Metabolism of immune cells in septic shock in children

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ABSTRACT
In septic shock the hyperimmune response and immunosuppression can occur at the same time, being defined as mixed antagonist response syndrome. Under standard conditions, mitochondria supports cellular energy metabolism through the citric cycle and the phosphorylation chain. Both NK and T cells produce an immediate effector response to inflammatory signals using the rapid production of energy in aerobic glycolysis (glucose-lactate). When the inflammatory signal is eliminated, they return to metabolism in the Krebs cycle. Septic shock in the children was accompanied by immunoparalysis (TNFα <200pg/ml), lower monocyte human antigen (HLA-DR), loss of peripheral non-naive CD4 T cells and low mitochondrial respiration. Children with septic shock had a in hospital mortality rate of 10.8-33.5%, high risk of hospitalization in the next 28 days and in the following months with predominantly respiratory infections. The treatment of mitochondrial dysfunction, insufficiently structured, is based on antioxidant medication, but the results await confirmation.

Keywords: septic shock, immunity, mitochondrial metabolism, children's evolution

INTRODUCTION
Shock is considered a hemodynamic and metabolic state produced by decreased tissue perfusion, decreased delivery of oxygen and substrates to vital organs, followed by incomplete elimination of metabolic products. The changes can be a consequence of the direct action of etiological factors in the case of hypovolemic, hemorrhagic or cardiogenic shock or mediated by active molecules in septic shock [1]. Over the years, the study of septic shock has switched to the cellular damage and molecular disorders characteristic of the syndrome. The most radical and important change was determined by the fact that the organism and not the germs lead the pathogenesis of sepsis [2]. Within the body’s global reaction, sepsis represents an uncontrolled immune response to infectious agents or their products. The activity of immune cells is amplified by the spread of germs and microbial products (bacteremia) through the proinflammatory response induced by chemokines and cytokines (Figure 1).

Systemic inflammatory response syndrome (SIRS)
Characteristics of SIRS, include changes in the expression of genes for the synthesis of pro-inflammatory cytokines, immune effectors and some proteins specific to the shock response (heat shock proteins, nitric oxide synthetase and others). Part of the response is the systemic inflammatory response, the activation of the immune system by T and B lym-
phocytes and other immune effectors produces the explosive release of numerous proinflammatory cytokines (IL-1, IL-6, IL-12, TNF-α).

The compensatory anti-inflammatory response syndrome (CARS)

The anti-inflammatory state is characterized by the reprogramming of the Toll-like receptors (TLRs) response, the decrease in the expression of the major histocompatibility complex, the long-term repression of the TNF-α and IL-8 genes, the increase in the production of anti-inflammatory cytokines, the apoptosis of lymphocytes and the increase in T cell anergy. Recently, it is considered that the hyperimmune response phase and the immunosuppression phase occur at the same time, being defined as the mixed antagonist response syndrome which includes SIRS and CARS [3]. The simultaneous activation of pro-inflammatory and anti-inflammatory pathways leads to the emergence of a set of persistent inflammatory, immunosuppressive and catabolic changes, frequent of patients who stay in the ICU for several days [3].

**TABLE 1.** The host response to sepsis

<table>
<thead>
<tr>
<th>Pro-inflammatory response</th>
<th>Immune suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPS (lipopolysaccharide)</strong></td>
<td>HSPs (heat shock proteins)</td>
</tr>
<tr>
<td><strong>LTA (lipoteichoic acid)</strong></td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td>DNA, RNA</td>
</tr>
<tr>
<td>Flagella</td>
<td>IL-1a</td>
</tr>
<tr>
<td>ADN</td>
<td>IL-33</td>
</tr>
<tr>
<td>ARN</td>
<td></td>
</tr>
</tbody>
</table>

↓

- Leucocyte activation: cytokines (IL-1, IL-6*, TNF-α, IL-12), proteases, reactive oxygen species
- Complement activation
- Necrotic cell death

