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Cytokines and the Abdominal Surgeon

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CHAPTER 13

Secondary Peritonitis and Cytokines

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Introduction

Peritonitis is still a life threatening complication for every surgical patient, which can occur after trauma, intra-abdominal operations or spontaneously.[1] Depending on localization or cause the mortality rate of peritonitis is between 40-70% according to literature. The principles of peritonitis treatment established by Kirschner, e.g., source control, debridement and lavage, are still valid.[2-4] Even the development of new, potent antibiotics within the last twenty years did not dramatically reduce the mortality rate of peritonitis. Intensive care therapy has influenced the survival of peritonitis patients. However, the treatment was focused on symptomatic treatment of organ dysfunction.[5] With the identification and recombinant development of cytokines the possibility to influence the course of peritonitis before organ dysfunction seem to available soon. Our knowledge of the pathophysiological immune response during peritonitis has been enlarged during the last 10 years. However, a uniform, efficient immunological concept of peritonitis treatment is not yet available. The purpose of this chapter is to summarize the main developments in cytokines in secondary peritonitis with regard to experimental and clinical studies.

Peritonitis and Cytokines in Experimental Models

Endotoxin which is released from cell walls of disintegrating gram-negative pathogens is a major trigger for cytokine release in peritonitis, although it may not be the only cause for macrophage cytokine production.[6] Peritonitis results in protease activation and protease inhibitor consumption, especially in the peritoneal fluid.[7] This may lead to a breakdown of C3 complement and IgG in peritonitis exudate.[8] Bacterial components other than endotoxin may induce dysfunction in the peritoneal macrophages capacity to produce proinflammatory cytokines during sepsis and peritonitis.[9,10]

However, endotoxin can be frequently detected in the peritoneal exudate and plasma of patients with peritonitis.[11,12] The activation of macrophages in peritonitis leads to a complex release of cytokines.[13] This activation occurs via specific LPS receptors, e.g., CD14, CD11/18 family, LPS receptor, scavenger receptor. The identification of specific cell membrane targets for LPS has important implications for immunotherapy.[14,15]

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It has been demonstrated that the infusion of either LPS or TNF can mimic the effects of sepsis.[16] Proinflammatory cytokine levels correlated with the prognosis of peritonitis and treatment failure. Reduced TNF plasma levels correlated with increased survival;[17] constantly increased peritoneal levels correlated with poor outcome.[18] The demonstration of detrimental TNF effects lead to the concept to block endotoxin or TNF. However, there was early evidence that I.V. anti-TNF antibodies decreased mortality only in I.V. sepsis and not in peritonitis.[19] Anti-TNF-antibodies reduced IL-1 and IL-6 plasma levels in I.V. sepsis and not in peritonitis. Anti-LPS-antibodies, however, were able to induce protection in peritonitis and to reduce pathogens, plasma TNF, IL-1 and IL-6 levels. The peritoneal cytokine levels remained unchanged.[20] Conversely, peritoneal cytokine production correlates with outcome in peritonitis and pretreatment with monophoryl lipid A as well as induction of endotoxin tolerance may reduce cytokine production.[21]

Dosage of Cytokines

The effect of cytokines on the immune response—beneficial or detrimental—may depend on the dose and the concentration of cytokines.[22] Anti-TNF-antibodies administration after CLP induction increased the mortality, while the addition of TNF increased the survival rate.[23] Interferon-γ increased TNF and IL-6 plasma levels and subsequent mortality. Blocking interferon-γ resulted in increased survival without any effect on the cytokine levels.[24] It seems to be likely that Interferon-γ increases sensitivity of cells to endotoxin.[25]

