Transfer of Primitive Stem/Progenitor Bone Marrow Cells from LT α−/− Donors to Wild-Type Hosts: Implications for the Generation of Architectural Events in Lymphoid B Cell Domains

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Transfer of Primitive Stem/Progenitor Bone Marrow Cells from LTα−/− Donors to Wild-Type Hosts: Implications for the Generation of Architectural Events in Lymphoid B Cell Domains

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To analyze whether the phenotypic abnormalities observed in lymphoxygenin-α−/− (LTα−/−) mice are intrinsic to the hemolymphoid system itself or dependent on stromal elements, wild-type (WT) mice were reconstituted with bone marrow (BM) cells enriched for hemopoietic stem cells from LTα−/− animals. WT mice reconstituted with LTα−/− c-kit+Lin−Sca-1+ BM cells do not maintain follicular dendritic cell (FDC) networks and do not form primary follicles, while clear segregation of B and T cells could be observed. Furthermore, IgM+IgD− B cells, MOMA-1 (anti-metallophilic macrophages), ERTR-9 (anti-marginal zone macrophages), and MEC-367 (anti-MAdCAM-1) were all absent from the splenic marginal zone. Surprisingly, however, the expression of MAdCAM-1, MOMA-1, and ERTR-9 in lymph nodes are not. Our results also suggest that the disturbed B-T cell segregation in LTα−/− mice is unrelated to defects in the marginal zone. The Journal of Immunology, 1998, 161: 3836–3843.

Members of the TNF superfamily of regulatory molecules TNF-α and lymphotoxin-α (LTα) are involved in both inflammatory and immune responses (1). Both TNF-α and LTα can exist as soluble homotrimers and can bind to both TNFR-1 (p55) and TNFR-II (p75) (2–4). In addition, LTα can exist as a membrane-bound heterotrimer with LTβ, which provides the transmembrane domain (5). This LTαβ2 complex can be specifically recognized by the LTβR, but not TNFR-I or TNFR-II (6). Thus, TNF-α and LTα have overlapping, yet unique, signaling functions depending on which ligand/receptor combination is used.

Recently, studies in mice deficient for the TNF/LT receptors or ligands have shown that these molecules also play important roles in the development and organization of the immune system (7–10). In contrast, the lymph nodes and Peyer’s patches are completely absent in LTα-deficient mice (13, 14), although rudimentary mesenteric lymph nodes can be observed in rare animals (14, 15). Furthermore, the normal T cell/B cell segregation was disrupted in the spleens of LTα−/− mice, and they were reported to be missing FDC and GC (7, 13). Part of these abnormalities are due to lack of signaling through the lymphotoxin-β receptor (LTβ-R) ligand, since blocking of this ligand during gestation results in the absence of Leydig’s patches and most of the peripheral lymph nodes, and Peyer’s patches are present (9, 10).

To determine whether all defects in LTα−/− mice could be attributed to the lack of LTα expression in cells of the hemopoietic lineage, we reconstituted lethally irradiated WT mice with LTα−/− BM cells, highly enriched for hemopoietic stem cells, separated using FACS into c-kit+Lin−Sca-1+ cells (KLSC). Using a population of purified hemopoietic progenitors, not contaminated by BM-derived stromal cells or their precursors, allowed us to study the effect of the absence of LTα in hemopoietic cells only.
Injection of Ly-5.1 LTα−/− cells into Ly-5.2 WT mice permitted us to follow the progeny of LTα−/− progenitor cells at the single-cell level in situ. We also made mixed chimeras, reconstituted with KLSC from both LTα−/− and WT mice, to determine which defects observed in LTα−/− KLSC-reconstituted mice were abolished in the presence of WT hemopoietic cells and whether LTα−/− cells were at a competitive disadvantage, in particular lymphoid structures. Here we show that although LTα−/− B cells are incapable of forming discrete primary follicles, they can form GC, similar to the phenotype of the LTβ−/− mice (17).

Materials and Methods

Animals

C57BL/Ka-Ly-5.2 mice were maintained in the laboratory animal colony at Stanford University School of Medicine, Stanford, CA. LTα−/− mice (backcrossed to C57BL/6 (Ly-5.1) generations F6 and F7) were kindly provided by Dr. D. Chaplin (Washington University, St. Louis, MO) via Dr. P. R. Streeter (Monsanto, St. Louis, MO). All animals were kept under routine laboratory conditions.

