REGULATORY T CELLS 2
Wednesday, July 8

OR.81. Gut CD103+ Dendritic Cells Express Indoleamine 2,3-Dioxygenase that is Responsible for T Regulatory Cell Differentiation and Oral Tolerance

Gianluca Matteoli, Elisa Mazzini, Maria Rescigno
IFOM-IEO CAMPUS, Milan, Italy

Background and Aims: The gut is a site for the de novo conversion of adaptive T regulatory cells. CD103+ gut dendritic cells (DCs) have been shown to be required for T regulatory cell differentiation. Indoleamine 2,3-dioxygenase (IDO) is involved in tryptophan catabolism. Expression of IDO by DCs under pathological conditions correlates with suppression of T cell activity. The aim of this study was to investigate IDO expression in different subtypes of intestinal DCs and its role in their tolerogenic properties. Methods: The expression level of IDO in CD103+ and CD103- DCs isolated from mesenteric lymph nodes and from lamina propria was analysed. The role of IDO in the conversion of T regulatory cells and Th17 cell development by gut DCs was evaluated by IDO inhibition *in vitro*. Oral tolerance and T regulatory cell differentiation *in vivo* was assessed in the presence or absence of a specific IDO inhibitor. Results: We show that exclusively mesenteric lymph node and lamina propria CD103+ but not CD103- DCs express IDO whose inhibition results in reduced CD4+Foxp3+ T regulatory cell conversion and enhanced T cell proliferation. Nevertheless, IDO inhibition does not restore the development of Th1 T cells while it fosters Th17 differentiation from CD103+ DCs, presumably due to previous environmental education received in the lamina propria. Finally, *in vivo* IDO blockade strongly impacted on the development of T regulatory cells specific for orally administered antigens and abolished the induction of oral tolerance. Conclusions: We identified a new pathway leading to acquisition of tolerogenic functions in mucosal CD103 expressing DCs that is IDO dependent. This study was funded by the Crohn's and Colitis Foundation of America, by the European Research Council and by the Italian Association for Cancer Research.

OR.82. Complementary Function of Plasmacytoid Dendritic Cells and CD4+ Regulatory T Cells in Oral Tolerance

Bertrand Dubois, Anne Goubier, Mercdes Gomez de Agüero, Marie Gouanvic, Dominique Kaiserlian
INSERM U851, Lyon, France

It is currently believed that peripheral T cell tolerance to oral Ag involves dendritic cells (DC) and is due to either deletion of Ag-specific T cells or active suppression by CD4+CD25+ regulatory T cells (Tregs). We recently showed that plasmacytoid DC (pDC) are essential for oral tolerance of Ag-specific systemic DTH responses mediated by CD4+ or CD8+ T cells (Goubier *et al*. Immunity 2008). Antibody depletion of pDC prevents induction of tolerance by Ag feeding and allows for oral T cell priming. Adoptive transfer of *in vivo* oral Ag-loaded pDC from MLN or liver induces Ag-specific suppression of CD4+ and CD8+ T cell responses to protein and hapten, respectively. More recently, we analyzed the respective contribution of pDC and Tregs in oral tolerance using the model of skin contact hypersensitivity mediated by CD8+ effectors. Hapten feeding enhances the suppressive function of Tregs and strikingly reduced the capacity of CD8+ T cells from liver and MLN to differentiate into DTH effector cells. Tolerant CD8+ T cells are not suppressive and remain hypo-responsive for prolonged periods of time even in the absence of Ag-re-exposure, indicating that deletion rather than anergy is induced by hapten feeding. Orally induced T cell deletion is dependent on pDC, but not Tregs nor IL-10. Residual circulating Ag-specific CD8+ T cells conditioned by this mucosal step are fully susceptible to suppression by Tregs activated by Ag gavage, which completely prevented their differentiation into DTH effectors upon re-exposure to the allergen via the skin. Altogether, our data demonstrate that oral tolerance is initiated by a mucosal step of partial deletion of Ag-specific T cells mediated by pDC and completed systemically by CD4+CD25+ T cells.