Function of Peritoneal Cells

Peritoneal macrophages may change their function during the course of peritonitis and therefore may influence the immune modulation. While antigen recognition is the main function during the early phase, cytokine production prevails during later stages of peritonitis. TNF plasma levels may be lower during peritonitis compared to I.V. induced sepsis.[26] Anti-TNF-antibodies reduced migration of granulocytes and monocytes into the peritoneum with increased number of pathogens.[27] Inflammatory cytokines may induce adhesion and fibrinogen production by stimulation of plasminogen activation inhibitor in mesothelial cells.[28,29] The mortality decreasing effect of Pentoxifyllin which is known to decrease TNF production in peritonitis may be due to reduced adhesion and fibrinogen production.[30] Peritoneal macrophages and mesothelial cells are responsible for initiation, amplification and termination of the inflammatory response. The surface of mesothelial cells, where bacteria colonize, may be an important place of interaction between macrophages and mesothelial cells.[31] Microbial colonization of the peritoneal mesothelial surface is a rapid and stable phenomena following penetration injury to the distal bowel. Mesothelial populations are resistant to intraperitoneal lavage.[32] *S. aureus* adherence to mesothelial cells is increased following preincubation of mesothelial cells with IL-1. Treating mesothelial cells with IFN-γ reduces adherence of *S. aureus*.[33] Other cell population in the peritoneum have not been studied until recently. Mast cells in the vicinity of blood vessels may be important for the synthesis of leukotrienes which are responsible for PMN recruitment.[34,35]

Local Response

Local TNF production is an important factor for tissue damage and organ dysfunction. Peritoneal macrophage TNF mRNA increased after CLP and i.p. LPS injection; however, TNF mRNA decreased faster after i.p. LPS injection. Both forms of

infection are able to induce TNF production in lungs and liver.[36] Bacterial infection of the peritoneal cavity may induce a slow release of cytokines which are important for the local immune response. IL-1 may induce the lethal effects of sepsis and TNF may be more important to launch the local immune response.[37] Increased local, intestinal IL-6 production has been demonstrated after CLP[38] and TNF levels were higher in portal vein than in hepatic veins.[39]

Within encapsulated abscess LPS binding protein (LBP) and bactercidal/permeability increasing protein (BPI) have been measured. LBP binds to the lipid A component of the bacterial endotoxin and facilitates its delivery to the CD14 antigen on the macrophage where inflammatory cytokines are released. The neutrophil granular protein bactericidal/permeability increasing protein (BPI) competes with LBP for endotoxin binding and functions as a molecular antagonist of LBP-endotoxin interactions. Within abscess cavities BPI is available in sufficient quantities for effective competition with LBP for endotoxin. BPI may attenuate the local inflammatory response and the systemic toxicity of endotoxin release during gram-negative infections.[40] Endotoxin derived from enteric bacteria might play an important role in the pathogenesis of lung injury and anti-endotoxin agents, such VVN1 222-5 appear to protect against endogenous bacterial endotoxin related disorders in severe hemorrhagic shock.[41,42]

Effect of Cytokines and Growth Factors in Peritonitis

Different results were reported when cytokines or growth factors were added in peritonitis. G-CSF increased survival rate in peritonitis, probably by reducing TNF levels.[43-45] High dose G-CSF decreased endotoxin and TNF, increased peripheral neutrophils and improved cardiopulmonary function.[46] Conversely, macrophages, which produce G-CSF, were not pivotal after bacterial translocation or septic shock in the knock-out mouse model.[47] GM-CSF, which was administered after the onset of peritonitis, was not beneficial and inhibited the neutrophil migration into the peritoneal cavity.[48] IL-1, an inflammatory cytokine, downregulated TNF and IL-6 plasma levels, decreased organ dysfunction and mortality when added before the induction of peritonitis.[49] This is supported by the finding that blockade of Kupffer cells decreased IL-1 and survival.[50] IL-2, which is known to be essential for immune response after thermal injury and CLP[51] induced influx of neutrophils in peritonitis and increased survival.[52] IL-2 administration and induction of peritonitis should be performed simultaneously to achieve a protection by IL-2.[53]

Anti-Endotoxin-Antibodies

The notion that anti-endotoxin-antibodies may be beneficial in peritonitis by reduction of cytokines[54] is further supported by other studies. Pretreatment with anti-endotoxin-antibodies were protective in peritonitis and reduced plasma TNF levels and splenocyte TNF production.[55] E5 monoclonal antibodies reduced mortality, endotoxin and TNF in peritonitis, but not endothelin.[56] Endotoxin neutralizing protein (ENP) decreased endotoxin and TNF; however, mortality was only reduced when ENP was added together with gentamicin.[57]