Antibodies

The following Abs were used to label for either FACS analysis or immunohistochemistry: 6B2 (anti-B220); KT-31 (anti-CD3); M1/70 (anti-Mac-1); 8C5 (anti-Gr-1); NK1.1 (anti-NK cell marker; Pharmingen, San Diego, CA); AL-4A2 (anti-Ly-5.1); A20.1.7 (anti-Ly-5.2); 104 (anti-Ly-5.2; Pharmingen) (mAb 6B2, KT-31, M1/70, 8C5, AL-4A2, and A20.1.7 were affinity purified from culture supernatant from hybridoma cells with protein G-Sepharose (Pharmacia, Uppsala, Sweden) and labeled in our laboratory); FDC-M2 (anti-FDC; provided by M. Kosco); ED31 (anti-MARCO) (19); provided by L. van der Laan); ERTR-9 (anti-marginal zone macrophages); MOMA-2 (anti-macrophages) (mAb ERTR-9, and MOMA-1 were provided by G. Kraal); MECA-367 (anti-MAcAM1; hybridoma was provided by E. C. Burcher); b.7.6 (anti-mouse IgM) (20); 1.19 (anti-mouse IgD) (21) (mAb b.7.6 and 1.19 were provided by G. G. B. Klaas).

Isolation and injection of populations highly enriched for hemopoietic stem cells

BM was obtained by flushing femurs and tibia from C57BL/Ka-Ly-5.2 or LTα−/− (Ly-5.1) mice with PBS containing 2% calf serum. The BM suspension was filtered by nylon mesh and stained for 20 min on ice with a lineage mixture of unlabeled Abs containing M1/70 (anti-Mac-1), 8C5 (anti-Gr-1), GK1.5 (anti-CD4), 53-6.7 (anti-CD8), KT-3.1 (anti-CD3), 53-7.3 (anti-CD5), 6B2 (anti-B220), and Ter119 (anti-erythroid), and most positive cells were removed using the MACS magnetic bead system (Miltenyi Biotech, Sunnyvale, CA) as described elsewhere (22). The lineage− fraction was subsequently stained with anti-Sca-1 (E13) and anti-c-kit (3C11), and the Sca-1 c-kit− lineage− (KLSC) population was sorted.

Long term reconstitution assays

C57BL/Ka-Ly-5.2 mice were lethally irradiated with 930 rads using an x-ray source operated at 200 kV, delivering 85 rads/min. Mice were reconstituted in two doses, of 465 rads each, 3 to 5 h apart. Sca-1−, c-kit−, lineage− hemopoietic stem cells (1000) from either LTα−/− (Ly-5.1) (KLSC-LTα−/−) or WT (C57BL/Ka-Ly-5.2) (KLSC-WT) mice or a combination of 1000 KLSC-LTα−/− and 1000 KLSC-WT (mixed KLSC) were injected retroorbitally into irradiated hosts (C57BL/Ka-Ly-5.2). Five mice were reconstituted per group. After irradiation, mice were maintained on antibiotics present in the water (1.1 g/L neomycin sulfate and 100 μL/L polymyxin B sulfate).

Analysis of reconstituted mice

For analysis of reconstitution, mice were bled from the tail and assayed for the presence of Ly-5.2+ cells of each lineage. To do so PBL were stained for lineage markers, anti-B220 to identify B cells, anti-CD3 to identify T cells, and a combination of anti-Mac-1 and anti-Gr-1 to identify myeloid cells, in combination with anti-Ly-5.2. Mice were killed to analyze spleen, lymph nodes, and Peyer’s patches by immunohistochemistry at different time points (13–27 wk) after reconstitution.

Immunohistochemistry

Cryostat sections of spleens or lymph nodes (5 μm thick) were allowed to dry for 15 min before fixation in acetone (2 min) after which they were air dried for another 15 min (12). The sections were incubated with various anti-mouse mAbs at saturating concentrations in PBS containing 0.1% BSA (PBS/BSA) for 45 min. After thorough washing in PBS, sections were incubated with the appropriate peroxidase-labeled second reagent (Jackson Immunoresearch, San Francisco, CA) in PBS/BSA.

