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IMMUNOLOGY ABSTRACTS

Poster Sessions

Poster Session: Authophagy

P0001

Apoptotic parasites silence macrophages by misusing the autophagy machinery

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Purpose/Objective: An appropriate T cell response to *Leishmania* (Lm) infection is critical for an effective immune response. Human macrophages (MF) can present antigen to T lymphocytes and at the same time serve as host cells. Upon macrophage infection the virulent inoculum of Lm promastigotes consists of apoptotic and viable promastigotes. The viable promastigotes enter a maturing phagolysosome were they can survive and grow as amastigotes; the fate of apoptotic parasites is unclear.
Materials and methods: In this study, we hypothesize that the apoptotic promastigotes use the MFs«autophagy machinery to down regulate MF antigen presentation and T cell activation.
Results: Upon promastigote uptake by human primary MFs, we found apoptotic promastigotes to enter a compartment positive for the autophagy marker LC3. This LC3 compartment matured over time and became LAMP positive. 24 h later the compartment resolved after highly efficient parasite degradation. When co-incubated with autologous T lymphocytes, MFs infected with viable promastigotes induced a strong CD4-positive T cell proliferation. Compared to viable parasites a significantly lower T cell reactivity was observed in response to MFs inoculated with apoptotic or a mixed population of apoptotic and viable parasites. Subsequently, preliminary results suggest that only in the presence of apoptotic promastigotes and human T cells Lm infection could be sustained in human MF over a period of 7 days.
Conclusions: We found that apoptotic promastigotes enter a maturing LC3 compartment. Our data suggest that degradation of parasites in this compartment could be involved in a down regulation of T cell activation. We now further investigate and characterize the proliferating T cell subsets and how the autophagy machinery and apoptotic promastigotes may dampen immune responses in human primary macrophages.

P0002

Autophagy is activated in the B cells of patients with SLE and correlates with disease activity

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Purpose/Objective: Autophagy is increasingly appreciated as an important immune surveillance and effector mechanism, but understanding of its dynamic function in human autoimmune disease is limited. We sought to evaluate its role in the B and T lymphocytes of patients with SLE compared with healthy controls.

Materials and methods: Patient samples were collected with informed consent, and disease activity quantified by the SELENA-SLEDAI index. Multispectral imaging flow cytometry was performed using an Amnis ImageStream^X instrument. LC3-positive autophagosomes were enumerated in viable, non-apoptotic cells using the Bright Detail Intensity algorithm implemented in IDEAS 6. Autophagic flux was determined by incubation with chloroquine. As an alternative measure of autophagy, uptake of the novel autophagosomotropic dye CytoID (Enzo) was analysed using conventional flow cytometry. Autophagy was assayed in negatively selected B cells stimulated with combinations of anti-IgM and anti-CD40 antibodies, and interferon-α.
Results: Autophagy was significantly increased in the CD19$^+$ B cells of patients with SLE compared with healthy controls

($P = <0.001$, $n = 22$ patients, 15 controls), and there was a positive correlation with SLEDAI score ($r = 0.67$, $P = <0.002$). There was however, no association in CD4$^+$ T cells ($P = 0.49$). There was no statistical evidence of confounding due to patient age or medication use. Assessment of autophagic flux using the autophagosome-lysosome fusion inhibitor chloroquine revealed an accumulation of autophagsomes following treatment.

Analysis of *ex vivo* viable, annexin V negative human B cells demonstrated a significant increase in autophagy in unstimulated compared with anti-IgM stimulated cells, with further decreases observed with the addition of anti-CD40 and interferon-α.
Conclusions: The process of autophagy has not been previously examined in *ex vivo* human B cells from patients with systemic autoimmune disease. We demonstrate that autophagy is enhanced in this context. Given our *in vitro* data, we may advance the hypothesis that autophagy is acting as a survival mechanism for auto reactive B cells lacking adequate survival signals. An alternative explanation requiring further investigation is that autophagy is acting to promote presentation of self-antigens by B cells. Autophagy is readily inhibited by many common pharmaceutical agents and may therefore represent a new treatment target in SLE.

P0003

Role of autophagy in the immunopathogenesis of leprosy

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Purpose/Objective: Leprosy is a chronic infectious disease that can present different clinical forms and there is evidence that the establishment of different clinical forms is driven by host innate mechanisms. Macrophages from tuberculoid (BT) and lepromatous (LL) patients seem to have a different behavior in relation to the mycobacteria. While in LL patients there are highly infected macrophages, in BT rare or few bacilli are found. Electron microscopy studies showed the presence of phagosomes with double membrane in macrophages exposed to *M. leprae* (ML), suggesting a possible involvement of

Results: It was compared the three groups with relation to the expression of IL-17. The Kruskal-Wallis test was used with the significance of 5%. The hypothesis of equality between the groups was rejected. The comparison was performed between groups of two. It was used the Wilcoxon test with Bonferroni correction (significance level = 0.05/3 = 0.0167). It was observed difference between the groups CARD and NAHD (P-value < 0.001) and between NI and NAHD (P-value = 0.008). There was not significative difference between the groups CARD and NI (P-value = 0.376). The expression of IL-17 was higher in patients of the NAHD group, If compared with patients in the other groups, median of 11.81 (5.79–22.86) versus 5.38 (3.87–10.55) of CARD group and 5.715 (4.25–11.31) of the NI group.
Conclusions: Therefore, IL-17 expression seems to be associated with a protective cardiac function in human Chagas disease.