Further studies with anti-endotoxin antibodies were published with controversial results. Some of them, however, revealed important pathophysiological mechanisms of the function of anti-endotoxin-antibodies. Type specific anti-endotoxin-antibodies were protective in peritonitis by Fc-mediated clearance of both bacteria and endotoxin.[58]

IgG and IgM anti-LPS mAbs exert protective capacity by extracellular neutralization of LPS, while Fc-receptor mediated cellular uptake also may serve to bypass macrophage activation and TNF secretion by promoting internalization and intracellular neutralization.[59] While some recent reports demonstrated significant activity of antibodies, not all anti-endotoxin antibodies have been demonstrated to bind to endotoxin or to be beneficial in peritonitis. Selected models may have a clear influence on the results.[60-63]

Pathogens

Treatment failures of intra-abdominal infection may be due in part to the presence of resistant pathogens at the site of infection.[64] Enterococcus plays an important role in the mechanisms of bacterial synergism in experimental peritonitis.[65,66] The LPS induced cytokine immune response and the bacterial surface characteristics may be more important for the killing of invading pathogens than previously thought.[67] In vitro studies have revealed that antibiotics may release different amounts of endotoxin depending on the type of Penicillin binding protein.[68] There may be important functional relationships between the immune response and resistant pathogens not yet clarified.[69,70]

Anti-Inflammatory Cytokines

There is a growing body of information available on the onset of inflammatory response in secondary peritonitis. However, there is less information available on the termination of inflammation and the role of anti-inflammatory cytokines. It was generally believed that the anti-inflammatory response is launched after the inflammatory response. However, we and others have demonstrated that both inflammatory and anti-inflammatory cytokines are released simultaneously during the inflammatory response.[71] Anti-inflammatory cytokines seem to suppress inflammatory cytokines. IL-10 administration prolonged survival in septic mice[72] and reduced mortality in severe peritonitis;[73] anti-IL-10-antibodies given before CLP increased mortality rate.[74] Pretreatment with anti-IL-10 increased plasma TNF levels, whereas IL-1 and IFN-γ could not be detected. IL-10 mRNA was observed in liver, spleen and lungs after CLP. The increased mortality rate with anti-IL-10 pretreatment could not be influenced by anti-TNF-antibodies.[75]

TNF exerts its effects by two cell surface receptors, TNF R I and II, also referred to as p55 and p75 receptors, respectively. TNF-R are transmembrane proteins which on cleavage of their extracellular domains result in the release of soluble fragments sTNF-R. sTNF-R increases markedly during infection and may serve to modulate TNF bioactivity. In endotoxin sensitive and resistant mice it was demonstrated that upon infection with LPS or live gram-negative bacteria there may be 2 separately regulated pathways that control sTNF-R shedding. Peritoneal macrophages of endotoxin sensitive mice responded to LPS stimulation; in contrast macrophages of resistant mice showed only a modest response.[76] Endotoxin induces downmodulation of monocyte and granulocyte TNF surface receptors in humans in vivo which may represent a mechanism to reduce excessive activity of TNF during systemic infection.[77]

Second Hit

Trauma may prime macrophages in such a way that a second hit to the immune system by infection or sepsis may lead to inadequate immune response. Trauma induces changes in endotoxin kinetics and PMN function in a model of trauma and posttraumatic peritonitis.[78] Synergism between trauma and infection was observed

for IL-1, but not for TNF.[79] After thermal injury and CLP macrophage production of inflammatory cytokines and arachidonic acid production has been downregulated and was associated with increased mortality.[80]

Therapy

Therapeutic intervention may influence the immune response. Resuscitation with fluids influenced TNF mRNA and IL-1 mRNA production in liver and intestines in intraabdominal sepsis.[81] Topical applied antiseptics or antibiotics may influence cytokine release in the peritoneal cavity or compounds used for other indications than sepsis may affect cytokine production, coagulation disturbances and mortality.[82]

