PhD thesis

Experimental and bioinformatical investigation of the human antimicrobial immune response

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Introduction

Despite the rapid development of intensive care medicine, life-threatening infections are still a serious problem regarding both medical and financial points of view. A better understanding of sepsis immunology would be of a high importance to develop new therapeutic tools and improve survival.

The gut as a motor of sepsis was first described in the 80s when the translocation of bacteria through the injured intestinal mucosa in the immunocompromised, critically ill host was belived to be a major problem. Since then, our knowledge on the intestinal compartment in sepsis has improved a lot. Recent research has discovered that some bacteria react sensitively to danger signals in their surrounding and activate virulence genes in response. Studies on intestinal ischemia-reperfusion also pointed out, that under certain stress situations the gut mucosa may contain harmful mediators that are drained via gut lymph and play a role in the development of multiple organ dysfunction.

It is very well known that the antigen specificity of lymphocytes is not absolute, since similar or identical epitopes may be present on unrelated structures and may be recognised be the same lymphocyte clone. This phenomenon is called molecular mimicry, and turned out to be – regarding at least linear epitopes

and T-cell mimicry – pretty frequent. Considering the new data on the importance of the intestinal mucosa in the pathophysiology of sepsis, we hypothesized that pathogen-specific T-lymphocytes might potentially induce a secondary inflammation in the gut mucosa based on immunological similarity between pathogens and gut commensals, what may have a harmful effect on disease course and outcome.

Apart from the gastrointestinal tract, immunological exhaustion and anergy are also relevant problems in septic patients since these may trigger secondary infections. The accurate immunological pathomechanism for this phenomenon has not yet been described in details, however, certain studies have pointed out the tolerogenic differenciation of monocyte-derived dendritic cells in human sepsis, that induce anergic or regulatory T lymphocytes. This problem is of exceptional importance, considering that dendritic cells are alone able to prime naive T lymphocytes and they may shape the entire adaptive immune response this way. The molecular mechanism of the tolerogenic differenciation of dendritic cells is obscure, however, some studies raised the possibility that the CR3 complement receptor might play a role.

Objectives

- We mathematically estimated the possibility of T-cell molecular mimicry between gut commensals and the identified pathogenic flora of patienst with pneumonia induced sepsis.
- We calculated „inflammatory quotients” for identified pathogen or pathogenic flora, which represent the predicted ability of pathogen-specific T-lymphocytes to induce a secondary inflammation in the intestinal mucosa based on the immunological similarity between pathogens and commensals (molecular mimicry).
- We investigated the relationship between the estimated pathogen versus gut comensals mimicry possibilities and APACHE II disease severity scores along with disease outcome.
- We investiganed the relationship between the estimated mimicry tendency of various pathogens and the sepsis mortality rates by causative agent obtained from the results of the SOAP study.

- We characterized the expression of complement receptors on human mature and immature monocyte-derived dendritic cells.
- We analyzed the uptake of iC3b and iC3b-opsonized particles by dendritic cells, along with the role of CR3 and CR4 in this process.
- We investigated the effect of CR3- and CR4-derived signals on the cytokine production and T-cell activating capacity of dendritic cells.

Methods

- Analysis of clinical data of patients with pneumonia-induced sepsis
- Mathematical estimation of molecular mimicry via a method published by Ristori et al.
- Human cell culture
- FACS
- Determination of phagocytic index using an invertoscope

- RNA-silencing
- Confocal laser scanning microscopy
- MLR
- ELISA
- Statistical analysis

Results

- We found a correlation between inflammatory quotients (calculated based on the identified pathogens of patients with pneumonia induced sepsis) and APACHE II disease severity scores.
- In patients with pneumonia induced sepsis, pathogenic floras identified in the survivor group had significantly lower inflammatory quotients compared to pathogenic floras in non-survivor patients.
- We found a correlation between sepsis mortality rates by causative agent (based on the results of the SOAP study)

and the corresponding inflammatory quotients of the pathogens.

- We demonstrated that the expression of CR3 decreases while that of CR4 increases during the maturation of human monocyte-derived dendritic cells (MDCs).
- Our results point out the dominant role of CR3 over CR4 in the phagocytosis of iC3b-opsonized particles by human MDCs.
- Our experiments show, that although CR3 has a dominant role in the phagocytosis of complement-opsonized particles, it does not have an influence on the differentiation, cytokine production and T-cell activating capacity of dendritic cells.

Publications connected to the thesis

Kristóf K, Madách K, Sándor N, Iványi Z, Király A, Erdei A, Tulassay E, Gál J, Bajtay Z Impact of molecular mimicry on the clinical course and outcome of sepsis syndrome. ***Molecular Immunology*** **49**(3)**:** *pp. 512-517.* (2011)
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* These authors contributed equally

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