Commentary

Mutant Mice without B Lymphocyte Follicles

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The immune system requires the cognate interactions of T cells, B cells, and antigen-presenting cells to respond to invading antigens/pathogens. However, the peripheral lymphoid organs where immune responses occur are not simply made of a random mixture of T cells, B cells, and antigen-presenting cells. Rather they are organized into microanatomic compartments that are composed mainly of T cell zones and B cell follicles. T cell zones are found in the paracortex of lymph nodes, the periarteriolar lymphoid sheaths (PALS) of spleen (Fig. 1) and the dome area of Peyer's patches. They contain both $CD4^+$ and $CD8^+$ T cells as well as interdigitating dendritic cells (IDC). B cell areas can be found in the form of either resting primary follicles or activated secondary follicles. A primary B cell follicle contains $sIgM^+IgD^+$ resting recirculating B cells and follicular dendritic cells (FDC) (Fig. 1). A secondary B cell follicle is composed of a follicular mantle containing $sIgM^+IgD^+$ resting B cells and a germinal center composed of centroblasts, centrocytes, activated $CD4^+$ memory T cells, $CD4^+$ $CD11c^+CD3^-$ germinal center dendritic cells (GCDCs) and FDC (1, 2). In addition, a third compartment, the marginal zone, observed in the spleen, contains a subset of non-recirculating $sIgM^{high}IgD^{low}$ B cells (Fig. 1) (3, 4), marginal macrophages as well as marginal metallophils (5–7). What are the functional advantages for peripheral lymphoid tissues to be structured into complex T and B cell zones? What are the molecular mechanisms underlying the segregation of T cell zones and B cell follicles? In this issue of *The Journal of Experimental Medicine*, Pasparakis et al. report that the spleens of TNFα knock-out mice lack primary follicles and mature FDC (8). These mice display impaired humoral immune responses and are unable to form germinal centers in response to T cell–dependent antigens. This observation, together with other recent reports, highlights the fundamental role of members of the TNF-TNFR superfamily in the development of peripheral lymphoid organs and germinal centers.

The Importance of TNFRI in Germinal Center Development

TNFα and LTα bind to the same receptors TNFRI (P55/CD120a) and TNFRII (P75/CD120b)(9). In addition, when one LTα monomer trimerizes with two identical LTβ subunits, the heterotrimers bind a third receptor, TNFRβ (10). In an earlier report, Chaplin's group has shown that LT-deficient mice display an abnormal splenic architecture and an inability to form germinal centers (11). However, in contrast to the TNFα-deficient mice reported herein by Pasparakis et al., LT-deficient mice lack visible peripheral lymph nodes and Peyer's patches (12). In the recent 6th International TNF congress, several groups have reported the successful blocking of signaling through TNFRI, TNFRII, and TNFRβ, respectively in various in vivo mouse models either by gene inactivation or by producing high concentrations of soluble receptors (13–16). It appears that while signaling through TNFRI is involved in germinal center development in the spleen (11, 13), signaling through TNFRβ is essential for the development of lymph nodes and Peyer's patches (15) as well as the development of normal splenic T cell zones, B cell zones and marginal zones (16). Interestingly, introduction of a human TNFα transgene into mTNFα KO mice restores the ability of those mice to form germinal centers (8). As human TNFα binds to mTNFRI but not mTNFRII, the results confirms the critical role of TNFRI in the induction of germinal center formation.

Previous studies have shown that mice that are deficient for the key molecules involved in T cell–B cell interactions such as CD40 (17), CD40-ligand (18), and MHC class II (19) or transgenic mice that produce large amounts of soluble CTLA-4 (20) also lack the ability to form germinal centers. However, in contrast to the mice lacking LTα, LTαβ, TNFα, and their receptors, these mice display normal peripheral lymphoid organs. Thus, mice that are deficient for LTα, LTαβ, TNFα, or their receptors may not directly inform us about the molecular controls of germinal center reaction during T cell-dependent immune responses, but rather about the molecular mechanisms underlying the development and organization of peripheral lymphoid organs.

