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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

P1002

Modification of a single lysine in a CYP2E1 epitope induces immune-mediated DILI in BALB/c mice

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Purpose/Objective: Key steps in the pathogenesis of immune-mediated drug-induced liver injury (Im-DILI) have not been identified. After receiving halogenated anesthetics, anti-seizure medications, antibiotics or non-steroidal anti-inflammatory drugs, susceptible patients develop Im-DILI thereby increasing their morbidity and often their mortality. In anesthetic Im-DILI patients, granulocytic hepatitis, trifluoroacetyl chloride (TFA) and IL-4-mediated cytochrome P4502E1 (CYP2E1) IgG4 antibodies support the diagnosis, while CYP2E1 epitopes responsible for the pathogenesis of Im-DILI are unknown. We previously demonstrated a CYP2E1 epitope [Gly^{113}-Leu^{133} (JHDN5)] containing a single lysine that was recognized by sera from anesthetic DILI patients with specific MHC II haplotypes. We showed that JHDN5 was recognized by splenocytes from mice with experimental Im-DILI induced by immunizations with liver proteins covalently altered by TFA, a drug hapten formed during metabolism of halogenated anesthetics. We hypothesize that covalent modification of a single lysine in JHDN5 induces IL-4-mediated, Im-DILI in BALB/c mice.
Materials and methods: JDN5 was modified by TFA (TFA-JHDN5) using the methods of Goldberger and Anfinisen. We confirmed 81.5% modification of JHDN5 using the method of Habeeb. BALB/c mice were immunized with 100 μg of an unrelated CYP2E1 epitope or JHDN5 ± TFA emulsified in CFA or CFA alone on days 0 and 7 and killed on day 21. IL-4 deficient (KO) mice were similarly treated with CFA ± TFA-JHDN5. Histology scores, antibodies and cytokine levels were analyzed using Mann−Whitney *U*-test. A P value <0.05 was significant.
Results: TFA-JHDN5 induced more granulocytic hepatitis ($P < 0.01$) as well as anti-TFA and anti-CYP2E1 antibodies ($P < 0.05$) than CFA-immunized BALB/c or KO mice. Granulocyte and macrophage attractants KC, MIP-2, G-CSF, M-CSF, MCP-1, MIP-1a, MIP-1b and VEGF as well as IL-7 and IL-9 were elevated in BALB/c but not KO livers ($P < 0.05$). Unmodified epitopes did not induce Im-DILI.
Conclusions: We confirm that covalent modification of a single lysine in a CYP2E1 epitope induces Im-DILI in BALB/c mice with features reminiscent of anesthetic Im-DILI in patients. Future studies of this epitope may uncover unidentified mechanisms of Im-DILI from other drugs and help to develop targeted agents to either treat or prevent this disease.

P1004

Overexpression of SMAD7 protects liver from TGFb/Smad-mediated fibrogenesis

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Purpose/Objective: SMAD7 is a negative regulator of TGFb/activin pathway. Recently, animal studies have shown that SMAD7 induction ameliorates TGFb/Smad-mediated fibrogenesis, suggesting a protective mechanism against liver injury. This study was scheduled to examine the role of SMAD7 in liver inflammation, fibrosis and the possible effect of antiviral treatment.
Materials and methods: Liver biopsies from 67 patients with hepatic diseases were studied: (1) 18 with chronic HCV hepatitis (CHC); (2) 19 with chronic HBV hepatitis at diagnosis (CHB/d); (3) four with CHB after antiviral treatment and relapse (CHB/non-r) (4) 14 with CHB after antiviral treatment response and remission for >5 years (CHB/r); (5) 12 with non alcoholic fatty liver disease (NAFLD). Three liver samples with a mild increase of aminotransferases but without histological changes, served as controls. Histological activity index and staging of fibrosis were also assessed. RNA was extracted and cDNA was synthesized using standard protocols. mRNA expression of TGFb isoforms (*TGFB1, 2, 3*), activins (*A, B, C, E*), *ALK4*, *ALK5*, SMAD molecules (*SMAD2, 3, 4, 7*), and *CTGF*was examined using quantitative real time PCR. Statistical analysis was performed using SPSS and P values < 0.05 were considered significant.
Results: Patients with CHB/r exhibited a significant increase of *SMAD7* and *ALK4* mRNA expression compared to CHB/d patients, and reduced levels of *TGFB1, SMAD2, SMAD3*, and *CTGF*. A significant increase of *SMAD7* was also found in NAFLD patients compared to untreated viral hepatitis patients and those who did not respond to any treatment. Moreover, NAFLD patients were presented with elevated levels of *TGFB1, TGFB3, INHβC, ALK5*, and *SMAD4*. Considering the intensity of inflammation, *SMAD7, ALK5*, and *INHβC* exhibited a significant increased expression from absent to minimal inflammation with a gradual reduction as inflammation exacerbates.
Conclusions: Our data indicate that in cases with low grade fibrosis, as NAFLD (characterized by a lower incidence of severe liver complications and fibrosis progression) and CHB/r, *SMAD7* overexpression might be a mechanism limiting the fibrogenic effect of TGFb suggesting that its induction may provide a target for novel therapeutic approaches.

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P1005

Peritoneal macrophage inflammatory profile in cirrhosis is dependent on the etiology and is related to ERK phosphorylation level

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Purpose/Objective: The aim of this work is to identify functional differences in the inflammatory profile of monocyte-derived macrophages (M-DM) from ascites in cirrhotic patients of different etiologies, alcohol- and hepatitis C virus (HCV)-related cirrhosis, trying to extrapolate studies from liver biopsies to immune cells in ascites.
Materials and methods: We studied 45 patients with cirrhosis and non-infected ascites, distributed according to disease etiology, HCV ($n = 15$) or alcohol ($n = 30$). Cytokines and cellular content in ascites were assessed by ELISA and flow cytometry, respectively. Cytokines and ERK phosphorylation level from peritoneal monocyte-derived macrophages isolated and stimulated *in vitro* were also determined.
Results: A different pattern of leukocyte migration to peritoneal cavity and primed status of macrophages in cirrhosis is observed depending on the viral or alcoholic etiology. Whereas no differences in peripheral