T-cell Co-inhibitory Molecules in Sepsis-induced Immunosuppression: From Bench to Bedside

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Sepsis, which is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, is a leading cause of death in the Intensive Care Unit (ICU).[1] Epidemiological studies have shown that the incidence of sepsis has been gradually increasing during the past decades.[2,3] It still remains a major challenge for clinicians to find potential targets to improve the outcome of septic patients.

Numerous of studies showed that host immunological response played an important role in sepsis. Immune dysregulation is often associated with sepsis death distribution.[4] Hyperinflammation, which contributes to organ failure, is often the cause of early death within several days. However, more septic patients die in the late course because of persistent inflammation and secondary infection which induced by innate immune dysregulation and adaptive immunosuppression.[5] Therefore, regulating adaptive immunity may improve the outcome of septic patients. In sepsis, upregulated expression of CD80, CD86, CD25, and CD69 and the increased concentration of serum proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-1β indicate immune activation. By contrast, downregulation of the CD28 and monocyte human leukocyte antigen-DR (HLA-DR)-mediated activation pathway, upregulated expression of programmed cell death protein 1 (PD-1), and expansion of regulatory T-cell (TReg) and myeloid-derived suppressor cell populations indicate sepsis-induced immunosuppression.[6]

T-cells play an important role in adaptive immunity. The balance of co-stimulatory and co-inhibitory molecules, which are expressed on T-cells control the immunological response, is crucial in the adaptive immunosuppression induced by sepsis.[7,8] Upregulated expression of co-inhibitory receptors on T-cell surface inhibit T-cell function by inducing cell exhaustion and apoptosis. A postmortem study from Boomer *et al*. indicated that these co-inhibitory molecules may be potential targets in the treatment of sepsis.[9]

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Immunosuppression in Sepsis

Conventionally, intense inflammation, or which called "cytokine storm," was considered to be the most important host response to infection in the possible induction of organ dysfunction. Therefore, anti-inflammatory treatment was thought to be an effective strategy in sepsis. Results in animal experiments showed that TNF, IL-1β, IL-6, etc., monoclonal antibodies could greatly improve survival in the sepsis model. However, clinical studies of anti-inflammatory therapies failed to improve the outcome of septic patients. These results directed researchers to start a new direction of study in the immunological response to sepsis.

Recent studies showed that both pro- and anti-inflammatory response occurred in sepsis simultaneously, even in the early stages. Van Dissel *et al*.[10] measured circulatory cytokines in febrile patients and found that in addition to an increased level of proinflammatory cytokine TNF-α, the anti-inflammatory cytokine IL-10 showed elevated levels. Furthermore, the high ratio of IL-10 to TNF-α was found to be associated with fatal outcome in the community-acquired infection. Later, Monneret *et al*.[11] measured cytokines

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and monocyte HLA-DR expression in septic shock patients and found that all included patients had elevated IL-10 concentrations and decreased HLA-DR expression. Interestingly, the proinflammatory cytokine TNF-α was not dissimilar between survivors and nonsurvivors. However, there were significantly higher IL-10 concentrations and lower HLA-DR expression in nonsurvivor patients. These results indicate that not only does the anti-inflammatory response occurred in sepsis but is also associated with the outcome.

It has also been demonstrated that there is a significant loss of lymphocyte cells and other immune cells during sepsis which is associated with programmed cell death.[12] Hotchkiss *et al*.[13] reported that caspase-3-mediated lymphocyte apoptosis in sepsis contribute to immunosuppression. Besides immune cell depletion, lymphocyte exhaustion is also common during sepsis as Boomer *et al*.[9] found upregulated expression of exhaustion markers such as T-cell immunoglobulin mucin domain-containing protein 3 (TIM-3), and lymphocyte activation gene 3 (Lag-3) on T-cells in septic patients. Thus, sepsis directly or indirectly impairs almost all categories of immune cells.[4] The study has demonstrated that sepsis results in immunosuppression which induces secondary infection and death.

With a better understanding of the pathophysiology mechanism of immunological response during the different stage of sepsis, immunosuppression, but not hyperinflammation, is considered to be a more critical factor affecting the outcome of septic patients.[6,14] Otto *et al*.[15] reported that the late phase of sepsis was associated with significantly increased positive blood culture results and incidence of opportunistic infection which indicates that immunosuppression is the predominant factor to cause high mortality in sepsis. In addition, Boomer *et al*.[9] performed a postmortem study concerning immune function in sepsis. They harvested the spleen and lung cells from patients who died of septic and nonseptic and tested the function of immune cells. The results showed some of the mechanisms of immunosuppression in sepsis which involves both the innate and adaptive immune system. They found that when compared with nonseptic patients, the expression of co-stimulatory molecules such as CD28 was downregulated and the expression of co-inhibitory receptors such as PD-1 was upregulated in T-cells in septic patients. These results indicate that targeted immune-enhancing therapy may be an effective strategy in septic patients.

