Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Advances in researches on the immune dysregulation and therapy of severe acute pancreatitis*

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Received Aug. 30, 2008; Revision accepted May 5, 2009; Crosschecked Apr. 27, 2009

Abstract: During the development and progression of severe acute pancreatitis (SAP), conspicuous immune dysregulation develops, which is mainly manifested as excessive immune response in the early stage and immunosuppression in the late stage. This process involves complex changes in a variety of immune molecules and cells, such as cytokines, complements, lymphocytes, and leukocytes. With the gradual deepening of studies on the development and progression of SAP, the role of immune dysregulation in the pathogenesis of SAP has attracted more and more attention. In this article, we review the advances in research on the immune dysregulation in SAP and the immunotherapy of this disease through exploring the formation of excessive immune response and immune suppression as well as their mutual transformation.

Key words: Severe acute pancreatitis (SAP), Immune, Dysregulation, Treatment
doi:10.1631/jzus.B0820265 **Document code:** A **CLC number:** R58

INTRODUCTION

The causes of severe acute pancreatitis (SAP) mainly include gallstones, alcohol, iatrogenic, medications, and some rare diseases such as mitochondrial cytopathy. Although its origins vary, the development of this disease has a close relationship with the imbalance of immune system (DiMagno and DiMagno, 2007; Skipworth and Pereira, 2008; Frossard *et al*., 2008). The nature of immune dysregulation is the dysregulation of T helper 1 (Thl)/T helper 2 (Th2) cytokines during the development and progression of diseases (Pietruczuk *et al*., 2006). Th1 and Th2 cells, which can secrete different cytokines, are derived from Th0 cells that are generated from the differentiation of $CD4^+$ T cells stimulated by antigens. Thl cells are often associated with cellular immune responses, while Th2 cells are often involved in humoral immune responses. Thl/Th2 cytokines form an interactive network whose equilibrium is crucial for maintaining the body's homeostasis. Some studies show that, in the early stage of SAP, the expression of peripheral blood lymphocyte activation markers, such as CD69, CD25, CD28, CD38, and CD122, is enhanced. Additionally, cytokines such as interleukin-2 (IL-2), IL-4, IL-5, IL-10, Interferon gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) also increase substantially and induce a cascade reaction, thereby triggering excessive immune response. In contrast, in the late stage of SAP, the number of peripheral blood lymphocytes decreases. Particularly, the number of B lymphocytes decreases more significantly than that of T lymphocytes. As a result, humoral and cellular immune responses are inhibited to varying degrees, thus decreasing immunity of SAP patients and increasing their bodies'

* Project supported by the Technological Foundation Project of Traditional Chinese Medicine Science of Zhejiang Province (Nos. 2003C130 and 2004C142), and the Foundation Project for Medical Science and Technology of the Health Bureau of Zhejiang Province (No. 2003B134), China

susceptibility to external infection (Pietruczuk *et al*., 2006).

EXCESSIVE IMMUNE RESPONSE

When SAP develops, the massive release of Th2 cytokines can exacerbate the transformation of Th0 cells into Th2 cells. The increase in the number of Th2 cells can in turn promote the production of numerous pro-inflammatory cytokines and intensify systemic inflammatory response (Pietruczuk *et al*., 2006). Excessive immune response-induced multiple organ dysfunction in SAP patients, to a large extent, contributes to strong inflammatory response. Other immune factors, such as complement activation and enhanced activity of leukocytes, can also aggravate excessive immune response to a certain extent.

