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Igazolás

Mint a Magyar Immunológiai Társaság 2015. évi Vándorgyűlés szervezőbizottságának tagja, és mint témavezető is igazolom, hogy Trojnár Eszter a következő címmel előadást tartott a jelzett konferencián: COMPLEMENT FACTOR H AUTOANTIBODIES B CELL EPITOPE ANALYSIS IN AUTOIMMUNE HAEMOLYTIC UREMIC SYNDROME

Szívélyes üdvözlettel,


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concentration of cytokines (IL-1 β , TNF α , IL-6) in cell culture supernatants was measured by ELISA. The amount of pro-IL-1 β mRNA was determined by qRT-PCR. The concentration of pro-IL-1 β protein was measured by ELISA following the lysis of THP-1 cells by repeated freeze-thaw cycles. Intracellular ROS was detected by DCFDA fluorescent assay.

Results: PBMCs stimulated with *C. parapsilosis* produced similar quantities of TNF α and IL-6, but much lower amounts of IL-1 β , compared to *C. albicans*-stimulated cells. In PMA-induced THP-1 monocytes, *C. albicans* induced the release of IL-1 β after 24 hours already at an MOI of 0.01, while a 100-times higher dose of *C. parapsilosis* cells (MOI of 1) was needed for the induction of IL-1 β secretion. This marked difference in secreted IL-1 β levels originated from the differential processing pro-IL-1 β , as we found no difference in the level of IL-1 β mRNA and pro-IL-1 β . As it has been associated with inflammasome activation, we also measured the amount of intracellular ROS and found that *C. albicans* induced higher ROS production in THP-1 cells compared to *C. parapsilosis*. We also found that both *C. albicans* and *C. parapsilosis* induced pyroptosis in macrophages after 24 h, and the production of IL-1 β in response to both species was dependent on Caspase-1, Caspase-8, Syk, NADPH oxidase and TLR4.

Conclusions: Our results strongly suggest that although *C. parapsilosis* is able to induce inflammasome activation when added in a high dose, it is very inefficient in inducing pro-IL-1 β processing in comparison to *C. albicans*. These findings contribute to the better understanding of the pathogenesis of *Candida* infections, and highlight the importance of studies focusing on different *Candida* species rather than *C. albicans* alone when investigating the immunity against these pathogens.

EFFECTS OF GENETIC MODIFICATION OF LACTOBACILLUS CASEI BL23 CELL WALL ON HUMAN MONOCYTE-DERIVED DENDRITIC CELL FUNCTIONS

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Introduction: Lactobacilli are commensal bacteria, which can be found in the human gut. They are facultative anaerob, rod-shaped Gram-positive bacteria with pro-biotic potential. Several strains of Lactobacilli have attracted intense interest due to their immunomodulatory properties. One of the most extensively studied strain is Lactobacilli rhamnosus GG which mediates anti-apoptotic activity in intestinal epithelial cells (IEC). The secreted factors responsible for this effect were identified as proteins with peptidoglycan-hydrolase (PGH)

activity. The two identified proteins are able to stimulate Akt signaling in host IEC, prevent these cells from damage and cytokine-induced apoptosis. Genes encoding homologues enzymes are present in closely related strains of *L. rhamnosus* such as *L. casei* BL23 which was the target of our experimental model.

Methods: Monocytes were separated from human buffy coats and differentiated to dendritic cells (DCs) in vitro in the presence of GM-CSF and IL-4 for 5 days. *L. casei* BL23 wild type and two peptidoglycan-hydrolase mutant bacteria ($\Delta 2770$ and $\Delta 2770/p45$) were added to the monocyte-derived DCs (moDCs) for 24 hours. The phosphorylation status of the three major families of mitogen-activated protein kinases (MAPK) in moDCs was studied with MAPK array. Phagocytic activity of the stimulated moDCs was monitored by flow cytometry. Culture supernatants were collected on day 6 of moDC differentiation and the concentration of the secreted cytokines was measured by ELISA. The number of IFN- γ and IL-17 producing T cells was detected by ELISPOT assays.

Results: Our experimental results showed that the phagocytic activity and the secretion of cytokines increased after bacterial stimulation and the genetic modification of the *L. casei* cell wall could modulate the phosphorylation status of selected MAP kinases. These experiments also revealed that the most efficient uptake by moDCs could be detected with the wild-type bacteria that also triggered the highest cytokine levels secreted by moDCs. *L. casei* BL23 wild-type and PGH negative mutants were able to induce the polarization of T lymphocytes by moDCs to Th1 or Th17 directions.

