SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

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TIPOLOGIA DI BORSA RICEVUTA: Borsa di studio SIF-MSD Italia

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): Finale

TITOLO DELLA RELAZIONE: Study of the role of dendritic cells in the endotoxin tolerance

RELAZIONE:

Sepsis and septic shock are frequent complications in the hospitalized patients that can result in multiple organ failure and death. Therefore, sepsis is an important public health problem, with increasing incidence and persistently poor outcome. In 2012, over 20 million people have been affected by sepsis worldwide [1], and the mortality from septic shock and severe sepsis both in Europe and in USA is around 30%. Sepsis is defined as the systemic inflammatory response syndrome (SIRS) due to infection [2], which indicated that SIRS and infection are two important factors in determination of sepsis. Although in recent years outcomes in sepsis have greatly improved, because of an enhanced focus on early diagnosis and fluid resuscitation, morbidity and mortality are still unacceptably high.

Pathophysiologically, sepsis develops when the initial appropriate host response to an infection becomes amplified and then dysregulated leading to haemodynamic changes, and is characterized by an hyperinflammatory response in the early stage and followed by a period of immunosuppression. The complex cascade which results in the release of pro- and anti-inflammatory mediators conditions the early stages of sepsis and is crucial for prognosis. AhR is a ligand-activated transcription factor that is activated by dioxins and other environmental pollutants.

We now know that AhR can bind a broad variety of activating ligands that are disparate in nature, including endogenous [3] molecules and those formed in the gut [4] from food and bacterial products. Consequently, in addition to its classical role as a toxicological signal mediator, AhR is emerging as a transcription factor involved in the regulation of both innate and adaptive immune responses in various immune cell types, including lymphocytes and antigen-presenting cells (APCs). During the progression of sepsis, DCs take part in the aberrant immune response and are necessary for survival [5]. Therefore, defining DCs pathology may permit to better understand the early stages of sepsis, and will be undoubtedly beneficial to increase treatment options and improve survival of patients with sepsis.

Reducing the mortality and preventing severe organ failure from sepsis remain two of the most significant unmet medical needs of our current age. Severe lethality associated with infection in sepsis is a product of both intrinsic pathogen virulence and the overt innate immune response to the infection. There are two distinct host strategies that deal with an infection: 1. elimination of the pathogen (resistance) or 2. reduction of the negative impact of infection, resulting in an increased tissue fitness against damage (disease tolerance) [6]. Although recent studies, using gene-knockout mice, have led to an in-depth understanding of the innate sensors that detect pathogens in a variety of cell types [7, 8] we still lack basic knowledge on how the outcome of innate sensor stimulation may promote resistance vs disease tolerance. Notably, on this regard we have recently demonstrated that activation of one of these sensors, AhR, by specifc tryptophan metabolites in DCs, represents a pivotal mechanism in the activation of protective status of disease tolerance using an experimental animal model of Lipopolysaccharide (LPS)- induced septic shock. Activation of this pathway protected the mice against immunopathology both in Gram-negative and Gram-positive infections [3, 9, 10]. AhR is critically involved in maintaining appropriate barrier immunity, contributing potentially through DCs involvement, to the development of innate lymphoid cells (ILC)-3, producing tissue protective IL-22 particularly in the intestine [4].

In the previous 6 months, we found that LPS-primed splenic cDCs, treated with L-kynurenine promoted IDO1 expression at the mRNA and protein levels, both effects requiring AhR. Moreover, in the same setting, co-immunoprecipitation studies showed that AhR and RelB are able to interact in cDCs. Analysis of the *Ido1* promoter showed the presence of three mouse RelB responsive elements (mRelB), located at positions -3403, -3735, and -4473, relative to the start site of *Ido1* transcription. Chromatin-immunoprecipitation experiments showed specific binding of RelB to the *Ido1* promoter in LPS-primed cDCs treated with L-kynurenine. This effect was associated with the

maintenance of IDO1 enzymic activity over a prolonged timeframe (up to 48 h) in cDCs. Additionally, on examining *Ido1* transcription in *Relb*-silenced cDCs treated with LPS and L-kynurenine, we found that lack of functional RelB, but not of Arnt (aryl hydrocarbon receptor nuclear translocator), impaired *Ido1* transcription in cDCs. The occurrence those binding sites in the *Ido1* promoter suggested that RelB-AhR heterodimers regulate the induction of IDO1 at the transcriptional level, thus reinforcing the enzymic and signaling functions of IDO1 in cDCs. Altogether, these data suggest that LPS-primed DCs, rechallenged with L-kynurenine, participate in a feedforward loop enhancing *Ido1* trascription that may be instrumental in a stable immunomodulatory function of cDCs.

In the last 6 months, we provided the first in-depth understanding and characterization of the AhR system, specifically in DCs, as an important mechanism for healthy protective immune response in sepsis.

Several study demonstrated the loss of DCs occurring in spleens from patients with sepsis, no loss in macrophages was detected in spleens from patients with sepsis vs trauma. One possible explanation for the difference in the response to sepsis of these two cell types may be their propensity to undergo apoptosis [11]. Kenneth Murphy et *al* [12], in 2012, demonstrated that the expression of *Zbtb46* seemed to distinguish cDCs from macrophages in the intestine independently of their expression of the integrins CD103 (αEβ7) or CD11b(αM) and the administration of diphtheria toxin to *Zbtb46*^{DTR} mice should selectively eliminate cDCs but spare macrophages. Thus, we used this system to determine whether cDCs were required for protection against LPS sterile septic shock. We found that *Zbtb46*^{DTR} chimeras treated with diphtheria toxin were unable to recover after challenge with LPS and died within 2–6 d of infection. In contrast, wild-type mice reconstituted with wild-type bone marrow (wild-type chimeras) and *Zbtb46*^{DTR} chimeras not treated with diphtheria toxin survived beyond 7 d. These results demonstrated that cDCs, were required for early innate defense against LPS challenge, but did not indicate whether a particular cDC subset was required.

LPS is known to induce DC loss in vivo [13], but the mechanism is not well understood. In steady-state *Ahr*$^{-/-}$ mice, there was a statistically significant increase in the fraction of CD8^{+} DCs. Splenic DC populations were analyzed at 16 hrs following LPS challenge, a substantial loss of both CD11c^{+}MHCII^{+} and CD8^{+} DCs was observed in WT mice. Strikingly, in *Ahr*$^{-/-}$ mice, although the loss of CD8^{-} DCs resembled that in WT animals, the loss of CD11c^{+}MHCII^{+} and CD8^{+} DCs was completely prevented. These results demonstrate that endogenous AhR are responsible for LPS-induced CD8^{+} DC loss in vivo.

Based on the current research front and our research, we hypothesize that activation of AhR in selected subsets of host DCs, contribute to the protective transition from disease promoting immune responses (immunophatology) to a disease protective (disease tolerance) in sepsis. This hypothesis represents a fundamentally novel conception in understanding the cells and mechanisms involved in the transition from a friendly to a foe immune response in severe sepsis patients.

References

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