Excessive inflammation produce tissue injury

↓

- Decrease in the function of immune cells: apoptosis of T, B, DC (dendritic cells); the expansion of Treg and myeloid suppressors; decrease in phagocytosis
- Inhibition of transcription of pro-inflammatory genes; anti-inflammatory cytokines (IL-10, IL-6*, IL-17, IL-23); soluble receptors for cytokines; negative regulation of TLR signals; epigenetic regulation

Immune suppression: increased susceptibility to secondary infections and late mortality

Processed after Wiersinga Willem, Leopold Stije, Cranendonk Duncan, van der Poll Torfi. Virulence. 2014;5,1

**TABLE 2.** Components of the innate immune system [4]

<table>
<thead>
<tr>
<th>Barriers</th>
<th>Effects</th>
</tr>
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<tbody>
<tr>
<td>Skin</td>
<td>Prevents microbial entrance</td>
</tr>
<tr>
<td>Mucosa</td>
<td>Prevents microbial entrance, secretes proteins and enzymes, absorbs metabolic substrates</td>
</tr>
</tbody>
</table>

**Effector cells**

- Granulocytes: Phagocytosis, cytokine production, protein and enzyme secretion, destruction of pathogens
- Monocytes/macrophages: Phagocytosis, cytokine, protein/enzyme secretion, destruction of pathogens
- Dendritic cells: Phagocytosis, cytokine, protein/enzymes secretion, destruction of pathogens
- Natural killer(NK) cells: Lysis of infected and tumoral cells, activation of macrophages through cytokine production
- Innate lymphoid cells: Mediate immune response and regulate tissue homeostasis and inflammation
- Endothelial/epithelial cells: Microbial recognition, cytokine production

**Soluble mediators**

- **Cytokines**
  - TNF-α, IL-1, Chemokines: Mediate immune response and inflammation
  - INF-γ: Involved in resistance to viral infection
  - IL-12: Involved in resistance to intracellular pathogen infection and activation of macrophages
  - IL-10: Stimulate IFN-γ production by cells and T lymphocytes
  - TGF-β: Stimulate IFN-γ production by cells and T lymphocytes

- **Seric proteins**
  - Complement system: Opsonization, destruction of pathogens and T lymphocyte activation
  - Collectins: Opsonization of pathogens and complement activation
  - C reactive protein: Opsonization of pathogens and complement activation
  - Coagulation system: Localization of damage or infected tissue
Immunometabolism of T cells in sepsis

The advancement of immunology led to the development of immunometabolism and its cellular and tissue components [5]. In hyper-inflammatory phase of sepsis the effector cells of innate immunity have the role of identifying and eliminating pathogens. For this they have 3 specific needs: high energy support, activation of effector immunity and rapid cellular regeneration [6]. In the economy of the body, mitochondria represent the main energy factory under standard conditions. Almost all circulating cells (except red blood cells) possess mitochondria that process substrates from metabolism and produce energy in the presence of oxygen [3].

Apart from the energetic function, mitochondria represent a metabolic hub by being involved in the generation of reactive oxygen species (ROS), calcium homeostasis, regulation of immunity by succinate, itaconate and acetate [5]. The process of OXPHOS (the oxidative phosphorylation) depends on glycolysis, Krebs circle, fatty acid synthesis and its oxidation [5,6]. T cells in a naive state depend on the energy produced by OXPHOS following the degradation of substrates in the citric cycle (Krebs) [7]. Cytokines and Krebs cycle intermediates can activate proinflammatory gene expression [3,5]. Succinate that converts in succinate oxidase induce IL-1β production and acetate stimulate IFN-γ production in T cells [3]. During the activation of monocytes, macrophages and dendritic cells (CD) the amount of ATP provided by the Krebs cycle becomes insufficient, or is part of the acute cellular reorganization process [8]. Energy deprivation causes the metabolism to switch to aerobic glycolysis, with the transformation of glucose into pyruvate or later into lactate (Warburg effect). The energy yield of the citric cycle is 34 ATP/mol glucose, while glycolysis produces only 2 ATP/mol glucose. Although the efficiency of OXPHOS in the generation of ATP is very high, the process is slow, but glycolysis can provide energy very quickly to meet the demand imposed by the metabolic switch to hyperinflammation [3,6,7]. Both NK cells and T cells produce an immediate effector response to inflammatory signals, but when the inflammatory signal is eliminated, they return to basal functioning [7]. A shift from OXPHOS in naive T cells to glycolysis is believed to be an part of the initial activation of innate immune function [8] (Figure 3).

