SUMMARY

Lymphoid tissue: from Ontogeny to Function

Secondary lymphoid organs (SLOs), such as lymph nodes, spleen and Peyer’s patches, play a critical role in the homeostasis of the immune system. On the one hand, they form nuclei of immunosurveillance, where danger signals and antigens are scrutinized by the innate and adaptive immune system, and where immune responses are initiated. On the other hand, they ensure that unwanted immune responses do not take place as they purge the lymphocyte pool of autoreactive T cells and increase the threshold for B and T cell activation.

These seemingly antagonistic functions are particularly relevant at the body’s interfaces with the external world, given the continuous exposure to both harmless as well as pathogenic microorganisms and xenobiotics requiring disparate immune responses. In the present work, we have analyzed the development of SLOs at one of such interfaces – the colon. First, we describe a new methodology based on α-smooth muscle actin (αSMA) staining to discriminate between colonic patches and colonic solitary intestinal lymphoid tissue (SILTs) and show that the development of these tissues is independently and differentially regulated. Whereas colonic patch development during embryogenesis depended on CXCL13-mediated lymphoid tissue inducer (LTi) cell clustering followed by lymphotoxin α (LTα)-mediated consolidation, post-natal colonic SILT development seemed, as far as analyzed, chemokine-independent. Differentiation of gp38+VCAM1+ lymphoid tissue organizer (LTo) cells and dendritic cell accumulation within SILTs was, however, LTα-dependent; and B cell recruitment and follicular dendritic cell development dependent on microflora-independent MyD88 signaling. We subsequently extend our findings by showing that, in contrast to colonic patches, colonic SILT maturation (i.e. B cell recruitment) was dependent on Caspase1 activity (IL1 processing), IL1R/MyD88 signaling. This molecular axis was responsible for the accumulation of NKp46+ innate lymphoid cells (ILCs) within the maturing SILT. The presence of NKp46+ ILCs correlated with B-cell activating factor of tumor necrosis factor family (BAFF) expression, which may support the accumulation and survival of the SILT-infiltrating B cells. Focusing on the triggers that drive the early clustering of LTi cells at the colonic SILT anlagen, we determined that SILT development critically depended on the proper differentiation of the colonic epithelium. The combined action of repulsive and attractive factors secreted by differentiated villus intestinal epithelial cells and undifferentiated crypt intestinal epithelial cells, respectively, directed LTi cell clustering to the bottommost part of colonic intestinal crypts. In vivo interference with such migratory vector by conditional deletion of differentiated secretory intestinal epithelial cells or undifferentiated intestinal epithelial stem cells led to the development of reduced numbers of SILTs.

SLOs are structured by a population of non-hematopoietic stromal cells that was previously shown to strongly contribute to the function of the immune cells that reside within those SLOs. In this work, we present additional data implicating these scarce cells in both immune tolerance maintenance as well as the induction of immune responses. We show, that in addition to mediating tolerance within the CD8+ T cell repertoire, lymph node stromal cells also tolerize CD4+ T cells. MHC-II-mediated antigen presentation by lymph node stromal cells was required for the homeostatic maintenance of regulatory T cells (Tregs), and consequently for the maintenance of
immune quiescence and the enforcement of immune tolerance. In the selective absence of MHC-II expression on lymph node stromal cells, which was achieved by means of MHC-II-/- lymph node transplantation, the CD4+ and CD8+ T cell compartments became activated, ultimately resulting in transplant rejection. Conversely, Toll-like receptor (TLR) expression on lymph node stromal cells contributed to enhancement of immune responses. Stimulation of lymph node stromal cells with TLR ligands increased the expression of chemokines and adhesion molecules, and modified the patterns of immune cell recirculation leading to lymphocyte accumulation within the reactive lymph node. As a result, higher numbers of antigen-specific T cells were recruited into the developing immune responses, increasing its overall magnitude.

In conclusion, this work highlights the diversity of developmental pathways governing the formation of SLOs and the critical engagement of their stromal cell constituents in shaping immune function. The data presented opens new perspectives for the therapeutic manipulation of the immune system by identifying aspects of lymphoid tissue organogenesis that are unique for the development of some, but not other, SLOs. This may allow the targeting of local pathological processes while leaving systemic immunity unaltered. Furthermore, the identification of distinct immunomodulatory properties of lymph node stromal cells could be targeted to prevent unwanted immune responses, such as those occurring during auto-immune diseases, or to enhance desirable immune responses, such as those elicited by vaccination.