REGULATORY T CELLS
Wednesday, July 8

W.61. CD4+CD25+ Regulatory Cells Suppress Effector T Cells Independent of TCR Stimulation *in vitro*

Katharina Forster[1], Pengfei Zhou[2], Rajka Borojevic[2], Sheela Manek[1], Armando Heriazon[1], Kenneth Croitoru[1]
[1]University of Toronto, Toronto, ON, Canada; [2]McMaster University, Hamilton, ON, Canada

Introduction: OVA is not required for DO11.10 derived Treg cell inhibition of colitis induced by Balb/c CD45RBhi T cells. Transferred DO11.10 CD25+ cells proliferate *in vivo* in the absence of cognate antigen. This suggests that either DO11.10 Treg cells recognize other antigens via a second TCR or function independent of TCR stimulation. Methods: We stimulated DO11.10 CD4+CD25- T cells to proliferate with OVA pulsed APC. Increasing numbers of Balb/c, which do not recognize OVA, or DO11.10 CD4+CD25+ Treg were added to these cultures. In addition, we tested if DO11.10 CD4+CD25+ Treg were able to inhibit SEA induced proliferation of Balb/c T effector cells. SEA stimulates Vbeta 3,6 and 11 found on Balb/c but is not found on DO11.10 T cells which only express Vbeta8. Results: BALB/c CD25+ Treg suppressed the OVA induced proliferation of DO11.10 CD25-. DO11.10 CD25+ Treg cells suppressed SEA induced proliferation of Balb/c effector cells in a dose dependent fashion. Conclusion: These results indicate that cognate antigen is not required for Treg cells to suppress antigen induced T cell proliferation *in vitro*. We conclude that Treg cell function is antigen independent or once activated suppress other Ag responses in a non-specific bystander fashion.

W.62. Retinoic Acid-induced Gut Tropism Improves the Protective Capacity of Regulatory T Cells in Acute, but not in Chronic Gut Inflammation

Astrid Menning[1], Christoph Loddenkemper[2], Astrid Westendorf[2], Balint Szilagyi[1], Jan Buer[2], Alf Hamann[1], Jochen Huehn[3]
[1]Charité University Medicine Berlin, Berlin, Germany; [2]University of Duisburg-Essen, Essen, Germany; [3]Helmholtz Centre for Infection Research, Braunschweig, Germany

Being endowed with immunosuppressive activity, regulatory T cells (Treg) are regarded as an alternative target for the treatment of autoimmunity. Notably, the capacity of Tregs to suppress depends on Treg migration into the inflamed tissue. In an attempt to improve Treg-based therapeutic strategies, we tested whether priming Tregs *in vitro* to migrate to mucosal tissues would enhance their efficacy to suppress intestinal inflammations. Using retinoic acid (RA), gut-specific homing molecules α4β7 and CCR9 were induced on *in vitro* expanded Tregs. In fact, RA-treated Tregs were more potent suppressors of an acute intestinal inflammation compared with control Tregs. Conversely, the efficacy of Tregs to resolve an established chronic inflammation of the colon was not affected by RA-treatment. The latter finding was associated with a rapid decrease of RA-induced α4β7 expression and *de novo* induction of homing receptors on transferred Tregs *in vivo*. Furthermore, the use of β7-deficient Tregs revealed that α4β7 expression by Tregs is not essential for the cure of colitis. Our data show that conditioning Tregs with RA increases their protective potential in acute, but not chronic intestinal inflammations. Nevertheless, these results suggest that conditions leading to a sustainable site-specific Treg accumulation could improve therapy of organ-specific autoimmune diseases.

W.63. Paracrine IL-2-dependent CD4+ T Cells with Foxp3-independent Regulatory and Pathogenic Abilities

Atsuhiro Ogawa[1], Kiyotaka Nagahama[2], Yasuyo Shimomura[3], Svend Rietdijk[1], Ype de Jong[1], Emiko Mizoguchi[1], Cox Terhorst[1], Atul Bhan[1], Cathryn Nagler[1], Atsushi Mizoguchi[1]
[1]Harvard Medical School, Boston, MA; [2]Yokohama City University, Yokohama, Japan; [3]Fujita Health University, Nagoya, Japan

Foxp3 expression has generally been believed to represent an essential molecule to determine the regulatory ability of CD4(+) T cells. We herein describe a unique CD4(+)CD25(high) T cell population (termed exCD25 T cells) that can be generated *ex vivo* in the presence of high dose of IL-2. The exCD25 T cells possess regulatory abilities to suppress naïve T cell proliferation *in vitro* and inhibit the CD45RB(high) T cell-induced colitis *in vivo* even in absence of Foxp3 expression. The exCD25 T cells without Foxp3 expression retain some phenotypic characteristics with typical Foxp3(+) Treg (e.g. highly expressed CD25 and GITR) but lack expression of CTLA4 that is required for the function of Foxp3(+) Treg. In addition, similar to Foxp3(+) Treg, chromatin remodeling within the promoter region of the IL-2 locus remains impaired in exCD25 T cells. Of note, in contrast to Foxp3(+) Treg, exCD25 T cells have a potential ability to induce colitis and hepatitis. The dual roles (regulatory versus pathogenic) are determined by paracrine IL-2 supplied from non-CD4(+) T cells such as TCR gamma/delta T cells. These finding suggest a Foxp3-independent immune regulatory function that can be reversed depending on paracrine IL-2.

