



Samenvatting van het proefschrift
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"Formation and Function of Organized Gut Associated Lymphoid Tissue in Health and Disease"

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The intestinal tract is a unique tissue which contains a vast amount of commensal bacteria and food antigens. The intestine maintains a delicate balance of establishing tolerance to both commensal bacteria and food antigens, which are constantly present in the intestinal tract and defending the host against harmful pathogens. A shift in this balance can cause serious disease - a failure to induce tolerance to antigens present in the intestinal tract will lead to chronic inflammation (i.e. Celiac's Disease or Inflammatory Bowel Disease) and a failure to clear harmful pathogens will lead to persistent diarrhea, dehydration and in extreme cases even death. In order to maintain this delicate balance, the intestine contains different types of specialized organized gut associated lymphoid tissue (GALT) which provides a unique microenvironment bringing various immune cells and antigens into close contact with each other, allowing the induction of tolerance or alternately immune system activation.

Most research work to date focuses on the formation of organized GALT of the small intestine i.e. Peyer's Patches, Solitary Intestinal Lymphoid Tissue (SILT) and the draining mesenteric lymph node. Both Peyer's Patches and mesenteric lymph nodes have been shown to be present before birth, as clusters of lymphoid tissue inducer (LTi) and stromal organizer cells, and can thus be classed as true secondary lymphoid tissue. In contrast to this, SILT is not present before birth but forms after birth in the first two weeks of life and can thus not be classed as secondary lymphoid tissue. The formation of Peyer's Patches, mesenteric lymph nodes and SILT in the small intestine has been shown to be dependent on the lymphotoxin alpha (LT α) lymphotoxin beta receptor (LT β R) signalling axis. The triggering of LT β R on stromal organizer cells, by LT α produced by LTi cells, leads to the induction of chemokines (CXCL13, CCL21) and adhesion molecules (VCAM, ICAM,

MAdCAM). These cytokines and adhesion molecules are important for the subsequent attraction and retention of LT_i cells to the area and is responsible for the formation of organized GALT in the small intestine.

In chapter 2 of this thesis we investigate the different types of organized GALT of the colon. We show that two different types of organized GALT exist within the colon i.e. colonic patches and solitary intestinal lymphoid tissue (SILT). Colonic patches are present before birth at embryonic day 18.5 as clusters of LT_i and stromal organizer cells scattered along the antimesenteric border in the sub mucosa of the colon. Over the ensuing two weeks after birth these structures fill with T and B cells and give rise to a structure which contains more than one B cell follicle with distinct T cell areas. The B cell follicles which develop within these colonic patches contain follicular dendritic cells and germinal centres. Due to the fact that these structures are present before birth they can be classed as secondary lymphoid tissue. In the first two weeks of life a second type of organized lymphoid tissue forms within the colon which is referred to as SILT. SILT can be subdivided into five different classes based on their size and cellular composition. While the smallest SILT contains mostly clusters of LT_i cells, the larger clusters of SILT contain more B cell clusters. All classes of SILT are present within the lamina propria of the colon two weeks after birth. Thus in the normal adult mouse colon two different types of organized GALT exists which can be distinguished from one another. In this chapter we also show that colonic patches and mature SILT formation is dependent on LT α R signalling while the initial clustering of immature SILT development is regulated by other initiation cues.

In chapter 3 we investigate an alternative lymphotoxin independent pathway for the formation of secondary lymphoid tissue. We show in this chapter that during ontogeny neurons occur in close association with stromal organizer cells at predestined sites, were secondary lymph nodes forms, and that these neurons contain enzymes which convert vitamin A into its active metabolite retinoic acid (RA). RA leads to an induction of CXCL13 by stromal organizer cells, which is responsible for the initial attraction of LT_i cells to the area. Thus neurons providing a source of RA to stromal cells, which leads to the induction of CXCL13, may be an alternative pathway for the clustering of LT_i cells in a lymphotoxin independent manner.