Adaptive immunosuppression, often associated with significant morbidity and mortality is critical in sepsis. T-cells play a crucial role in adaptive immunity. Theoretically, the upregulated expression of co-inhibitory receptors and the downregulated expression of the co-stimulatory receptor is the sign of sepsis-induced immunosuppression. Therefore, the co-signaling molecules may be further targets for sepsis therapy.

Effect of T-cell Co-inhibitory Molecules in Sepsis-induced Immunosuppression

T-cell co-signaling receptors which transduce positive or negative signals into T-cells are all cell-surface molecules.[8] T-cell function is controlled by the balance of expressing co-signaling molecules. At present, few studies have reported on the effect of inhibiting the co-stimulatory receptors of T-cell in immunosuppression induced by sepsis. By contrast, numerous animal experiments have been performed and the results show that blocking co-inhibitory signals improves the outcome of sepsis.

It has been shown that there are more than ten co-inhibitory receptors expressed on the T-cell surface,[9] among which several have been well studied in sepsis-induced immunosuppression such as PD-1 and cytotoxic T-lymphocyte antigen 4 (CTLA-4). Strategies of targeting this co-inhibitory to reverse immunosuppression in sepsis have begun to be applied in patient care.

Programmed Cell Death Protein 1

PD-1, a member of the B7-CD28 superfamily, is one of the best characterized co-inhibitory molecules. It is shown that PD-1 plays an important role in host immunological response. In sepsis, PD-1 is not only upregulated on T-cells but also on monocytes/macrophages, impairing the function of these immune cells.[19] Signaling through PD-1 inhibits the ability of T-cells to proliferate and attenuates cytotoxic T-cell function. Animal studies showed that CD4 and CD8 T-cells of PD-1 expression increased by 48 h after cecal ligation and puncture (CLP). Blocking the PD-1 signal could inhibit lymphocyte apoptosis and improve 7 days survival in the CLP model.[17]

Recent clinical studies indicated that PD-1 upregulation on the T-cell surface was associated with mortality. Chang *et al*.[18] reported that CD8 expression of PD-1 was significantly higher in septic patients compared to non-septic patients. After blocking PD-1 *in vitro*, they found that anti-PD-1 decreased cell apoptosis and improved cellular functions of T-cells from septic patients. Shao *et al*.[19] showed that PD-1 of T-cell expression was significantly higher in septic shock patients than septic patients without shock. In addition, they also found significantly elevated PD-1 expression in nonsurvival septic shock patients compared to survival. These results indicate that anti-PD-1 may improve survival of septic patients. This is currently being investigated by a clinical study (NCT 02960854) in America.

Cytotoxic T Lymphocyte Antigen 4

CTLA-4, also known as CD152, is upregulated in T-cells after activation and plays an important negative regulatory role in the immune system. CTLA-4 is homologous to the T-cell co-stimulatory receptor CD28, which binds CD80 and CD86, transmits an inhibitory signal to T-cells and prevents T-cell activation. In the murine sepsis model, CTLA-4

expression increased not only on CD4 and CD8 T-cells but also on TReg cells.[20] Anti-CTLA-4 (aCTLA-4) therapy decreased sepsis-induced apoptosis. It has been shown that aCTLA-4 has a dosage-dependent effect on survival where high dose worsened survival while low-dose improved survival.[20] Furthermore, Chang *et al*.[21] revealed that aCTLA-4 therapy could also improve survival in primary and secondary fungal sepsis in animal experiments. In clinical studies, the CTLA-4 expression of CD4 T-cells was significantly higher in septic patients than nonseptic patients which indicate that it is a potential target for sepsis therapy.[22] However, further study needs to be performed to confirm the effect of the aCTLA-4 block in sepsis.

T-cell Immunoglobulin and Mucin Domain Protein 3

T-cell immunoglobulin and mucin domain protein 3 is another co-inhibitory molecule which regulates both innate and adaptive immune functions. Yang *et al*.[23] studied the effect of TIM-3 on immune function in sepsis. They found that Tim-3 is a negative regulator of the toll-like receptor-mediated immunological response. Interestingly, different from PD-1 and CTLA-4, when the authors blocked the Tim-3 pathway, they found that lymphocyte apoptosis was exacerbated and mortality increased. A possible reason for this contrasting result is that blocking the Tim-3 pathway might exacerbate the sepsis-induced proinflammatory response during the early phase of sepsis. From these results, it can be ascertained that timing is a crucial factor for immunotherapy in sepsis.