W.64. Regulatory Role of CD4+FoxP3+CD25- T Cells in the Lungs of Mice Infected with *Bordetella Pertussis*

Michelle Coleman, Barry Moran, Joseph Keane, Kingston Mills, Padraic Dunne
Trinity College Dublin, Dublin, Ireland

We have identified a novel subset of $CD4^+FoxP3^+$ regulatory T (Treg) cells in the lungs of naive mice which lack CD25, distinguishing them from $CD4^+FoxP3^+CD25^+$ natural Treg cells. These $CD4^+FoxP3^+CD25^-$ T cells predominate over $CD4^+FoxP3^+CD25^+$ T cells in the lung, liver and colon. Lung $CD4^+FoxP3^+CD25^-$ T cells also express CD44 and CD69 but not CD45RB, CD28 or CD154, suggesting an activated, memory phenotype. Pre-activated $CD4^+FoxP3^+CD25^-$ T cells isolated from the lung suppressed effector T cell responses *in vitro*, but enhanced IFN-γ production. This suppression was mediated via IL-10.

In a mouse model of infection with the respiratory pathogen *Bordetella pertussis*, we found a high frequency of antigen-specific IL-10-secreting $CD4^+FoxP3^+CD25^-$ T cells in the lung, which peaked 7 days post-challenge. Depletion of $CD25^+$ cells prior to *B. pertussis* infection did not significantly impair the immunoregulatory response or alter the course of infection. However, depletion of $CD25^+$ cells in IL-$10^{-/-}$ mice resulted in significantly enhanced bacterial clearance and increased *B. pertussis*-specific IFN-γ production. Collectively, these results indicate the presence of an additional immunoregulatory mechanism in the lung, mediated by IL-10-secreting $CD4^+FoxP3^+CD25^-$ regulatory T cells, which may function in place of $CD25^+$ natural Treg cells in regulating immunity to infection in the lung.

W.65. Colitis in P-glycoprotein Deficient Mice is not an Intrinsic Defect in Regulatory T Cells

Scott Tanner, Robin Lorenz, Elizabeth Staley
University of Alabama at Birmingham, Birmingham, AL

The lack of P-glycoprotein (P-gp), encoded by the multidrug resistance gene (*mdr1a*), in FVB.*mdr1a*$^{-/-}$ mice results in T cell mediated colitis. We hypothesize a defect in $CD4^+CD25^+Foxp3^+$ regulatory T cell (T_{reg}) function is resulting in colitis in these animals. To examine the regulatory capacity of P-gp deficient T_{reg}, $CD4^+CD25^-$ T effector cells (T_{eff}) and $CD4^+CD25^+$ T_{reg} from FVB.*mdr1a*$^{-/-}$ mice were cultured together utilizing anti-CD3 stimulation. Proliferation of T cells was then measured by ^{3}H-Thymidine incorporation. P-gp deficient T_{reg} displayed similar suppressive ability as wild type FVB control T_{reg}. To test FVB.*mdr1a*$^{-/-}$ T_{reg} function *in vivo*, T_{reg} and T_{eff} isolated from FVB or FVB.*mdr1a*$^{-/-}$ mice were adoptively transferred into FVB.*rag*$^{-/-}$. Recipients were monitored for colitis development, as measured by weight loss, diarrhea, or rectal prolapse. Initial weight changes indicate FVB and FVB.*mdr1a*$^{-/-}$ T_{reg} protect from colitis. Both the *in vitro* cell culture studies and *in vivo* adoptive transfer experiments suggest that there is no innate defect in P-gp deficient T_{reg} suppressive activity. Because P-gp is required for dendritic cell (DC) differentiation, and DCs are vital to successful T_{reg} activation, the defect may be in DC/T_{reg} interaction. Support: HHMI Med-to-Grad Fellowship, CCFA Student Fellowship Award, NIH P01 DK071176, UAB Digestive Diseases Research Development Center #P30 DK064400.

W.66. TGF-β and Retinoic Acid-induced Expression of CD38 on T Cells Reflects Mucosal Imprinting

M. Fleur du Pré[1], Lisette van Berkel[1], Femke Broere[2], Mariette ter Borg[1], Frances Lund[3], Georg Kraal[4], Edward Nieuwenhuis[1], Janneke Samsom[1]
[1]ErasmusMC, Rotterdam, Netherlands; [2]Utrecht University, Utrecht, Netherlands; [3]University of Rochester Medical Center, Rochester, NY; [4]VU Medical Center, Amsterdam, Netherlands

