Lymphotoxin-α-Dependent Spleen Microenvironment Supports the Generation of Memory B Cells and Is Required for Their Subsequent Antigen-Induced Activation

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Lymphotoxin-α-Dependent Spleen Microenvironment Supports the Generation of Memory B Cells and Is Required for Their Subsequent Antigen-Induced Activation

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Lymphotoxin-α-deficient (LTα−/−) mice show dramatically reduced IgG responses after either primary or secondary immunizations with sheep red blood cells (SRBC). When splenocytes from SRBC-primed wild-type donor mice were infused into irradiated naive wild-type recipient mice, they generated a robust memory IgG response, but not when infused into LTα−/− recipients, indicating that the microenvironment that develops in LTα−/− mice is incompetent to support the activation of this memory response. When irradiated wild-type mice were reconstituted with splenocytes from primed LTα−/− donors and then challenged with the same immunizing Ag, no memory response was observed, indicating further that memory cells could not be generated in the LTα−/− environment. To address which lymphocyte subsets were impaired in the LTα−/− mice, we performed reconstitution experiments using a hapten/carrier system and T cells and B cells from different primed donors. There was no detectable defect in either the generation or expression of memory T cells from LTα−/− donors. In contrast, B cells were not primed for memory in the microenvironment of LTα−/− mice. Additionally, primed wild-type memory B cells could not express a memory IgG response in the LTα−/− microenvironment. Thus, splenic white pulp structure, which depends on the expression of LTα for its development and maintenance, is needed to support the generation of memory B cells and to permit existing memory B cells to express an isotype switched memory Ig response following antigenic challenge. The Journal of Immunology, 2000, 164: 2508–2514.

Recent studies have shown that signals induced by membrane lymphotoxin (LT) are essential for the organogenesis of peripheral lymph nodes and Peyer’s patches and the formation of both the primary and secondary B cell follicles in the spleen white pulp (1–4). Membrane LT is a heterotrimer of LTα- and LTβ-chains with predominant stoichiometry LTαβ2 (5). Signaling by the LTαβ2 heterotrimer is mediated through the LTβ receptor (LTβR). Thus, membrane LT signals are independent of the type I or type II TNF receptors that mediate signals from the soluble LTα, homotrimer (5, 6). Mice rendered deficient in LTα, LTβ, or the LTβR are born with a dramatic impairment of lymph node and Peyer’s patch biogenesis. In addition, LTα+/−, LTβ−/−, and LTβR−/− mice each fail to form distinct splenic T cell and B cell zones, follicular dendritic cell (FDC) clusters, or germinal centers (GC). These structural disturbances are associated with impaired high affinity isotype-switched Ig responses following primary or secondary immunization with T cell-dependent Ags (such as sheep red blood cells (SRBC) or keyhole limpet hemocyanin (KLH)) when administered without adjuvants (7–10).

Long-term reconstitution of lethally irradiated LTα−/− mice with bone marrow cells from wild-type (wt) mice leads to restoration of the ability to form FDC clusters, GC, and adjuvant-independent strong IgG responses (8). We have recently shown that cells alone are required to deliver the LTα-dependent signals that restore the formation of FDC clusters, GC, and recovery of IgG responses in LTα−/− mice (11). Expression of LTα by T cells is not required. In contrast, when sublethally irradiated LTα−/− mice are reconstituted with spleen cells from wt donors rather than with bone marrow cells, no FDC clusters, GC, or T cell-dependent high affinity IgG responses are detected 2 wk after cell transfer and immunization with SRBC (8). These findings suggest that the microenvironment that develops in LTα−/− mice cannot support a high affinity isotype-switched Ig response, but that at least some aspects of this microenvironment are plastic and can be restored under the influence of sustained LT-dependent signaling through the LTβR. The ability to mount a high affinity isotype-switched Ig response appears to be correlated with the presence of clusters of FDC, which can be detected between 2 and 3 wk following transfer of LTα-expressing B cells into LTα−/− recipients. The present study was undertaken to determine whether the lymphoid tissue microenvironment that forms under the influence of LT is required to support either the induction or expression of memory responses.

