EP17-INFECTION AND AUTOIMMUNITY - MICROBIOME, INFECTOME AND INTERACTOME

Thursday April 7, 2016; From: 08:00To: 18:30

IMMUNE RESPONSES AGAINST HELICOBACTER PYLORI HEAT SHOCK 60 IN PATIENTS WITH MULTIPLE SCLEROSIS

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Introduction and Aim: In view of published data suggesting that *Helicobacter pylori* (Hp) is a trigger multiple sclerosis (MS), we investigated the role of anti-heat shock 60 (hsp60) Hp antibody responses in different clinical phenotypes of MS and compared them with those noted in patients with another autoimmune disease, namely systemic sclerosis (SSc) and healthy controls (HC).
Material and Methods: Serum samples from 53 anti-Hp(+) MS patients (39 with relapsing remitting, RRMS and 14 with secondary progressive, SPMS), 22 anti-Hp(+) SSc patients (12 with diffuse SSc and 10 with limited SSc) and 11 anti-Hp(+) HC were tested for IgG anti-hsp60 Hp by immunoblotting. IgG anti-Hp antibody positivity was also assessed by two independent anti-Hp ELISAs.
Results: All MS and SSc sera, irrespectively of their clinical phenotype, were reactive with anti-hsp60 Hp (53/53 and 22/22, respectively) compared to 8/11 HC ($p<0.05$ in both cases). However, the magnitude of anti-hsp60 antibody responses was stronger in MS compared to SSc (89.2 ± 33.7 *vs* 70.8 ± 34.6 AU, $p<0.05$). No significant difference of the anti-hsp60 response magnitude was observed between RRMS and SPMS (RRMS: 85.3 ± 33.4; SPMS: 100 ± 33.1) or diffuse and limited SSc (diffuse SSc: 65.5 ± 38.5; limited SSc: 77.2 ± 30 AU)
Conclusions: The pathogenic relevance of anti-hsp60 Hp antibody responses in MS is to be questioned in view of its universal presence in MS, SSc and HC. Stronger anti-hsp60 Hp antibody responses are observed in MS than in SSc, a finding which necessitates further investigation.

EP34-PULMONARY ARTERIAL HYPERTENSION & SYSTEMIC SCLEROSIS

Thursday April 7, 2016; From: 08:00To: 18:30

ALTERED B REGULATORY CELL HOMEOSTASIS IN AUTOIMMUNE RHEUMATIC DISEASE-RELATED PULMONARY FIBROSIS

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INTRODUCTION: Pulmonary fibrosis (PF) is a feature of systemic sclerosis (SSc), as well as (to a lesser extent) rheumatoid arthritis (RA). Though altered B cell homeostasis is found in both diseases, the quantitative or qualitative differences of B cell subsets in patients with SSc or RA with or without PF have not been investigated in detail.
AIM: To investigate phenotypic and functional alterations of B cells subsets in patients with SSc/PF and RA/PF patients.
METHODS: PBMCs were obtained from 40 individuals, including 15 patients with SSc-PF, 15 patients with RA-PF and 10 normal controls (NCs). The expression of CD19, CD27, CD24 and CD38 on B cells was examined by flow cytometry using conjugated antibodies (BD Biosciences). B regulatory cell function was assessed by measuring intracellular IL-10 expression following stimulation with ODN2006 (TLR-9).
RESULTS: Infrequent B cell subsets such as IL-10 expressing memory and transitional B regulatory cells (Bregs) are significantly decreased in SSc/PF and RA/PF patients compared to NCs. Significant loss of CD27posCD19pos and CD27posCD22pos B cells was noted in SSc and to a lesser extent in RA patients compared to NCs ($p<0.01$). The ratios of naive (CD19posCD27neg)/memory (CD19posCD27pos) were higher in SSc/PF>>RA/PF>>SScwithoutPF>>RAwithoutPF ($p<0.05$, for all groups).
CONCLUSIONS: An impairment of memory Bregs is a feature of pulmonary fibrosis in patients with systemic sclerosis or rheumatoid arthritis. The lack of this B cell subset is associated with diminished IL-10 production.