Oral tolerance is associated with regulatory T-cell (Tr) induction in the mesenteric lymph nodes (MLN). T cells in MLN differentially express the ectoenzyme CD38 whereas the molecule is not expressed during a non-mucosal response. We determined the role of CD38 in mucosal T cell responses and its potential to identify mucosal T cells within the systemic T cell pool. CD38 expression was induced on T cells after co-culture with MLN-DC and TGF-β. Treatment with retinoic acid (RA) receptor-antagonists revealed that endogenous RA production by MLN-DC stimulated CD38 induction. As TGF-β and RA induce mucosal Foxp3+ Tr differentiation we assessed whether CD38 is selectively expressed on Foxp3+ cells. However, when Foxp3+ Tr differentiation was blocked, CD38 expression was maintained. This implies that CD38 expression on T cells reflects mucosal imprinting irrespective of effector or regulatory function. To establish that CD38 identifies mucosal T cells in peripheral blood, CD62L-CD38+ T cells from healthy individuals were analyzed. CD62L-CD38+ T cells selectively expressed the gut-homing receptor CCR9 and preliminary data show the subset expressed high levels of the cytokine IL-10. In conclusion, CD38 expression identifies T cells that have been imprinted by encounter with mucosal DC. This restricted population may reflect ongoing intestinal immune responses and provide insights into the pathogenesis of intestinal disease.

W.67. Gut Mucosa and Mucosal Immunity as a Gateway for Autoimmunity in Type 1 Diabetes: The NOD Mouse Model

David Funda[1], Petra Fundova[2], Hana Kozakova[2], Milan Jirsa[3], Helena Tlaskalova-Hogenova[2], Karsten Buschard[1]
[1]Rigshospitalet, Copenhagen, Denmark; [2]Institute of Microbiology, Czech Academy Science, Prague, Czech Republic; [3]Charles University, Prague, Czech Republic

Gut mucusa parameters may play an important role in mediating the effects of environmental factors on development of type 1 diabetes (T1D). In this study we investigated intestinal permeability, gut maturation (activities of brush border enzymes) and lymphocyte subsets within the gut mucosa of NOD vs. BALB/c female mice. In addition, mucosal lymphocytes were also evaluated in NOD mice fed diabetes-preventive, gluten-free Altromin diet. Significantly decreased intestine permeability, measured as urine lactulose/rhamnose ratio, was found in NOD compared to BALB/c mice. Increased lactase and decreased sucrase activities were found in jejuna of NOD vs. BALB/c mice. Alkaline phosphatase values were substantially increased in NOD mice. No significant changes were observed in jejunal activities of DPP IV, glucoamylase and γ-glutamyltransferase. Using immunohistochemistry, significantly lower numbers of CD3+ , CD8+α, T-γδ+ cells and IgA+ but not IgM+ and CD4+ were observed in proximal and distal jejunum as well as ileum of NOD mice compared to BALB/c mice. Furthermore, a significantly lower ratio of intraepithelial to lamina propria CD3+ cells was found in NOD mice. Interestingly, NOD mice fed gluten-free diet displayed lower number of CD3+ cells with the lamina propria compartment. Altered villus architecture was documented in jejuna of NOD vs. BALB/c female mice. Our results showed a decreased intestine permeability, immature-activated enzyme

profile of the small intestine, and an impairment of gut mucosal immune system of pre-diabetic NOD compared to BALB/c mice as well as changes in lamina propria T cells in NOD mice on gluten-free diet. The impairment of gut mucosa in NOD mice may contribute to autoimmunity, e.g. by failing to shape the proper mucosal regulatory immune response to outer environmental stimuli.

W.68. Oral Anti-CD3 Plus Glucocerebroside Ameliorates Metabolic Syndrome in OB/OB Leptin-deficient Mouse Model of Type 2 Diabetes

Ann-Marcia Tukpah[1], Henry Wu[1], Yaron Ilan[2], Ruth Maron[1], Howard Weiner[1]
[1]Brigham and Women's Hospital, Boston, MA; [2]Hebrew University-Hadassah Medical Center, Jerusalem, Israel

Type 2 diabetes is characterized by increased blood glucose, and insulin resistance. The etiology of type 2 diabetes involves immune mediated metabolic dysregulation and characterized by inflammatory signaling, monocyte infiltration in adipose tissue and a lack of glucose regulation. We have previously shown that oral anti-CD3 suppresses EAE, lupus and type 1 diabetes by generating inducible CD4+LAP+ regulatory T cells. Administration of GC shows a marked reduction in hepatic fat content and improved glucose tolerance. We hypothesized that immune regulation by oral anti-CD3/GC may impact disease in the OB/OB mouse. We found that oral anti-CD3/GC decreased pancreatic islet cell hyperplasia and decreased accumulation of fat in the liver as well as lowered blood glucose and liver enzymes. In adoptive transfer studies these effects were shown to be mediated by CD4+LAP+ Tregs in a TGF-beta dependent manner. In addition, we found that treatment decreased the expression of inflammatory cytokines (IL-1B, TNF, IL-6) by adipocytes and the number of macrophages in white fat. There is also a marked decrease in production of inflammatory cytokines by splenocytes. These findings demonstrate the involvement of inflammatory immune mechanisms in type 2 diabetes and oral anti-CD3/GC treatment suppressed these mechanisms and improved disease.