Several studies suggest that memory B cells form primarily in GC and that their formation requires the presence of FDC clusters (12–15). A prominent contribution of GC to the formation of memory B cells is suggested by the observation that the B cell Ag receptors expressed by memory B cells carry a significant number of somatic mutations and generally show high affinity for the eliciting Ag. Thus, memory B cells appear to have passed through GC during their development. Like maturing B cells, Ag-specific T cell-dependent signals are required to support the organogenesis of the lymphoid tissue, and therefore the formation of memory B cells.
cells also undergo phenotypic changes within GC (16–18); however, it remains unclear whether these GC-dependent changes in T cells also represent an integral part of the program leading to the generation of memory T cells. It also remains unclear whether GC or FDC clusters play an important role in the expression of memory responses by established memory T and B cells.

Until recently, the lack of animal models in which the clustering of FDC and the formation of GC could be regulated has limited our understanding of the role of these structures in the generation of memory cells and in the maintenance of memory responses. LTα−/− mice fail to form clusters of FDC and are unable to generate GC following immunization with T-dependent Ags (7). These mice provide a model in which to study whether these structures contribute to the generation of memory T or B cells and to the functional expression of the memory response. We demonstrate here that the microenvironment of LTα−/− mice supports the generation of memory T cells but not of memory B cells. Furthermore, the microenvironment in LTα−/− mice cannot support the expression of functional memory IgG responses, even when sensitized memory cells from wt mice are provided by adoptive transfer. Of interest, when sublethally irradiated LTα−/− mice are reconstituted with splenocytes from wt mice, immunization elicits the formation of clusters of peanut agglutinin + (PNA +) cells, apparently in the absence of associated FDC, suggesting that normal B cells can be activated to form GC-like structures without FDC; however, these clusters of PNA + cells fail to differentiate into functional memory cells in the splenic microenvironment of LTα−/− mice. The dysfunctional nature of these GC-like structures is underscored by their dissociation from the apoptotic process that normally appears to be a consequence of B cell selection in these structures (15, 19).

Materials and Methods

Mice

C57BL/6J, 129Sv, and B cell receptor (BCR)−/− (C57BL/6J-Igh-2a-momCon) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). LTα−/− mice (1) were maintained on a mixed 129Sv/C57BL/6 background and were bred under specific pathogen-free conditions.

Preparation of NP-haptenated SRBC

Four milliliters of 10% SRBC (Colorado Serum, Denver, CO) in PBS were incubated with 4 ml of NP-Osu (1 mg/ml, Biosearch Technologies, San Rafael, CA) in 0.15 M NaHCO3 for 2 h at room temperature. The NP-conjugated SRBC were washed with PBS and resuspended in 8 ml of PBS. Immunization was with 0.1 ml of this suspension injected i.p. NP25-KLH was also purchased from Biosearch Technologies.

Measurement of Ag-specific Ig

Specific Abs were measured and analyzed as previously described (8). For measurement of anti-SRBC Abs, 96-well Falcon plates (Becton Dickinson, Lincoln Park, NJ) were coated with SRBC (150 μl of 0.1% SRBC in PBS per well). For anti-NP Abs, 96-well Immunul 4 plates (Dynatech Laboratories, Chantilly, VA) were coated with NP25-BSA (10 μg/ml, Biosearch Technologies) for 1 h. Unbound Abs were removed by washing with PBS. Diluted mouse sera were then added and incubated at 4°C for 1 h. Bound Abs were detected using 100 μl of 1:2000 diluted alkaline phosphatase-conjugated goat anti-mouse IgG-specific antiserum (Southern Biotechnology Associates, Birmingham, AL), followed by the addition of the alkaline phosphatase substrate p-nitrophenyl phosphate (Sigma, St. Louis, MO) at 1 mg/ml. The mean OD405 from triplicate wells was compared to a various dilutions of a standard anti-NP immune serum to calculate the relative units (RU) using linear regression analysis. The results are reported as means ± SEM.

Transfer of splenocytes

Whole spleen cell suspensions were prepared from single donor mice and were injected i.v. into recipients that had been irradiated with 750 rads 3 h earlier. When indicated, SRBC were injected i.v. together with the spleen cell suspensions. Each recipient received all of the cells derived from a single donor spleen.

