which immunonutrition helps in the recovery of critically
ill and septic patients. The mechanisms by which glutamine
prevents the occurrence of infection are still unclear, but it is
well known that in surgical or burn patients glutamine
decreases the production of pro-inflammatory cytokines [44]
and improves the bactericidal function of neutrophils [46].

The anti-inflammatory action of unsaturated fatty acids
(mainly n-3 PUFAs) and their application in treating
surgical infections and 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 [23, 47, 48]. Enhanced survival and reduced lung failure after enteral or parenteral usage of n-3 lipids was observed in experimental models of sepsis [49-51]. Interestingly, by incorporation into various membrane (phospho)-lipid pools, n-3 fatty acids may affect lipid-signaling events in different cell types [52, 53]. The omega-3 fatty acids have also an ability to selectively suppress the signaling cascade associated with innate antibacterial response (mainly leukocytes and macrophages), independently at sub-

**Fig. 1.** Schematic diagram of TLR4, MyD88 and TRAF6 down-regulation in rats intestinal mucosa following glutamine administration and LPS-induced endotoxemia (A). N-3 omega acids inhibition of TLR signaling pathway at the extracellular (DHA interfere with TLR4 receptor) and intracellular level: inhibition of the phosphorylation and degradation of the IκB, inhibition of the NF-κB activation and inflammatory cytokines production in LPS-stimulated human leukocytes and macrophages (B). TLR-independent signaling via the NODs cytoplasmic sensors of LPS does not require members of the MyD88 adaptor family (interrupted lines)
sequent stages: a) endotoxin interaction with TRL4, b) activation of inhibitor phosphorylation kinases of the NF-kB (IκB) transcription factor and c) translocation to nucleus and connecting NFκB to an appropriate DNA sequence (suppressing the transcription of inflammatory response mediator genes) (Fig. 1) [54-61].

It was indicated that the enteral administration of diet enriched in unsaturated fatty acids (EPA) and glutamine in septic patients treated in intensive care units reduced the inflammatory response and mortality rate caused by acute lung injury (acute respiratory distress syndrome – ARDS) [62, 63]. The enteral administration of n-3 acids in septic patients modulated the functions of neutrophils, 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 [64, 65]. These findings indicate that immunomodulating nutrition may be an effective means of influencing the inflammatory response, particularly for those pathways affected by TLR4 signaling.

Our previous study has clearly indicated that the anti-inflammatory mechanisms are activated early in malnourished patients after pancreaticoduodenectomy receiving enteral immunonutrition [66]. Early enteral immunonutrition (with glutamine, arginine and n-3 fatty acids) in comparison to standard nutrition has an immunomodulatory effect on the changes in the immune response after extensive surgical trauma. These consist in selective stimulation of IL-6, IL-8, IL-10 and IL-1ra production and down-regulation of IL-1 beta and TNF-α production. The temporary increase in IL-1ra concentration between post-operative days 7-14 obtained as a result of enteral immunonutrition decreases the inflammatory response to extensive surgical trauma and shortens its duration; this accelerates the wound healing process/tissue regeneration and may help avoid late complications (fistulas, abscesses).

The above-presented results show that to improve outcomes in the group of malnourished surgical patients suffering from severe infections more attention should be devoted to explaining the molecular mechanisms regulating the innate antibacterial response. One of the preconditions to provide progress in treating the most severely ill patients is to find out more about the impact of the state 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 applying immunonutrition are promising and indicate that in the future it can be a valuable supplement of the therapy using a blockade of selected signaling pathways to reduce the life-threatening effects of massive infection, including mainly the increased inflammatory response.

References

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