Quiescent Th cells rely mainly on oxidative phosphorylation (OXPHOS) to maintain homeostasis, while the effector subsets are characterized by increased glycolytic metabolism (Glc) as well as a differential reliance on glutaminolysis (Gln), fatty acid oxidation (FAO) and fatty acid synthesis (FAS) to support effector functions [9] (Table 3).

### TABLE 3. Th subsets display distinct metabolic programs

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Metabolism</th>
</tr>
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<tbody>
<tr>
<td>Naive CD4+ T cells</td>
<td>OXPHOS</td>
</tr>
<tr>
<td>Pro inflammatory</td>
<td></td>
</tr>
<tr>
<td>Th1</td>
<td>Glc↑↑ FAO↓ Gln↓</td>
</tr>
<tr>
<td>Th2</td>
<td>Glc ↑↑↑↑ FAO ↓</td>
</tr>
<tr>
<td>Th17</td>
<td>Glc ↑↑↑ FAO ↑ FAS ↑ Gln ↑</td>
</tr>
<tr>
<td>Anti inflammatory</td>
<td></td>
</tr>
<tr>
<td>T reg</td>
<td>Glc ↑ FAO ↑ FAS ↓</td>
</tr>
<tr>
<td>Memory</td>
<td>Glc ↓ FAO ↑↑↑↑</td>
</tr>
</tbody>
</table>

**FIGURE 3.** The citric cycle and its metabolic precursors

http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy
Mitochondrial dysfunction in sepsis was demonstrated by the reduction of biogenesis (the production of new mitochondria), an increased generation of reactive oxygen species and reducing ATP production by 50% [10]. Depletion of energy during hyperinflammatory response induce anti-inflammatory phase in septic shock state [11]. In this stage a drastic decrease occur in the resistance to infections by affecting innate and adaptive immune cells [6].

Immunoparalysis – clinical investigations. The persistent anti-inflammatory response in sepsis increases the risk for other infections and mortality. In clinical conditions it is difficult to estimate when this transition occurs but it is possible that it takes place early in the mixed pro- and anti-inflammatory response. A lethal dose of *Escherichia coli* endotoxin administered to rodents under experimental conditions induced ultrastructural mitochondrial damage within 2-3 hours [12]. In the sepsis state in 14 children and 7 control patients, Lindell et al [13] studied the changes in mononuclear cells from the peripheral blood, TNF-α production, monocyte expression and mitochondrial respiration. At sepsis onset immunoparalysis (defined as TNF-α production capacity <200pg/ml) was present in 39% of sepsis patients. Compared to controls, sepsis patients demonstrated lower monocyte human leucocyte antigen DR (HLA-DR), loss of peripheral non-naive CD4+ T cells, and reduced peripheral blood mononuclear cell (PBMC) mitochondrial spare residual capacity (4.0 pmol/s/106 cells vs. 8.4 pmol/s/106 cells) [13]. Weiss et al [8] studied the mitochondrial dysfunction PBMCs from the peripheral blood of 161 children hospitalized with sepsis and possible association with immunoparalysis and systemic inflammation. Immunoparalysis was defined as whole-blood ex vivo lipopolysaccharide (LPS)-induced TNF-α < 200pg/ml and monocyte antigen DR (mHLA-DR) <30%. Mitochondrial respiration in PBMCs was lower in children with immunoparalysis compared to controls in serial determinations in the first week of the disease. The subset with immunoparalysis and low mitochondrial respiration exhibited the highest level of systemic inflammation [8]. Ex vivo measurement of oxygen consumption and membrane potential in PBMCs on day 1 and 5-7 were studied on 13 children in septic shock and in controls [14]. Mitochondrial bioenergetic reserve (spare respiratory capacity) was lower on day 1 and normalized by day 5-7, suggesting mitochondrial uncoupling early in sepsis [14]. Leucocytes from septic adults with diminished cytokine secretion after stimulation by LPS exhibit broad defects in glycolysis and mitochondrial oxidative phosphorylation [15]. Recent data confirm the utility of PBMC mitochondrial function as a diagnostic, prognostic or predictive biomarker in children with sepsis [16].