EP41-T CELLS AND B REGULATORY CELLS (TREG, BREG) – TOLERANCE

Thursday April 7, 2016; From: 08:00To: 18:30

IMPAIRED P38 MAPK ACTIVATION IS A CHARACTERISTIC FEATURE OF B REGULATORY CELLS IN SYSTEMIC SCLEROSIS

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INTRODUCTION: Memory (CD27+) B regulatory cells bearing the stimulatory CD19, are deficient in early and established systemic sclerosis (SSc) and IL-10 expression is impaired. Whether the altered homeostasis and signaling of naïve (CD27-) B cells favors anti-inflammatory gene expression is currently unknown.
AIM: To characterize signaling events downstream of BCR and other co-receptors in B cell subsets from SSc patients and to examine the activation of p38 MAPK, a known regulator of IL-10 gene expression.
METHODS: PBMCs were collected from 40 individuals, including 30 patients with SSc and 10 normal controls (NCs). The expression of CD19, CD27, CD24 and CD38 on B cells was examined by flow cytometry using conjugated antibodies (BD Biosciences). Naïve and memory B cells were magnetically sorted using B cell negative selection and CD27 kits (Miltenyi Biotech). BCR-induced and TLR-9 induced phosphorylation of p38 MAPK was measured using phoshoFlow.
RESULTS: Phosphorylation of p38MAPK was significantly decreased in total B cells from patients with early and established SSc compared to NCs. More than 60% reduction in p-p38positive B cells was found following either BCR or TLR9 stimulation. P38 activity was also impaired in fractionated naïve and memory B cells irrespective of the stimulus and the stage of the disease. Hydrogen peroxide which deactivates phosphatases and other non-specific p38 activators such as sodium arsenite were unable to rescue p38 phosphorylation in SSc B cell subsets.
CONCLUSION: Systemic sclerosis is a disease characterized by impaired p38 MAPK activation of IL-10 producing Bregs.

EP17-INFECTION AND AUTOIMMUNITY - MICROBIOME, INFECTOME AND INTERACTOME

Thursday April 7, 2016; From: 08:00To: 18:30

HELICOBACTER PYLORI CAGA AND VACA ANTIBODY REACTIVITY IN MULTIPLE SCLEROSIS PATIENTS FROM CENTRAL GREECE

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Introduction: Contradictory studies indicate higher or lower prevalence of anti-*Helicobacter pylori* (Hp) antibodies against immunodominant proteins, such as cytotoxin-associated protein (CagA) and vacuolating-cytotoxin (VacA), in patients with multiple sclerosis (MS) compared to controls. Our aim was to determine the prevalence of anti-CagA and anti-VacA antibodies in MS patients from Central Greece.

Material and Methods: A total of 129 MS patients, 102 with relapse remitting MS (RRMS) and 27 with secondary progressive MS (SPMS), and 25 healthy controls (HC) were assessed for IgG anti-Hp antibodies by ELISA and for CagA and VacA specific antibodies by line immunoassay (Euroimmun, Germany).

Results: Overall, 53/129 (41.1%) MS patients (39 RRMS/14 SPMS) and 8/25 (32%) HCs were Hp seropositive by ELISA ($p>0.05$). Anti-CagA(+) and anti-VacA(+), were found in 39/53 (73.6%) and 16/53 (30.2%) MS patients, respectively, compared to 75% and 25% HCs, respectively ($p>0.05$, for both). The prevalence and magnitude of anti-CagA was comparable between RRMS and SPMS. Anti-VacA antibodies were more prevalent in SPMS (7/14, 50%) compared to RRMS (8/39, 20.5%), respectively, $p<0.05$). All but one anti-VacA(+) patients were also anti-CagA(+) (15/16, 94%), and there was a statistical trend of double anti-CagA(+)/anti-VacA(+) (7/14) than anti-CagA(+) alone (4/14) ($p=0.068$) in SPMS patients.

Conclusions: Antibodies to CagA and VacA Hp are equally prevalent in Hp(+) MS patients and HC from Central Greece. Within MS, the prevalence of anti-VacA antibodies is more frequent in SPMS patients, with all anti-VacA(+) SPMS patients also being anti-CagA(+). The role of VacA immune responses in distinct MS phenotypes must be explored further.

EPD45-ADDITIONAL ASPECTS OF GASTROINTESTINAL AUTOIMMUNITY

Saturday April 9, 2016; From: 16:00To: 16:05

MYCOBACTERIUM AVIUM INDUCED PARATUBERCULOSIS AND CROHN'S DISEASE SHARE COMMON ANTIBODY PROFILES

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Introduction: Crohn's disease (CrD) patients are characterized by ASCA and pancreatic autoantibodies (PABs) targeting GP2 or CUZD1. *Mycobacterium avium* (MAP)-induced paratuberculosis (ptb), the ruminant model of CrD, is characterized by PABs targeting GP2 but not CUZD1. What has not been investigated so far is the extent of ASCA in ptb.
Aim: To assess ASCA in ruminants with (anti-MAP antibody positive) or without (anti-MAP negative) ptb.
Material and Methods: ASCA were tested by ELISA in 72 serum samples from 14 anti-MAP positive and 58 anti-MAP negative cattle, pre-characterized for PABs by indirect immunofluorescence (IIF), anti-MAP and anti-GP2 antibodies by ELISA using anti-cattle specific conjugates.
Results: Strong ASCA reactivity was found in 19% ptb sera while medium or high ASCA were present in 44% of the sera. ASCA were more frequently found in anti-GP2 positive than anti-GP2 negative ruminants (50% *vs* 33%, p=0.07). ASCA reactivity was higher in anti-GP2 positive than anti-GP2 negative ruminants.
Conclusion: ASCA's presence in ptb with or without CrD-specific GP2 PABs indicates an antibody profile similar to that seen in patients with CrD, further supporting that ptb and Crohn's disease share common pathophysiological origins.