Roles of Th2 cytokines in the development of excessive immune response in SAP

In the early stage of SAP, the inappropriate activation of phospholipases, elastases, and other digestive enzymes activates the cascade of Th2 cytokines such as TNF-α (Malleo *et al*., 2007), IL-6 (Pooran *et al*., 2003), and other mediators of inflammation (Al Mofleh, 2008). Among of them, IL-6 seems to be the most promising parameter for clinical assessment (Schütte and Malfertheiner, 2008). In contrast, during the phase of excessive immune response in SAP, the release of some anti-inflammatory factors such as IL-4 and IL-10 is also intensified. However, these anti-inflammatory factors show no effect in reducing inflammatory reaction, which is mainly manifested as the following three aspects. First, although the secretion of anti-inflammatory factors increases in absolute levels during the phase of excessive immune response, their increase in relative levels is far less than that of pro-inflammatory factors. Ohmoto and Yamamoto (2005) found that, before treatment, the ratio of serum IL-10 and IL-6 significantly decreased in SAP patients. After treatment, this ratio increased back. These results suggest that excessive pro-inflammatory reaction is the main contributing factor of immune dysregulation in SAP patients. Second, the expression of cytokines such as IL-4 and IL-10 can promote the expression of Th2 cytokines in various ways, thereby enabling the equilibrium to move towards Th2 cells. For example, in the early stage of SAP, IL-4 expression can promote the differentiation and development of Th2 lymphocytes (Romagnani, 2000), while IL-10 is involved in inducing the apoptosis of Th1 lymphocytes. These effects can ultimately cause the dysregulation of Thl/Th2 cytokines (Ayala *et al*., 2001). Third, the interactions among cytokines can form a complex network, in which changes in the levels of one or several anti-inflammatory factors cannot play a decisive role.

Roles of other immune factors such as the complement system and leukocyte activity in the development of excessive immune response in SAP

The complement system, as an important component of non-specific immune system, plays an important role in inducing excessive immune response in SAP. As complement components C3 and C5 can be activated directly by pancreatic enzymes and thus decomposed, the massive release of pancreatic enzymes in SAP has an important effect on the activation of the complement system (Roxvall *et al*., 1991). After being activated, complements and their active fragments can promote macrophage recruitment, enhance their phagocytic and digestive abilities, activate neutrophils and vascular endothelial cells, and alter fibrinolytic system activity and intravascular coagulation process, thus being involved in the lung injury in SAP (Gloor *et al*., 2003). Hartwig *et al*. (2006) found that injecting soluble complement receptor-1 into SAP rats could effectively inhibit the activation of leukocytes and endothelial tissue by the complement system and reduce pancreatic necrosis and lung injury. In addition, the activities of leukocytes also increase significantly. Paulino *et al*.(2007) found that, under normal circumstances in in vitro experiments, the endothelial tissue injury induced by neutrophils needed the activation of endothelin (ET) via ET receptors. In contrast, the neutrophils isolated from SAP patients could induce more severe endothelial tissue injury in an ET-independent manner. In the present studies, the platelet-activating factor (PAF) has been considered as a key mediator in the progression of SAP, which can lead to severe complications and high mortality rate (Chen *et al*., 2008).

IMMUNOSUPPRESSION

With the massive release of various cytokines during the phase of excessive immune response in SAP, peripheral blood lymphocytes are urgently mobilized. However, after experiencing the processes of activation, reaction, and apoptosis, the number of these cells gradually declines. Accordingly, the levels of pro-inflammatory and anti-inflammatory factors also decrease (Han *et al*., 2003). Subsequently, a large number of spleen lymphocytes enter into the blood circulation to maintain immune response. At this time, the decrease in the total number of peripheral blood lymphocytes is not significant, and immunosuppression is not obvious. However, with the intensification of inflammation, endogenous anti-inflammatory factors cannot effectively mitigate inflammatory response. Thus, more spleen lymphocytes enter into the blood circulation. Ultimately, the spleen becomes overburdened. As a consequence, the number of spleen lymphocytes declines sharply. Due to the insufficiency of the number of peripheral blood lymphocytes, the suppression of the immune system is caused (Yasuda e*t al*., 2002).

