ImmunoTools *Special* Award 2013

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HOST-MICROBIOTA INTERACTIONS: EFFECTS OF *Nod2* DEFICIENCY

My PhD studies are related to the mucosal response to increased intestinal permeability. The increase in intestinal permeability, with consequent increased exposure to intestinal microbiota condition, is commonly believed a condition contributing to the pathogenesis of inflammatory bowel diseases (ulcerative colitis and Crohn's disease). Indeed it has been reported that the polymorphism of *NOD2* gene, considered a risk factor for the development of Crohn's disease, is associated with the presence of increased intestinal permeability, not only in patients but also in healthy relatives carrying the same gene polymorphism. In my studies, I made use of a mouse model where an increase in intestinal permeability with intrarectal administration of agents capable of altering the epithelial barrier was induced and verified the results obtained in this model in *Nod2-/-* mice. Data obtained from these studies demonstrated that the induced increase in the intestinal permeability, was associated with a regulatory response characterized by the local expansion of lamina propria CD4+Foxp3+ regulatory T cells and the expansion of a CD4+LAP+ Foxp3- (a TGF-beta producing, IL-10 dependent- regulatory T cell population that shows on the cell membrane the TGF-beta linked to its Latency Associated Peptide) regulatory T cells (Boirivant M. et al, 2008)(1). *Nod2-/-* mice that do not develop a spontaneous colitis, showed a selective increased of lamina propria CD4+LAP+ Foxp3- T cells and were protected from TNBS-induced experimental colitis, a colitis resembling Crohn's Disease (Amendola A. *et al*, 2012)(2). Thus, the increase in intestinal permeability rather than representing a risk factor for the onset of intestinal inflammation seems to be associated to an increased regulatory response to the microbiota components suggesting that it may represent a mechanism to reinforce mucosal tolerance toward the microbiota. The data also demonstrated that environmental factors are necessary to uncover the effects of the genetic background on the development of inflammation. Since adoptive transfer of CD4+Foxp3+ regulatory T cells, as opposite to the transfer of CD4+LAP+ Foxp3- regulatory T cells, was unable to protect the recipient mice from TNBS colitis, the expansion of CD4+LAP+ Foxp3- regulatory T might be of clinical relevance in the management of IBD. In previous studies we have shown that the generation of LP CD4+LAP+ regulatory cells was dependent on the presence of an intact intestinal microbiota (1). Thus, it seemed possible that *Nod2 -/-* mice harbor a changed microbiota that affects the development of regulatory cells in the *Nod2*-deficient host. To examine this possibility, we conducted co-housing studies in which *Nod2 -/-* mice were maintained in the same cages with *Nod2 +/+* mice or maintained in separate cages at weaning for 4 weeks before induction of TNBS colitis. Co-housed *Nod2 -/-* mice exhibited more severe colitis and decreased LP CD4+LAP+ T cells than non-co-housed *Nod2 -/-* mice. Reciprocal studies of *Nod2 +/+*

showed that *Nod2* +/+ mice did not exhibit a change in baseline LP CD4+LAP+ cells. Thus, the *Nod2* -/- mice develop an intestinal microbiota that supports regulatory responses that contribute to the protection of mice from development of TNBS-colitis (Amendola A. *et al*, 2013) (3). I am now involved in "*in vitro*" studies to fully characterize the requirements for the expansion of CD4+LAP+ Foxp3- regulatory T cells. In these studies I am using different recombinant cytokine produced by epithelial cells, dendritic cells and T lymphocytes.

References:

1) Boirivant M, Amendola A, Butera A, Sanchez M, Xu L, Marinaro M, Kitani A, Di Giacinto C, Strober W, Fuss IJ. A transient breach in the epithelial barrier leads to regulatory T-cell generation and resistance to experimental colitis. GASTROENTEROLOGY. 2008; 135: 1612-1623.e5.

2) Amendola A, Butera A, Boirivant M. NOD2 Deficiency is Associated With Increased Mucosal Regulatory Response to Commensal Microorganisms. GASTROENTEROLOGY. 2012; 142: S12-S12

3) Amendola A, Butera A, Sanchez M, Strober W and Boirivant M. *Nod2* deficiency is associated with an increased mucosal immunoregulatory response to commensal microorganisms. Mucosal Immunology 2013. doi: 10.1038/mi.2013.58. [Epub ahead of print]