For in situ analysis of donor-derived cells in the mixed chimeras, sections were stained for Ly-5.1 or Ly-5.2, in combination with several rat anti-mouse mAb. The sections were incubated with biotin-conjugated Ly-5 and one of the rat anti-mouse mAb for 45 min at room temperature, followed by peroxidase-labeled avidin-biotin complex (DAKO, Carpenteria, CA) and alkaline phosphatase-labeled goat anti-rat Ig in the presence of 5% mouse serum (Sigma, St. Louis, MO). Peroxidase activity was visualized by staining with 3,3′-diaminobenzidine tetrahydrochloride (Sigma) at a concentration of 0.5 mg/ml in Tris buffer (pH 7.2–7.6) containing 0.01% H2O2 for 10 min at room temperature. Alkaline phosphatase was detected using Fast Blue (Sigma), as described earlier (23). Double labelings for IgM and IgD were performed as described elsewhere (9, 24).

Results and Discussion

LTα−/− B and T cells segregate normally in WT spleens

In addition to the absence of lymph nodes and Peyer’s patches in LTo-deficient mice, there is a failure of the normal B and T cell segregation (12) in the spleen (13, 14). However, it is unclear whether this abnormality is due to an intrinsic B or T cell defect or a defect in lymphocyte development, or whether stromal cells in the spleen are lacking the necessary molecules to keep the B and T cells in place. Previous studies, in which LTα−/− splenocytes or bone marrow cells were transferred to WT recipients, suggest that the defect of B/T cell segregation in LTo−/− mice lies at the level of the stromal cells within the spleen (18). However, these studies were done with mixtures of cell populations (total spleen or bone marrow cells), and we therefore repeated these studies with a highly enriched precursor population isolated from the bone marrow. We reconstituted lethally irradiated C57BL/6 mice with KLSC from either LTα−/− mice or wild-type (WT) C57BL/6 mice using the congenic Ly-5 system to be able to trace the transferred cells. Hemolymphoid cells derived from the LTo−/− mice all express the Ly-5.1 allelic form of CD45 and can easily be distinguished from cells of WT mice, which express the Ly-5.2 form.

We sorted the c-kit+ lineage− Sca-1+ (KLSC) population of cells, which contains the most primitive hemopoietic stem cells with long term reconstituting potential (22), from the bone marrow of both LTo−/− and WT mice. The Sca-1+ c-kit+ lineage− BM cells were found at a similar frequency in both strains of mice, indicating that the absence of LTo had no effect on the maintenance of primitive hemopoietic progenitors. Lethally irradiated WT mice were then reconstituted with 1000 Sca-1+ c-kit+ lineage− cells from either LTo−/− or WT mice. In addition, five mice were also reconstituted with a mix of KLSC from both LTo−/− and WT mice, to test for any competitive defects in the cells derived from the LTo−/− KLSC.

At different times after reconstitution, the mice were analyzed by FACS analysis of peripheral blood for the percentage of LTo−/−-derived cells, as well as by immunohistochemistry for their cellular distribution in situ. At all time points, the reconstituted mice had the expected percentages of LTo−/− and WT cells in peripheral blood. Thus, mice reconstituted with LTo−/− KLSC had >90% LTo−/−-derived PBLs in all lineages, while mice reconstituted with mixed KLSC had between 30 and 60% LTo−/−-derived PBLs in all lineages. As expected, mice reconstituted with WT KLSC had 100% WT PBLs. Furthermore, the degree of reconstitution was stable throughout a period of 27 wk, when the last animals were killed. Thus, the hemopoietic compartment of the bone marrow appeared normal in LTo−/− animals, and we did not observe any selective defects in the production of circulating T, B,
or myeloid cells even when LTα−/− and WT cells were in a competitive situation (not shown).

At 13, 23, and 27 wk after reconstitution, tissues were analyzed by immunohistochemistry. Labelings for B220 and CD3 were performed to determine whether distinct B and T cell areas could be found. In mice reconstituted with WT cells, B and T cell areas were segregated normally, as expected (Fig. 1, A, C, and E). In mice reconstituted with KLSC derived from LTα−/− mice, distinct B and T cell areas could be observed in both spleen and lymph nodes (Fig. 1, B, D, and F), although the B cells tended to be organized in a ring around the T cell area rather than in discrete follicles. Therefore, although well-defined B and T cell areas are absent in LTα−/− mice, LTα−/− cells can clearly find their final destination in a WT host.

Our results suggest that the defect in initial formation of segregated T and B cell areas in LTα−/− mice is not a consequence of the failure of hemolymphoid cells and that either these domains, once established in WT mice, are stable in the absence of local LTα expression, or that radioresistant cells in the spleen are producing LTα/β ligands, necessary for the organization of B-T cell compartments. The recent observation that injection of LTβ-R-Ig chimeras in adult WT mice causes a loss of B cell follicles and a disappearance of the B and T cell compartments suggests that radioresistant cells in the spleen are producing LTβ-R ligands continuously (25). This production of LTβ-R ligands is apparently not sufficient for the organization of B cells into follicles.