Decrease in the numbers of peripheral blood and spleen lymphocytes

The vital roles of immunosuppression in the development and progression of SAP mainly attribute to the decrease in the numbers of peripheral blood and spleen lymphocytes. After examining 101 SAP patients with or without infection, Ueda *et al*.(2006) found that the levels of serum IgG and IgM as well as the numbers of lymphocyte-activated killer cells and natural killer cells were lower than the normal values by 50.0%, 65.0%, 45.5%, and 42.4%, respectively. Moreover, the numbers of $CD4^+$, $CD8^+$ and $CD20^+$ T lymphocytes were also significantly lower than the normal ranges. The numbers of $CD4^+$ and $CD8^+$ T lymphocytes in patients with infection decreased more significantly. With the continuous development of immunosuppression, SAP can cause serious secondary infections and damages to multiple organs (Beger and Rau, 2007). Additionally, through examining the numbers of spleen and peripheral blood lymphocytes in SAP rats, Yasuda *et al*.(2002) found that, at 12 and 24 h after the onset of SAP, the weight of the spleen declined and the number of spleen lymphocytes decreased. At 6, 12 and 24 h after the onset of SAP, the number of peripheral blood lymphocytes decreased significantly, accompanied by an increase in nuclear debris and deoxyribonucleic acid (DNA) fragments. In contrast, the apoptosis of spleen cells could not be detected. These results suggest that a large number of spleen lymphocytes enter into the blood circulation to compensate for the loss of peripheral blood lymphocytes.

Roles of Th1 cytokines in the development of immunosuppression in SAP

IFN-γ secreted by Th1 cells can not only protect tissues against virus-mediated injury but also induce self-damage to the body's tissues. In SAP, IFN-γ secretion is inhibited, thus aggravating the disease. Ma *et al*.(2006) found that stimulation of spleen cells with antigen in SAP rats could lead to a significant decrease in the secretions of IL-2, IL-12, and IFN-γ by these cells when compared with those in the control group. Rau *et al*.(2006) found that treatment of SAP rats with recombinant rat IFN-γ could postpone the process of pancreatic tissue necrosis, reduce neutrophil infiltration and decrease the levels of IL-1 in pancreatic tissues. These results suggest that inadequate release of Thl cytokines, such as IL-2, IL-12, and IFN-γ, can aggravate the immunosuppression in SAP.

Roles of other immune factors in the development of immunosuppression in SAP

The levels of immunosuppression in SAP patients can be evaluated using the expression level of monocyte human leukocyte antigen HLA-DR, which is mainly expressed on the membranes of B lymphocytes, monocytes and endothelial cells. Kylänpää-Bäck *et al*.(2001) found that the expression level of HLA-DR showed a sustained decline in SAP patients. Patients surviving after SAP showed a back elevation in the expression level of HLA-DR, while dead patients showed no back elevation. Through observing 57 cases of SAP patients, Krivoruchko *et al*.(2003) found that only 51.2% of SAP patients complicated with pancreatic necrosis and infection had HLA-DR-positive lymphocytes. Moreover, the activities of T cells were inhibited significantly and the activities of neutrophils were very low. In addition, the expression of toll-like receptors (TLRs) on the

surface of macrophages was also inhibited in SAP (Matsumura *et al*., 2007). Furthermore, the above-mentioned Thl cytokines, such as IL-4 and IL-10, also play important roles in the development of immunosuppression in SAP. These factors work together with pro-inflammatory factors to form an inflammatory mediator network system that can ultimately lead to greater damage to the body.

IMMUNOTHERAPY OF SAP

Considering that immune dysregulation is present in SAP, some researchers have suggested that immunotherapy is adopted to treat this disease by using cytokine- or endotoxin-specific antagonists to block the actions of inflammatory mediators or inflammatory cytokines. At present, anti-tumor necrosis factor monoclonal antibodies and soluble IL-1 receptor have been successfully developed. However, although these drugs show prominent effects in antagonizing excessive immune response in animal experiments, the majority of them yield no expected effects in clinical trials. Instead, some of them may increase the mortality rates of patients, which is perhaps due to the fact that the treatment is too unitary and limited (Iwagaki *et al*., 1997). Since the immune system is a whole system, there are complex feedback interactions among the cytokines that are involved in inflammatory reaction. Once inflammatory reaction is started, a single factor cannot terminate the immune reaction of the entire network. Inappropriate immune intervention may further destroy the immune equilibrium.