Expanded Splenic Marginal Zones, Normal T Cell Zones, and Absence of Primary Follicles and Follicular Dendritic Cells in TNFα-deficient Mice

An important observation of Pasparakis et al. in TNFα-deficient mice is the expanded splenic marginal zone B cell compartment that contrasts with the lack of primary follicle and follicular dendritic cells (8). An explanation for such a pattern is that the recirculating B cells in these mice may not cross the marginal zone sinuses and migrate into the PALS to form primary follicles. In this context, the TNFRI-deficient mice reported by Chaplin et al. appear to have IgD^+ recirculating B cells in their splenic marginal zones (11). Since the splenic marginal zone in normal mice (as in normal rats and humans) contains a subpopulation of $sIgM^+IgD^-$

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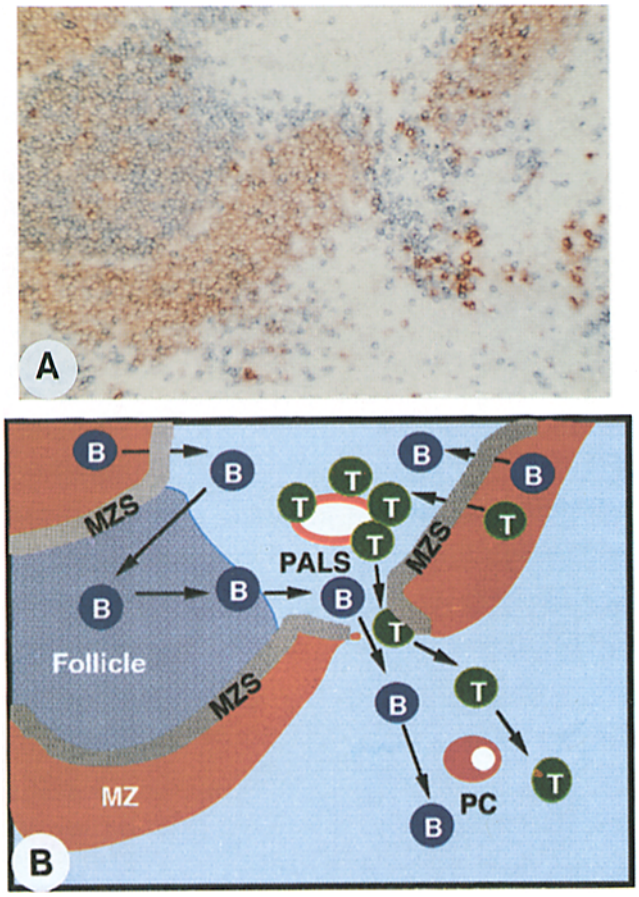

Figure 1. The structure of splenic white pulp and the migration pathways of B lymphocytes and T lymphocytes (1). (*a*) shows the double anti-IgM (*red*) and anti-IgD (*blue*) immunohistological staining of a splenic section from a non-immunized rat spleen. *b* shows the schematic representation of *a* and the migration pathways of non-activated B and T cells within the spleen. The primary follicle (*blue*) contains IgM^+IgD^+ recirculating B cells, which enter the spleen through the marginal zone (*MZ*, *red*). These cells cross the marginal zone sinuses (*MZS*) and then migrate along the outer edge of periarteriolar lymphoid sheaths (*PALS*) into the primary follicle. The follicular B cells subsequently migrate into the red pulp through the gaps known as bridging channels (*BC*), that break the marginal zone. Recirculating T lymphocytes also enter the spleen through the marginal zone. They migrate into the periarteriolar area, forming the so called PALS. Then these T cells migrate into the red pulp through the bridging channels. The cells with strong IgM staining (*red* in *a*) are plasma cells (*PC*).

non-recirculating B cells (3, 4), the presence of large numbers of IgD^+ B cells in the splenic marginal zone of TNFRI-deficient mice suggests that the migration of IgD^+ B cells may be blocked at that level. By double anti-IgM and anti-IgD staining, Pasparakis et al. here indeed show that IgD^+ recirculating B cells are localized within the inner part of the splenic marginal zone of TNFα-deficient mice (8). The splenic B cell compartments (primary B cell follicles and marginal zones) in TNFRI KO mice seem to require a more careful re-examination (11, 21). The possible failure of recirculating IgD^+ to cross the marginal sinuses to form primary follicles could be due to missing adhesion receptors on the marginal zone endothelial cells, since both LTα and TNFα have the ability to induce the expression of adhesion molecules, such as ICAM-1, VCAM-1, MadCAM-1, and PNAd on endothelial cells (22–25). In keeping with this, TNFRI KO mice were shown to lack the expression of MAdCAM-1 in splenic marginal zone sinuses (26). Alternatively, it may be due to the lack of chemotactic factors released by cells localized within the T cell zones, such as IDCs. In keeping with this, it has been demonstrated that TNFα is required for the generation of DCs from $CD34^+$ hematopoietic progenitor cells (27) and for the migration (28) and survival of Langerhan's cells (29). The spleen of relB-deficient mice, that lack dendritic cells, shows a disorganized architecture and no germinal center formation during T cell–dependent immune responses (30).