OR.83. Wiskott-Aldrich Syndrome Protein (WASP) is Critical for the Inducible Generation of FoxP3+ Regulatory T Cells

Elisa Boden[1], Vineet Ahuja[2], Christopher Moran[1], Deanna Nguyen[1], Scott Snapper[1]
[1]Massachusetts General Hospital, Boston, MA; [2]All India Institute of Medical Sciences, New Delhi, India

The Wiskott-Aldrich syndrome (WAS) is an immunodeficiency that results from mutations in WASP. WASP transduces cell surface receptor signals to the cytoskeleton via ARP2/3, a protein recently linked to ulcerative colitis. WASP-deficient (WKO) mice develop spontaneous colitis that is associated with quantitative and qualitative defects in CD4+CD25+Foxp3+ regulatory T cells (Tregs) that control autoimmunity. As inducible Tregs (iTregs) are believed to control organ-specific inflammation, we asked whether reduced numbers of peripheral Tregs reflected impaired iTreg generation in WKO mice. iTregs are generated by dendritic cells (DCs) in a process that is dependent on TGFβ and T cell receptor (TCR) stimulation. Therefore, CD4+CD25- T cells from WT or WKO mice were cultured in the presence of TGFβ and anti-CD3ε. After 72 hours, cells were analyzed by flow cytometry for the expression of FoxP3. The induction of FoxP3 was strikingly low in WKO T cells (3.3 +/- 1.3%) compared to WT T cells (29.5 +/- 9.3%, p<.04). WKO T cells had no significant alterations in TGFβ signaling. Addition of the costimulatory antibody anti-CD28 enhanced iTreg generation from WT T cells (80.8 +/- 7.9%), but did not rescue WKO iTreg generation (1.8+/- 0.77%). However, stimulation with concanavalin A partially rescued FoxP3 expression in WKO T cells (20.9+/- 3.2%) (Figure 1). These data suggest that the early, WASP-dependent steps in TCR signaling are necessary for induction of FoxP3. Impaired peripheral generation of Tregs likely contributes to colitis in WKO mice, and may be operative in human IBD. Further understanding the mechanisms that control Treg homeostasis may allow for targeted therapeutics for IBD that favor increased Treg numbers.

OR.84. Respiratory Infection or Depletion of Regulatory T Cells Break CD8+ T Cell Tolerance to Alveolar Self Antigen in Autoimmune Prone Mice

Milena Tosiek, Marcus Gereke, Dunja Bruder
Helmholtz Center for Infection Research, Braunschweig, Germany

Using a novel mouse model for CD8+ T cell-mediated autoimmune lung disease that shares major features with lymphocytic interstitial pneumonia in human patients and that offers a relevant experimental tool to dissect the basic mechanism underlying CD8+ T cell mediated autoimmune pulmonary disorders we investigated the specific requirements for priming of lung-specific CD8+ T cells, the regulatory mechanisms that control such autoimmune reactions as well as the conditions leading to the loss of immune tolerance to pulmonary self antigen. We demonstrate that auto-reactive CD8+ T cells can reside quiescently in close proximity to their specific self-antigen in the lung and that this steady state is labile. We identified circumstances leading to abrogation of self-tolerance mechanisms in the lung. Among them viral infection, simulation of bacterial infection by application of a TLR-4 ligand, as well as direct targeting of immune cells, i.e. licensing of dendritic cells or depletion of regulatory T cells, resulted in failures in immune regulation, activation of formerly quiescent lung-specific CD8+ T cells and consequently autoimmune pathology in the lung. To our knowledge, this is the first report directly providing detailed insights into the mechanisms underlying loss of CD8+ T cell tolerance to pulmonary self antigen in an autoimmune predisposed individual. Data presented here may contribute to a better understanding of the immunological processes leading to exacerbation of lung diseases following respiratory infections or other kind of immune activation in patients with chronic pulmonary disorders.