B and T Lymphocyte Attenuator

B and T lymphocyte attenuator (BTLA), expressed on T-cells, B-cells, natural killer cells, macrophages and dendritic cells is upregulated in macrophages, inflammatory monocytes, dendritic cells and CD4 T-cells and induces immune cell dysfunction in the murine sepsis model. BTLA-deficient mice that received CLP had reduced lymphocyte apoptosis and lower mortality when compared to the wild-type.[24] Shubin *et al*.[25] reported that an increased $BTLA^+$ $CD4^+$ lymphocyte frequency in nonseptic critically ill patients was associated with a subsequent infection. These results indicate that BTLA could be used as a biomarker and mediator of sepsis-induced immunosuppression.

However, different results were later found in a clinical study. A prospective clinical study involving 336 septic patients from Shao *et al.* showed that the percentage of $BTLA^+$ $CD4^+$ T-cells was higher in healthy volunteers than in septic patients.[26] In addition, a lower percentage of $BTLA^+$ $CD4^+$ T-cells in the early stages of sepsis is associated with the severity and the mortality. The contradictory results between the two clinical studies may be explained by the utilization of different populations in varying phases of sepsis during the periods when BTLA expression was measured. Therefore, further studies need to be performed to confirm the function of BTLA in sepsis.

Other co-inhibitory molecules expressed on T-cells are also studied in other conditions than sepsis. For example, Lag-3 and T-cell immunoglobulin and ITIM domains (TIGIT) are well studied in autoimmunity disease, cancer, and chronic viral infections.[27] However, these co-inhibitory molecules may also play an important role in sepsis which needs to be investigated in the future.

Immunotherapy in Sepsis-induced Immunosuppression: One Size Does Not Fit All

In addition to antibiotic and other sepsis management strategies, immunotherapy may be an effective approach to improve the outcome of septic patients. Several small clinical studies with therapy by granulocyte macrophage colony stimulating factor, IL-7 and interferon-γ showed beneficial effects in sepsis.[28-30] However, the immunological responses of sepsis are too complex to be characterized by a simple immune method. While intensive inflammation and immunosuppression both induce poor outcome in sepsis, it is difficult to decide between decreasing excessive inflammation or boosting host immunity.

Targeted immunotherapy on co-signaling molecules involved in sepsis has resulted in different effects. For example, in the early phase of sepsis, during which host has hyperinflammation, blocking the co-inhibitory molecule Tim-3 can increase mortality in experimental models,[23] while blocking the co-stimulatory molecule CD28, significantly decreased mortality.[31] As recent study describes, immunotherapy should be based on immune system status. Shakoory *et al*.[32] reanalyzed the data of a Phase III study concerning the role of an IL-1 receptor antagonist in sepsis. In that study, the IL-1 receptor antagonist failed to improve 28-day survival in sepsis patients. However, when they analyzed the data of a subgroup patients with macrophage activation syndrome, the IL-1 receptor antagonist clearly reduced mortality. Thus, we believe that boosting host immunity offers increased effectiveness in patients with dominant immunosuppression but not intensive inflammation.

The immune system provides a diverse array of responses in the different stages of sepsis. In the early phase of sepsis, the proinflammatory response may be dominant while immunosuppression is dominant in the late stage.[6] However, it is difficult to pinpoint the exact phase of sepsis in the clinic. In addition, several factors such as age, microorganism load, virulence, and comorbidities of patients also affect the host response in sepsis. Therefore, determining the immune status of sepsis patients is crucial to guide immunotherapy. At present, HLA-DR has been the best studied and may be used as an optimal clinical marker to determine the immune status in sepsis patients.[33]

In conclusion, immunosuppression induced by sepsis is very common and co-inhibitory molecules expressed on the T-cell surface play a crucial role in this mechanism. Several studies have shown that targeting these molecules could improve

survival in sepsis which indicates that these co-inhibitory molecules may be potential targets for sepsis treatment. However, precision immunotherapy based on the immune status of patients is needed while we reverse the immunosuppression. Only in the right patient and at the right time, immunotherapy improves the survival of patients with sepsis.

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Conflicts of interest

There are no conflicts of interest.

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