It is known that lymphoid tissue can form in adult life in a variety of different tissues if the tissue is chronically inflamed. The de novo formation of lymphoid tissue which forms in adult life, as a result of inflammation, is referred to as tertiary lymphoid tissue. Due to the fact that two types of lymphoid tissue already exists within the healthy adult colon, the identification of the third type of lymphoid tissue i.e. tertiary lymphoid tissue is a challenging task. In chapter 4 we propose a definition for all three types of lymphoid tissue in the adult colon which allows for them to be identified: (1) Colonic Patches consist of more than one B cell follicle with distinct T cell areas and occurs in the sub mucosa of the colon, (2) SILT consist of a single

B cell follicle with scattered T cells which occurs in the lamina propria of the colon and (3) tertiary lymphoid tissue which consists of more than one B cell follicle with distinct B cell areas but occurs in the lamina propria of the colon. We also show that the formation of tertiary lymphoid tissue is only partially dependent on lymphotoxin signalling as $LT\alpha^{-/-}$ mice have the ability to form tertiary lymphoid tissue however this tertiary lymphoid tissue lacks follicular dendritic cells and germinal centres. Interestingly, we show the expression of the vitamin A conversion enzymes (RALDH 1 and 2) by neurons present within the tertiary lymphoid tissue of both wild type and $LT\alpha^{-/-}$ animals along with expression of CXCL13 by stromal cells. Thus it is plausible that neurons may also provide a lymphotoxin independent mechanism for the induction of CXCL13 by stromal cells which could initiate the initial attraction and clustering of B cells marking the beginning of tertiary lymphoid tissue formation within the inflamed colon.

Retinoic acid (RA) is known to have numerous effects on the mucosal immune system. It has been shown that RA leads to the upregulation of gut homing molecules on lymphocytes, enhances the differentiation and gut homing of T regulatory cells to the intestine and is needed for IgA production by B lymphocytes. It has also been shown that RA suppresses TH1 responses and enhances TH2 responses. It is known that BALB/c mice predominantly have a TH2 response and C57/BL6 mice predominantly have a TH1 response thus we investigated if there was a difference in the amount of RA signaling taking place in the intestine of these two mice strains. Indeed in chapter 5 we show that there is an increase in vitamin A conversion enzymes and RA signaling within the small intestine and draining mesenteric lymph node of BALB/c mice compared to C57BL/6 mice. This increase in RA signaling is accompanied by an increase of immune cells, i.e. regulatory T cells, T cells and B cells, in the intestine. Furthermore increased RA signaling in BALB/c mice was also accompanied by increased IgA production. Therefore, the level of RA production and consequently the degree of RA-mediated signaling is crucial for the efficiency of the mucosal immune system.

BALB/c mice are reported to be resistant to dextran sulfate sodium (DSS) induced colitis when compared to C57BL/6 mice. DSS is a chemical compound which disrupts the epithelial barrier integrity allowing microflora present within the colon to enter the lamina propria giving rise to inflammation which strongly resembles ulcerative colitis. To show if the inherent increase in RA signaling, within the intestine of BALB/c mice, was beneficial in dealing with inflammation DSS colitis was induced in both BALB/c and C57BL/6 mice. Indeed, in chapter 5 we confirm that BALB/c mice are resistant to DSS induced colitis and that they have higher levels of vitamin A conversion enzymes and RA signaling in the colon. BALB/c mice also have more organized GALT than C57BL/6 mice which may be a result of increased RA signaling. To show the effects of vitamin A and/or RA signaling within the colon during inflammation vitamin A deficient animals were

created. Indeed we showed that vitamin A deficient animals suffered from more severe colitis than controls.

Taken together this research works shows the importance of vitamin A, and its active metabolite RA, in the formation and function of organized GALT of the intestine. Furthermore, the conversion of vitamin A into RA by neurons may be responsible for the formation of tertiary lymphoid tissue during chronic inflammation of the colon. This tertiary lymphoid tissue may be of benefit to the host in the context of inflammatory bowel disease as tertiary lymphoid tissue contains regulatory T cells and also B cells which are responsible for the production of IgA. Thus administration of vitamin A, or its active metabolite RA, may serve as a potential new therapeutic strategy for the treatment of inflammatory bowel disease. ◀