P1171

Evaluation of serum cytokine concentrations of patients in different clinical stages of Chagas' disease

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Purpose/Objective: The aim of this study was to evaluate serum concentrations of cytokines IL-4, IL-10, IL-12, TNF-α and IFN-γ in patients in different clinical forms of Chagas' disease.
Materials and methods: We conducted a case-control study of 115 individuals. Infected individuals were divided into four stages as the Brazilian consensus of Chagas' disease: indeterminate patients or without apparent heart disease (normal electrocardiogram and echocardiogram) cardiac patients on the stage A (electrocardiogram changes and normal echocardiogram), cardiac patients on the stage C [electrocardiogram and echocardiogram altered with compensable congestive heart failure (CHF)] and cardiac patients on the stage D (electrocardiogram and echocardiogram changed with refractory CHF). Also were included uninfected individuals with *T. cruzi*. We included 30 indeterminate patients, 31 cardiac patients on the stage A, 14 cardiac patients on the stage C, 11 cardiac patients on the stage D and 29 uninfected individuals.
Results: Among the pro-inflammatory cytokines, IFN-γ showed higher serum levels in relation to IL-12 and TNF-α. The cardiac patients on the stage A had higher concentrations of TNF-α, however, there was a significant decrease in the concentrations of this cytokine in the same time that we observed the later stages of the chronic Chagas cardiomyopathy (CCC). Both indeterminate and cardiac patients showed high levels of TFN-α and IFN-γ and low levels of IL-4 and IL-10, demonstrating a dominant Th1 profile with an imbalanced immune response.
Conclusions: This study demonstrated a direct proportionality in concentrations of pro-inflammatory and anti-inflammatory cytokines with respect to the left ventricular ejection fraction in all groups of patients, suggesting that this correlation could be used as a marker of progression to CCC.

P1172

IgG and IgM anti-toxoplasma gondii antibodies class in high risk pregnant women in Brazil

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Purpose/Objective: Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, an apicomplexa obligate intracellular protozoan parasite, which infects different species including humans. Fetuses can be infected by transplacental transmission, a condition that may cause significant sequelae in babies. The knowledge of the antigenic profile of IgM and IgG anti-*T. gondii* antibodies during the gestational period gives clinical support towards preventing the fetal infection by this parasite. This study aimed to verify the serological profile of the pregnant women from S'o Jos* do Rio Preto, S'o Paulo State, Brazil, which received medical attention in a public health service from a tertiary school hospital.
Materials and methods: The medical records of 793 pregnant women, attended in the High Risk Antenatal Care and Fetal Medicine, Gynecology and Obstetrics Outpatient Clinic of the Hospital de Base in S'o Jos* do Rio Preto, S'o Paulo State, Brazil, between 2001 and 2004, were analyzed. The serology tests performed to determine specific IgG and IgM anti-*T. gondii* antibodies were based in a commercial immunoenzymatic assay kit (ETI – TOXOK * IgG, ETI – TOXOK * IgM DiaSorin, Italy).
Results: From the overall data, 503 (63.4%) were reagent and 290 (26.6%), non reagent. Among the reagent pregnant women, 32 (4.0%) presented a serology profile with both anti-*T. gondii* antibodies IgG and IgM positive and 471 (59.4%), with anti-*T. gondii* antibodiesIgG positive and IgM negative. The profile of anti-*T. gondii* antibodies IgG negative and IgM positive was not found.
Conclusions: The prevalence of *T. gondii* infection is high in the region where this study was carried out and the majority of the pregnant women with positive serology tests are not under risk of gestational transmission of this parasite. However, considering that 4.0% of the pregnant women are under risk of gestational transmission, these results attract the attention for the necessity to implement a protocol for serological and molecular screening of mother and newborn babies, to contribute with the clinical guidance and to prevent babies sequelae.

P1173

IL-10 secreting, type 1 regulatory T cells and naturally occurring regulatory T cells differently modulate IgG secretion by B cells in human hypo-responsive onchocerciasis

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Purpose/Objective: Onchocerciasis or river blindness is the second leading infectious cause of blindness after trachoma. The disease is caused by infections with the filarial nematode *Onchocerca volvulus* and usually present two distinct pathological forms: the hyper-reactive or Sowda form and the hypo-reactive or generalized form. The hypo-reactive form is characterized by anergy and tolerance to the parasite,