Both T and B cells enter the spleen through marginal zones (31). In TNFα-deficient mice, while B cells are blocked within the marginal zones, T cells migrate normally through the marginal zone sinuses to form the T cell zones (8). This finding represents the first experimental evidence indicating that the molecular mechanisms controlling the entries of B and T cells into the splenic white pulps are different.

The lack of FDC in both TNFα and TNFRI-deficient mice may very well be explained by the absence of follicular B cells, as mice depleted of B cells by neonatal anti-IgM treatment also lack FDC (32). In keeping with this, SCID mice also lack mature FDC, but FDC develop in their peripheral lymphoid organs after B and T cell transfer (33).

Transplantation of Wild-type Bone Marrow Corrects the Developmental Defect of Peripheral Lymphoid Organs and Germinal Centers

Chaplin's group has further demonstrated that transplantation of wild-type bone marrow into LT-deficient mice restores the formation of peripheral lymph nodes and splenic germinal centers (11). A similar experiment by Müller et al. shows that the abnormal splenic structure in TNFα-LTα–deficient mice can also be corrected by wild-type bone marrow transplantation (34). These two experiments indicate that the splenic structure is not fixed during the early fetal development as the defect can be corrected by bone marrow derived LTα and/or TNFα producing cells in postnatal development.

Ectopic Expression of LT and TNFα May Be Responsible for the Ectopic Development of Lymphoid Follicles and Germinal Centers in Autoimmune Diseases

Ectopic development of lymphoid follicles and germinal centers has long been observed within the thymus of patients with myasthenia gravis (35) and within the synovial tissues of patients with rheumatoid arthritis (36). The mechanisms underlying this phenomenon may now be uncovered by observations made in transgenic mice in which either LT is expressed under the insulin promoters or TNFα is expressed under the CD2 promoter. These mice devel-

oped ectopic lymphoid follicles and germinal centers within the kidneys and pancreas (37) or within the thymus and lungs (38), respectively. In this context, the critical role of TNFα in the pathogenesis of rheumatoid arthritis is further shown by the spectacular clinical improvement observed after anti-TNFα monoclonal antibody therapy (39, 40).

Future Prospective

As discussed above, the impairment of germinal center formation may not be a direct consequence of knocking out TNFα, LTα, LTαβ, and their receptor genes. Therefore, there is now a need to specifically understand the early cellular and molecular mechanisms that lead to defects in the development of primary follicles, follicular dendritic cells and splenic marginal zones. For example, it will be informative to carry out: (*i*) an in situ analysis of cell types such as Langerhan's cells, interdigitating cells, tissue macrophages and T cell subsets; (*ii*) a thorough analysis of the expression of adhesion and homing molecular pairs in peripheral lymphoid organs and in circulating leukocytes.

Since bone marrow transplantation can correct the developmental defects in TNFα and TNFα-LTα-deficient mice, it will be useful to determine which cell type (T cells, B cells, dendritic cells, macrophages?) actually permits the reconstitution.

The absence of primary follicles and follicular dendritic cells in TNFα-deficient mice may provide important models to address several fundamental questions in immunology: (*i*) Do primary follicles and FDC play an important role in the survival and recruitment of newly generated non-self reactive B cells into the recirculating B cell pool (41–43)? (*ii*) Do follicular dendritic cells play any function in the maintenance of memory B cell clones (44)? (*iii*) What are the contributions of follicular B cells versus marginal zone B cells to the T cell dependent and T cell–independent antibody responses (45, 46)?

Somatic-hypermutation and affinity maturation without GCs in lymphotoxin KO mice just comes out as another striking finding (47). It opens a new area of investigation on the kinetics and sites of antigen-specific B cell activation in all these interesting mutant mice.

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