Immunosuppressants

Since excessive immune response is the main immune event present in the early stage of SAP, appropriate short-term use of immunosuppressants, under the prerequisite of using potent antibiotics, may be a safe, effective and economical way for the therapy of SAP. Dexamethasone, as a glucocorticoid hormone, is able to significantly lower the levels of inflammatory mediators, mitigate pancreatic inflammation, and reduce the incidence rates of complications and transit operations. Therefore, it is an effective drug for comprehensive treatment of SAP (Ma *et al*., 2002). Some studies have found that dexamethasone can promote the secretion of IL-10 by either peripheral blood monocytes or $CD4^+$ T cells cultured in vitro (Fushimi *et al*., 1997). IL-10, as an important anti-inflammatory cytokine, has strong immunosuppressive effect. It can effectively inhibit not only the production of cytokines by monomacrophages, such as TNF-α, IL-1, IL-6, and granulocyte-macrophage colony stimulating factor (GM-CSF), but also the expression of major histocompatibility antigen major histocompatibility complex (MHC)-II by monocytes. An animal experiment (Chen *et al*., 2002) indicated that immunosuppressive drugs such as 5-fluorouracil (5-FU), cyclophosphamide, and methotrexate could significantly inhibit the expression of inflammatory cytokines TNF-α, IL-1 and IL-6 in SAP. Simultaneous with the decrease in the expression of inflammatory cytokines, the expression of anti-inflammatory cytokines transforming growth factor-β (TGF-β) and IL-10 also decreased. This result suggests that immunosuppressive drugs have bidirectional effects on the immune dysregulation in SAP, through which abnormal immune status can return to normal one, the illness is mitigated, and the aggravation of SAP is prevented. Currently, it has been confirmed that other drugs such as ethyl pyruvate can improve survival rate, ameliorate distant organ injury, and significantly decrease serum TNF, IL-1 and IL-6 levels in experimental models during SAP, therefore having some potential clinical values (Fink, 2008).

Immunomodulators

Currently, the use of immunomodulators in the therapy of SAP is still in the experimental stage. Immunomodulators, of which thymosin alpha 1 (TA1) and IFN-γ are more often used, are mainly used in the immunosuppressive stage of the disease to improve immunity, prevent infection, and reduce the mortality rate.

TA1, an extract from the thymus, has hormone-like activities. It has been increasingly used in clinical practice, since it has anti-aging, anti-tumor and anti-infection effects and is able to auxiliarily treat autoimmune diseases and acquired immunodeficiency syndrome (AIDS) as well as modulate the neuroendocrine system. Some studies (Sjogren, 2004; Armutcu *et al*., 2005) have found that TA1 is also able to activate macrophages, dendritic cells,

mitogen-activated protein kinase (MAPK) signal transduction system and innate immune system, and inhibit the activity of lipid peroxides. It has been found that, in SAP rats subcutaneously injected with TA1, TA1 can improve tissue injury and reduce the mortality rate through modulating $CD3^+/CD4^+$ T cell ratio and reducing the release of inflammatory factors and cytokines (Yao *et al*., 2007).

IFN-γ can upregulate the expression of MHC-1 molecules in nucleated cells, activate macrophages, promote the secretion of IL-12 by macrophages, and inhibit the proliferation of Th2 cells, thus enabling the differentiation towards Th1 cells. In SAP, the activities of IFN-γ are inhibited. As a result, the disease is aggravated. Hayashi *et al*.(2007) found that IFN-γ could exert anti-inflammatory effects through inhibiting nuclear factor-κB (NF-κB) induced pro-inflammatory response, thereby mitigating the disease. Through in vitro supplement of IFN-γ into the blood samples from 28 SAP patients, Kylanpaa *et al*.(2005) found that IFN-γ could significantly increase the level of TNF-α in a toxin-dependent manner and raise the percentage of HLA-DR positive monocytes.

CONCLUSION

To sum up, excessive immune response is related with the multiple organ failure present in the early stage of SAP, whereas immunosuppression is a potential factor contributing to the infection present in the late stage of this disease. At present, it is yet not fully clear what relationship exists between excessive immune response and immunosuppression. When immunomodulatory approaches are clinically applied to treat SAP, the treatment should be conducted according to doctors' experience. The efficacy of immunomodulatory therapy on SAP still needs to be validated by using large-sample, multi-center, randomized, double-blind studies. Immune interventions based on the changes in immune function in different stages of SAP have gradually become an important means for comprehensive treatment of this disease. We believe that immunotherapy of SAP represents an important direction for the treatment of this disease and therefore has broad prospects for application.

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