Enrichment for T and B cells

Nylon wool columns (Polysciences, Warrington, PA) were used to enrich spleen cell suspensions for T or B cells. Splenocytes were incubated on nylon wool columns with 10% FCS in DMEM for 1 h at 37°C. Nonadherent cells were eluted with 10% FCS in DMEM at 37°C. Enriched T cells in the fraction of nonadherent cells were further purified by panning on tissue culture dishes coated with goat anti-mouse Ig H/L chain (Southern Biotechnology Associates), yielding 75–85% T cells. The contamination by B cells was <5%. Cells that adhered to nylon wool were eluted using cold PBS. Eluted cells were 80–90% B cells. After panning on tissue culture dishes coated with anti-Thy1.2 Ab, contamination by T cells was reduced to <5%.

Evaluation of spleen follicle structure and apoptosis

Spleens were harvested, embedded in OCT compound (Miles, Elkhart, IN), and frozen in liquid nitrogen. Frozen sections (6–10 μm thick) were fixed, quenched, and stained as previously described (8) using 0.2% H2O2 in methanol. After washing, the sections were stained by first incubating with FITC-conjugated B220 (PharMingen, San Diego, CA), and biotinylated PNA (Vector, Burlingame, CA), all at 1:100 dilution. HRP-conjugated rabbit anti-FITC (Dako, Glostrup, Denmark; diluted 1:10) was added 1 h later. Sections were then incubated for 1 h with one drop of alkaline phosphatase (AP)-conjugated streptavidin (Zymed, South San Francisco, CA), and color development for bound AP and HRP was with an AP reaction kit (Vector) and diaminobenzidine.

Cells undergoing apoptosis were detected using a modified TUNEL method (20). Tissue sections were incubated with 2 mM digoxigenin-conjugated dUTP (Boehringer Mannheim, Indianapolis, IN) and 5U of TdT in 0.5 M cacodylate (pH 6.8), 1 mM CoCl2, 0.5 mM DTT, 0.05% BSA, and 0.15 M NaCl. After washing in Tris-buffered saline, sections were incubated with sheep anti-digoxigenin Ab (Boehringer Mannheim) in Tris-buffered saline, washed, and further incubated with HRP-conjugated anti-sheep Ig Ab (Jackson ImmunoResearch, West Grove, PA). Color development for bound HRP was with 100 μg/ml 3-aminobenzidine in 0.17 M sodium acetate (Sigma). Sections were then counterstained with 1% methyl green.

Results and Discussion

Impaired memory IgG response in LTα−/− mice

We previously reported that LTα−/− mice developed a dramatically reduced Ag-specific IgG response following immunization with the T cell-dependent Ags SRBC or KLH without adjuvant (8). We now extend these experiments to test the character of the IgG response in LTα−/− mice that received repeated immunizations intended to elicit immunological memory. Three months after initial priming, LTα−/− and wt mice were challenged by i.p. immunization with 108 SRBC without adjuvant. Anti-SRBC IgG was measured 5 days after the booster immunization (Fig. 1A). Measurements were made 5 days after challenge because at this time memory responses in wt mice show strong IgG production, whereas primary IgG responses remain undetectable or low. Wt mice mounted an anti-SRBC memory response that was more than 200-fold higher than that of LTα−/− mice, suggesting that there was a severe impairment of the memory response in the LTα−/− mice. We had previously shown that LTα−/− mice were not absolutely unable to generate an IgG response, but could do so following immunization with either NP-OVA (21) or SRBC (8) when the Ag was administered with an adjuvant. Therefore, we tested the memory IgG response following priming with IFA (Fig. 1B). Although LTα−/− mice showed a robust IgG response when immunized with SRBC in IFA, when challenged 3 mo later, they showed only a weak anti-SRBC memory response. These experiments suggest that LTα−/− mice either are unable to express a memory response or fail to generate long-lived memory lymphocytes.
immunized i.p. on day 0 with 10^8 SRBC without adjuvant (E). Strikingly, when lymphocytes were transferred from primed wt mice (expected to contain memory B and T cells) were transferred from naive wt mice. Groups of three to five 8-wk-old mice (wt, \( \text{LT}^{-/-} \), \( \text{LT}^{-/-} \)) were immunized i.p. on day 0 with 10^8 SRBC without adjuvant (A) or with IFA (B) and given a boost with the same dose without adjuvant 3 mo later. Serum was collected 10 days after the initial immunization (Primary) and 5 days after the booster immunization (Memory). SRBC-specific IgG was measured by ELISA as described in Materials and Methods. Data shown represent the means \( \pm \) SEM of triplicate determinations from three to five mice. Similar results were obtained in a replicate experiment.