The evolution of children and adults with sepsis and septic shock

Children who survived severe sepsis have an increased risk of death in the next few years. Excluding premature babies and the 0-1 month age group from the study, Czaia et al [17] reviewed over 7000 cases of children with severe sepsis, of which 6.8% died during the first or subsequent readmissions, within 28 days. Almost half of the children included in the study were readmitted at least once in the following months. In over 30%, the main diagnosis was related to respiratory infections, the risk of death being high in the next 2 years. In different studies, mortality in sepsis and septic shock varies depending on the year of data collection and the structure of the intensive care services. In adults hospitalized in intensive care services, mortality at 28 days increases from 10% in SIRS, to 20% in sepsis, to 20-40% in severe sepsis and to 40-60% in septic shock [18]. A meta-analysis of 170 studies on sepsis mortality in the period 2009-2019 showed: average mortality at 30 days was 34.7%, at 90 days was 38.5% in the USA, Europe and Australia [19]. The mortality rate ranges from 25-30% for severe sepsis and up to 30-40% in septic shock in Korea [20]. Recently, children with septic shock had an in-hospital mortality rate of 10.8% and 33.5% in higher – and lower – resource settings, respectively [21]. Critically ill adult patients often develop immunosuppression after a few hours/days of evolution followed by increased susceptibility to lung infection persisting for days or weeks [18]. These conditions are associated by following 20-40% mortality rate in the next 2 years [18]. The evolution of the patients suggests a significant association between the immunoparalysis group and a longer length of hospital stay [22]. Septic patients can present in evolution persistent inflammation, immunosuppression and catabolic syndrome. In a study carried out on the elderly, Quartin et al [23] found a 26% risk of death from nonseptic causes in a 1-year interval and a decrease in estimated life expectancy from 8 to 4 years.

Perspectives in the treatment of mitochondrial dysfunction in sepsis and septic shock

The excessive inflammatory response from sepsis triggers overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These compounds can produce effects through oxidative stress, lipid peroxidation, protein modification and DNA damage [24]. Intake of antioxidants in the treatment of sepsis can reduce cell damage, modulate the inflammatory response and potentially improve patient prognosis [24,25]. ROS/RNS are molecules signaling for antimicrobial host defense, but their complete eradication has deleterious con-
sequences [26]. Therapeutic trials have included vitamin E, Vitamin C, melatonin, N-Acetylcysteine or the combination hydrocortisone, vitamin C and thiamine [24,26,27]. Some clinical and especially experimental research seeks to build a strategy for the morphological and functional restoration of mitochondria during severe energy deficiencies from septic shock. Experimental mitochondrial therapy can focus on 5 parameters: substrate provision, co-factor provision, mitochondrial antioxidants, mitochondrial reactive species scavengers and membrane stabilizers [28].

REFERENCES


CONCLUSION

In sepsis and septic shock, the dynamic reprogramming of immune cells occurs simultaneously with the reorganization of energy metabolism. Impaired mitochondrial metabolism, energy depletion, inability to use oxygen, immunodepression and multi-organ dysfunction are associated with unfavorable evolution.

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