Mice reconstituted with LTα−/− KLSC have defects in the splenic marginal zone but not in the lymph node subcapsular sinus

Cell expressing the macrophage-specific Ag MOMA-1 have been reported to be missing in the marginal zone of the spleen of the LTα−/− mice (7). In addition, MAdCAM-1 expression could not be observed on the splenic sinus lining cells of either LTα and TNF-α doubly deficient mice (26) or LTβ-R-Ig infused mice (16). Here we found that although ERTR-9+ MOMA-1+, and MAdCAM-1+ (MECA-367) cells could be easily observed in mice reconstituted with WT KLSC (Fig. 2, A–C), neither MOMA-1+ metallophilic macrophages nor ERTR-9+ marginal zone macrophages could be detected in the spleens of animals reconstituted with LTα−/− cells (Fig. 2, D–E). Furthermore, there was a complete lack of MAdCAM-1 expression on the marginal sinus lining cells (as well as in germinal centers) in these animals compared with WT controls (Fig. 2 F). When spleen sections of mice reconstituted with LTα−/− cells were stained with acid phosphatase, a diminished population of macrophages in the marginal zone could be observed, suggesting that not only were the Ags recognized by...
MOMA-1 and ERTR-9 absent but also the whole splenic macrophage population was significantly reduced in size (data not shown). Analysis of the peripheral and mesenteric lymph nodes of mice reconstituted with LTα−/− KLSC, however, demonstrated that the Abs ERTR-9 and MOMA-1 were expressed in these organs (Fig. 2G and H). In addition, MAdCAM-1 was expressed without any abnormality on high endothelial venules in Peyer’s patches and mesenteric lymph nodes of mice reconstituted with LTα−/− cells (Fig. 2J). Therefore, in the spleen at least, the lack of MOMA-1, ERTR-9, and MAdCAM-1 in the marginal zone of the spleen must be caused by a lack of LTα from a BM-derived cell type. However, the LTα signal does not appear to be necessary for the presence of cells expressing MOMA-1, ERTR-9, or MAdCAM-1 in the lymph nodes. The signals that are involved in these regulatory events need to be clarified further.

Although the impact of the absence of MOMA-1−/− and ERTR-9−/− macrophages from the marginal zone on lymphocyte localization is unknown, it has been shown that fewer B cells enter the white pulp after elimination of marginal zone macrophages and marginal metallophilic macrophages (27). Furthermore, the significance of MAdCAM-1 expression on sinus lining cells in the marginal zone is unknown, since in blocking studies, MAdCAM-1 appeared not to regulate the entry of lymphocytes into the white pulp (28). Our results show that in the absence of macrophages and MAdCAM-1 expression in the marginal zone, B and T lymphocytes are still able to enter the white pulp. In addition, our results show that hemopoietically derived LTα controls expression of MAdCAM-1 and the presence of macrophages in the marginal zone of the spleen. T and B cells enter the spleen via the marginal sinus vessels, first attaching to the endothelial cells that line the marginal zone (opposite to the endothelial cells that overlie the white pulp), then migrating into the marginal zone, and then crossing into the white pulp (29). The lack of marginal zone-specific Abs does not seem to effect the ability of B and T lymphocytes to migrate to their own microenvironments within the white pulp.

**LTα−/− B cells do not form primary follicles**

Since formation of primary B cell follicles requires TNF-α (9), we were interested to see whether the primary B cell follicles were formed properly in mice reconstituted with LTα−/− KLSC. To analyze primary follicles in the spleens, lymph nodes, and Peyer’s patches, a double labeling with mAbs for IgM and IgD was performed. This allowed us to distinguish the B cells in the marginal zone from the B cells that form the follicles, since the IgM+ IgD− B cells in the marginal zone are stained only for IgM (brown) while the B cells in the primary follicles express both IgD (blue) and IgM (brown) (Fig. 3). Mice reconstituted with WT KLSC had IgM+ IgD− B cells properly located in the marginal zone, although the ring of B cells seemed thin at times, and primary follicles of dark IgM+ IgD− cells could be easily observed (Fig. 3A). However, when mice reconstituted with LTα−/− KLSC were analyzed, no IgM+ IgD− cells could be observed in the marginal zone, and IgM+ IgD− cells did not organize in follicles, but formed ring-like structures around the T cells in the PALS (Fig. 3, B and C). Primary follicles were also absent from the lymph nodes and Peyer’s patches of mice reconstituted with LTα−/− cells but appeared normal in WT controls (data not shown).