Conclusions: In our in vitro culture system we compared the functional activities of live wild-type *L. casei* BL23 bacteria as compared to their peptidoglycan-hydrolase mutants. Genetic modification of the bacterial cell wall modulated the activating potential and the cytokine secretion of moDCs and affected the outcome of T lymphocyte responses.

COMPLEMENT FACTOR H AUTOANTIBODIES B CELL EPITOPE ANALYSIS IN AUTOIMMUNE HAEMOLYTIC UREMIC SYNDROME

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Introduction: One of the most common factors evoking acute renal failure in children is hemolytic uremic syndrome (HUS). A rare form of HUS is mediated by autoantibodies against complement factor H (CFH). In patients with anti-CFH autoantibodies mutation of CFH related proteins (CFHR), most

often CFHR3-1 is observed. CFH domains 19-20 and the homologous ones of CFHR1 differ only in two amino acids, but these slight differences could affect the binding sites for the autoantibodies. To understand the origin of anti-CFH autoantibodies the exploration of their B cell epitope specificities would provide essential data, therefore our aim was to carry out the B-cell epitope mapping of anti-CFH autoantibodies, and to investigate the inhibitory effect of CFHR1 domains 4-5 on antibody binding to CFH.

Methods: We analyzed 5, anti-CFH IgG positive HUS patients' sera taken in the first episode of the disease. As control sera of healthy children and patients' sera from remission was used. The binding of antibodies to overlapping synthetic peptides representing selected regions of CFH and CFHR1 was measured by altered ELISA method. We used competitive ELISA technique to investigate antibody binding to CFH in presence of CFH domains 19-20 and CFHR1 domains 4-5.

Results: In healthy controls enhanced reactivity was measured in three regions of CFH (AA1142-1156, AA1182-1196, AA1202-1216). Serum samples from HUS patients contained antibodies to these 3 regions and to two additional (AA1157-1171, AA1177-1191) regions as well. Increased antibody binding was detected in patients' sera to CFHR1, too. Although in competitive assays CFH showed significantly stronger inhibition than CFHR1, both were capable to maximally inhibit antibody binding to CFH.

Conclusion: Our results corroborate a recent publication, in which anti-CFH autoantibodies' binding was also reported to multiple regions of factor H in healthy controls and in HUS patients. However, we describe here for the first time specific B-cell epitopes localized on the 19th and 20th CCP domains of factor H in aHUS patients. Further analysis of these data might facilitate the exploration of the autoantibody productions' mechanism and may potentially lead to diagnostic improvements. Besides, we found that multiple linear epitopes of CFH and CFHR1 determine the inhibition of antibody binding to CFH.

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AUTOANTIBODIES AGAINST THE COMPLEMENT REGULATOR FACTOR H IN NEUROMYELITIS OPTICA PATIENTS

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Introduction: Neuromyelitis optica (NMO) is an autoimmune inflammatory disorder of the central nervous system (CNS), characterized by pathogenic, complement-activating autoantibodies against the main water channel in the CNS, aquaporin 4 (AQP4), and lesions in the CNS that contain complement activation products. NMO is frequently associated with other autoantibodies and antibody-mediated diseases. Because complement is implicated in the pathogenesis of NMO, the aim of this study was to evaluate the presence of autoantibodies against the complement regulator factor H and the alternative pathway C3 convertase components C3b and factor B in the serum of NMO patients.

Methods: Serum samples of 45 NMO patients, who were all seropositive for AQP4 autoantibodies, were screened by ELISA for autoantibodies against C3b, factor B, and factor H. Recombinant fragments of factor H and recombinant factor H-related proteon 1 (FHR1), expressed in insect cells, were used in ELISA to map the binding sites of the autoantibodies. Serum FHR1 was analyzed by Western blot. The presence of factor H-antibody complexes in the sera were analysed after IgG precipitation and Western blotting. The avidity of the antibodies were measured in ELISA using NaSCN as chaotropic salt.

Results: Four of 45 NMO patients (~9%) had factor H autoantibodies and none had antibodies to C3b and factor B. The factor H autoantibody titers were low in three and high in one of the patients' sera. The avidity indexes calculated for the four autoantibodies were low. We could also detect factor H-IgG complexes using a monoclonal antibody to factor H as a capture antibody in ELISA and in the IgG fractions by Western blot. The autoantibodies bound to factor H domains 19-20, and also recognized the homologous protein FHR1. This binding pattern was similar to pathogenic factor H autoantibodies associated with atypical hemolytic uremic syndrome (aHUS), used as positive controls in the assays. However, in contrast to the majority of autoantibody-positive aHUS patients, these NMO patients did not lack FHR1.

Conclusions: Our results demonstrate that factor H autoantibodies are not uncommon in NMO. Our data also suggest