Secondary Peritonitis and Cytokines in Clinical Studies

Increased TNF and IL-6 plasma levels correlated with outcome and APACHE II scores in several studies.[84-86] However, the determination of plasma cytokines is hampered by the interference with plasma proteins and receptors. Cell-associated cytokines may give a more realistic picture of the inflammatory response.[87] The information on the kinetics of cytokines during peritonitis is incomplete. TNF and IL-6 plasma levels were decreased before death in peritonitis and may indicate an anergic response induced by T-cell suppression.[88] However increased elastase production (a marker for PMN activation[89]) and increased neopterin production (a marker for macrophage activation[90]) in peritonitis do not support this notion.

Plasma IL-1 and IL-6 were increased even after large abdominal operations reflecting more the operative trauma than the infection.[91-93] The significance of local cytokine production has been supported by several studies. After colectomy IL-6 levels were higher in portal vein than in systemic circulation[94] supporting the hypothesis of bacterial translocation in portal and lymphatic circulation.[95] Bacterial peritonitis induces local release of proinflammatory cytokines and secondary mediators with subsequent interaction of endothelial cells and neutrophils, microcirculatory dysfunction and tissue damage.[96] The peritoneal cavity may be already cleared from pathogens by lavage, while the local release of inflammatory cytokines may continue.[97] Peritoneal levels of endotoxin, TNF, IL-1, IL-6 and elastase may be several fold higher than systemic levels. Peritoneal TNF and elastase levels decreased in survivors and remain elevated in nonsurvivors.[80] This lead to the conclusion that the mechanisms in sepsis and peritonitis may be similar. However, the immune response occurs in two functional different compartments and the intensity in both compartments may influence outcome.[98] This is also supported by studies in other compartments of the body.[99]

Treatment interventions may influence and modulate cytokine production. IL-6 levels correlated with mean arterial pressure in severe peritonitis. Reoperation caused hypotension which may have induced an early increase in IL-6 plasma levels.[100] Antibiotics can release, according to the type of penicillin binding protein (PBP), different amounts of endotoxin.[101,102] In surgical intensive care patients, PBP 3 specific antibiotics induced more often endotoxin release than PBP 2-specific antibiotics.[103] Several clinical studies with anti-endotoxin-antibodies and anti-TNF-antibodies have been performed in septic patients including patients with peritonitis. The clinical significance of endotoxin is still disputed. Endotoxin may not be the trigger for proinflammatory cytokine release,[104] however, it may help in the early detection of anastomotic leaks by endotoxin determination.[105] HA-1A reduces mortality in septic patients with endotoxemia and lowers serum TNF levels.[106] Other studies were

not successful in reducing the mortality of septic patients. This may be due to conceptual and organizational weakness of some studies. The fact that results from animal experiments and clinical studies are not well reflected in the design of these studies is further supported by many investigators. High circulating levels of IL-1ra and sTNF-R and the relatively small proportion of patients developing Endotoxin Core Antibody depletion may contribute to the limitations of therapies to augment natural defenses against endotoxin or proinflammatory cytokines.[107]

Conclusions

Endotoxin and cytokines play an important role in secondary peritonitis and contribute to the outcome of this life-threatening complication. The effect cytokines may have on the immune response depends on concentration, location and other co-factors, e.g., BPI and LBP. The addition of growth factors and cytokines to the treatment arsenal in peritonitis may not be advocated at this time with regard to the controversial results in animal and clinical studies. Cells and pathogens in the peritoneal cavity interact with each other and cytokines play an important part in this communication. However, our information on this network is rather limited. Therapeutic interventions, e.g., resuscitation, antibiotics, surgery, modulate the cytokine levels and thereby the immune response. The blockade of cytokines and endotoxin was not successful in most clinical studies. Newly developed anti-endotoxin-antibodies and compounds which block the endotoxin-induced activation of cells seem to be more promising. The concept of anti-inflammatory response in humans is not yet clear and needs further investigations and well planned studies to reveal the pathophysiological consequences of cytokines in secondary peritonitis.

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