In mixed chimeras, B cells derived from LTα−/− KLSC could be distinguished from B cells derived from WT KLSC by the expression of either Ly-5.1 or Ly-5.2, since all cells derived from LTα−/− KLSC express Ly-5.1 and all WT express Ly-5.2. We observed that primary follicles could readily be detected in mice reconstituted with mixed WT and LTα−/− KLSC and contained cells derived from both WT KLSC and LTα−/− KLSC (Fig. 3, D and E). This suggested that LTα−/− B cells are able to participate in the formation of primary follicles, as long as a signal derived from WT hemopoietic cells is present. Furthermore, LTα−/− B cells are not at a competitive disadvantage compared with WT B cells in follicular entry as similar numbers of each population were found in the follicles.
We also analyzed reconstituted animals for the presence of FDC in the B cell areas using the mAb FDC-M2. Although FDC were easily visible in spleens of mice reconstituted with WT KLSC at 13 wk after reconstitution, only a few FDC could be found in mesenteric lymph nodes, but not peripheral lymph nodes or spleen, of mice reconstituted with LTα−/− KLSC (see Fig. 4 for spleen). Furthermore, by 27 wk postreconstitution, FDC networks were still present in mice reconstituted with WT KLSC (Fig. 4A); however, no FDC could be found in spleens of mice reconstituted with LTα−/− KLSC (Fig. 4B). The observation that FDC were detected in mesenteric lymph nodes of mice reconstituted with LTα−/− cells at 13 wk, but not at later time points, is suggestive of a model in which FDC are continuously renewed or whose survival depends on local LTα1 cells. Although some investigators propose that FDC are derived from locally present reticular cells (30–32), others have proposed that the precursors to FDC reside in the BM (33). Our data suggest that either the replacement or maintenance of existing FDC is dependent on LTα from a BM-derived cell type at some point during the differentiation pathway, although we cannot address whether the FDC themselves are BM or locally derived as FDC do not express Ly-5.

LTα−/− B cells can form germinal centers

Analysis for the presence of GC by peanut agglutinin labeling showed obvious GC in the mesenteric lymph node as well as spleen and Peyer’s patches of mice reconstituted with WT KLSC. To our surprise, however, even some of the mice reconstituted with LTα−/− KLSC had well-defined peanut agglutinin+ GC (Fig. 3C; Fig. 5G and H). However, the GC found in mice reconstituted with LTα−/− KLSC were formed in the absence of detectable FDC (Fig. 5H). The ability of LTα−/− B cells to produce GC in the absence of FDC has been reported recently; however, these GC could be found only in LN and not in spleen (15). In our studies, we observed GC in the absence of FDC not only at 13 wk after reconstitution in both spleen and lymph nodes but also at 23 and 27 wk after reconstitution when FDC had been absent for several month. The peanut agglutinin+ GC in mice reconstituted with LTα−/− KLSC were smaller than GC from mice reconstituted...
with WT KLSC, suggesting that these GC could be slowly disappearing in the absence of FDC, although the existence of GC in the absence of FDC has also been observed in the LTβ²/² mice as well (17).

GC were present in the mixed chimeras as well (Fig. 3D). When examined for the expression of either Ly-5.1 or Ly-5.2, GC were found to be predominantly, although not exclusively, derived from either LTα⁻/⁻ B cells (Ly-5.1) or WT B cells (Ly-5.2), suggesting that GC contain the progeny of a limited number of founding B cells (Fig. 3, D and E; Fig. 5, B, D, and F). Pauciclonality of GC has been suggested before (34–41). Furthermore, B cells are not required to express their own LTα to participate in the germinal center reaction (Fig. 5, B, D, and F).

FIGURE 5. Immunohistochemical labeling of germinal centers. Splenic sections of WT mice reconstituted with WT KLSC (A, C, E) or for WT mice reconstituted with mixed KLSC (B, D, F). Serial sections were stained for FDC-M2 (blue) in combination with Ly-5.1 (brown), showing the LTα⁻/⁻ KLSC-derived cells brown among an FDC network (A, B); FDC-M2 (blue) and peanut agglutinin (PNA) (brown) showing GC and FDC simultaneously (C, D); and FDC-M2 (blue) in combination with Ly-5.2 (brown), showing all WT KLSC-derived cells in brown (E, F). GC can be found that are completely Ly-5.1 positive and Ly-5.2 negative and are thus of LTα⁻/⁻ KLS cell origin. G and H were stained for FDC-M2 (brown) and PNA (blue) and show that peanut agglutinin (PNA) PNA⁺ GC can be observed in the absence of detectable FDC.

Summarizing our results we show that 1) LTα is involved in formation of primary B cell follicles; 2) LTα⁻/⁻ B cells can form GC and that GC can exist in the absence of FDC; 3) B and T cell segregation does not require LTα expression on these cells or an intact marginal zone; 4) LTα expression in a (subset of) hemopoietic cells is required for a proper development of the marginal zone.

The characteristics observed in LTα⁻/⁻ KLSC-reconstituted mice are similar to the specific defects seen in LTβ⁻/⁻ mice; i.e., GC formation without FDC, lack of normal splenic marginal zone, while lack of B T cell separation seems less severe in LTβ⁻/⁻ than in LTα⁻/⁻ mice. Therefore, the abnormalities seen in both LTβ⁻/⁻ mice and WT mice reconstituted with LTα⁻/⁻ KLSC could be due to lack of LTα,β₂ or LTα,β₁ on hemopoietic cells,
Ag-immunoglobulin complexes (Ag-Ig) are required, while FDC are not essential for the formation of IgM. For the induction of class switch, affinity maturation, and formation of memory B cells, FDC, Th1, or Th2 and Ag-immunoglobulin complexes (Ag-Ig) are required, while FDC are not essential for the formation of IgM+ GC.

Speculation

Perhaps the most striking finding in these experiments is the demonstration that the lymphoid organs of WT mice irradiated and reconstituted with KLSC from LTα−/− BM contain T and B cell networks, but that the B cell domains fail to aggregate B2-2 cells around FDC, which are absent, to form classical primary follicles (12). Even more surprising, the lack of FDC and primary follicles do not block the ability of T/B/APC interactions that generate GC and Ab formation. In our experiments, these GC are IgM+ IgD−, characteristic of GC in an immune response that has not undergone the IgH chain “class switch” (42). It is therefore reasonable to speculate that FDC do not play a role in the T/B2-2/APC interaction that leads to activated clones of B cells (43) to shed IgD (44–46) and L-selectin (47), enlager, and form the first phase of a GC at the T-B domain interface (48). Rather, FDC and activated Th cells (that locate in the cortical zones of those GC (12, 49)) are required for other GC events, namely the IgH class switch (50), affinity maturation via selection of somatically mutated V_{H}V_{S} (39, 40, 51–54), generation of class-switch memory B cells, and maturation of class-switch plasma cells (55–59). The roles of Th vs. FDC in these events, if this is an accurate speculation, should be amenable to study. But how are FDC formed in a LTα-dependent fashion? In our view, cells expressing surface LT, such as the unusual population of CD4+ CD3− (IgOPO2TCR) lymphocytes that seed early lymphoid organs via their surface Peyer’s patch homing receptors (integrin α4β7) (60, 61), are good candidates for the class of BM-derived cells that stimulate FDC maturation. FDC, once generated, form FDC networks that aggregate B2-2 cells around them, perhaps via FDC MadCAM-1 (62), interacting with α4β7 on B2-2 cells (63–67). These FDC-B2-2 follicles can then receive Ig-Ag complexes for long term display of antigenic determinants, and only B cells with surface Ig of higher avidity than the Ig on FDC will compete successfully to receive FDC (and Th) signals via Ig receptors and costimulatory receptors, respectively, leading to the events of somatic mutation, IgH class switch, B memory, and Ab formation that characterizes this later phase of a B cell immune response. These events are summarized in Figure 6.

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FIGURE 6. A model for LTα involvement in the maturation of FDC and for the role of FDC in the immune response. In this model, CD4+ CD3− cells are required for FDC maturation. For the induction of class switch, affinity maturation, and formation of memory B cells, FDC, Th1, or Th2 and Ag-immunoglobulin complexes (Ag-Ig) are required, while FDC are not essential for the formation of IgM+ GC.

while the defects unique to LTα−/− mice are caused by lack of LTα, independent of LTβ, in nonhemopoietic cells.