FIGURE 1. Impaired IgG anti-SRBC memory response in \( \text{LT}^{-/-} \) mice. Groups of three to five 8-wk-old mice (wt, \( \text{LT}^{-/-} \), \( \text{LT}^{-/-} \)) were immunized i.p. on day 0 with 10^8 SRBC without adjuvant (A) or with IFA (B) and given a boost with the same dose without adjuvant 3 mo later. Serum was collected 10 days after the initial immunization (Primary) and 5 days after the booster immunization (Memory). SRBC-specific IgG was measured by ELISA as described in Materials and Methods. Data shown represent the means \( \pm \) SEM of triplicate determinations from three to five mice. Similar results were obtained in a replicate experiment.

When memory lymphocytes are transferred into the disturbed lymphoid tissue microenvironment of \( \text{LT}^{-/-} \) mice, they fail to mount a memory response

To test whether the lymphoid tissue microenvironment that forms in congenitally \( \text{LT}^{-/-} \)-deficient mice can support the function of memory lymphocytes, we transferred splenic lymphocytes from either naive or SRBC-immunized wt mice into irradiated naive wt or \( \text{LT}^{-/-} \) recipients and then challenged these chimeric recipients with SRBC. In previously immunized wt animals, a strong memory IgG response can typically be detected as early as day 3–5 after i.p. challenge. When naive cells were transferred, development of a significant primary IgG response was not detected 5 days after immunization of the recipients, although it was by 10 days (Fig. 2). In contrast, when primed lymphocytes from SRBC-immunized wt mice (expected to contain memory B and T cells) were transferred into naive wt recipients, challenge with SRBC elicited a high titer IgG response on day 5 with a further increase by day 10. Strikingly, when lymphocytes were transferred from primed wt mice to \( \text{LT}^{-/-} \) recipients, rechallenge with SRBC failed to produce a high titer memory IgG response (even by day 10 after rechallenge). Thus, preformed memory cells elicited in wt mice were unable to express a mature memory response in the disturbed lymphoid tissue environment that exists in \( \text{LT}^{-/-} \) mice. The disturbed microenvironment in \( \text{LT}^{-/-} \) mice can support neither primary (8) nor memory responses, even when \( \text{LT}^{-/-} \)-expressing naive or memory cells are provided.

Failure to generate memory cells in \( \text{LT}^{-/-} \) mice

The studies described above showed that primed wt memory cells were unable to express a memory IgG response following transfer into an \( \text{LT}^{-/-} \) recipient mouse. To compare the ability of the lymphoid microenvironments of wt and \( \text{LT}^{-/-} \) mice to support the formation of memory lymphocytes, we immunized wt and \( \text{LT}^{-/-} \) mice with the T-dependent Ag SRBC to provide a stimulus for memory cell formation. Splenocytes from the primed mice were then transferred to sublethally irradiated wt recipients. These reconstituted animals were challenged with SRBC to stimulate memory cells that might have been transferred (Fig. 3). Five days after challenge, mice that had received cells from primed wt donors showed a brisk IgG anti-SRBC response characteristic of established B cell memory. In contrast, cells transferred from primed \( \text{LT}^{-/-} \) mice supported no IgG response, similar to cells transferred from naive \( \text{LT}^{-/-} \) donors. This clearly indicates that functional memory cells had not been induced in the \( \text{LT}^{-/-} \) environment.

Lack of memory B cells but not memory T cells in \( \text{LT}^{-/-} \) mice

Memory cells of both the B and T cell lineages are required to coordinate memory responses in vivo (14, 22). To address which
memory cell compartment(s) failed to form in the microenvironment of LTα+/mice, a hapten-carrier system was applied. To investigate whether LTα−/− mice could generate memory B cells, the following strategy was used. BCR−/− mice were primed i.p. with SRBC in PBS to generate anti-SRBC memory T cells that were free of contamination by naive or memory B cells. Wt mice and LTα−/− mice were primed i.p. with NP-KLH to provide potential sources of anti-NP memory B cells. Then, 10^7 SRBC-primed T cells from the spleens of BCR−/− mice were mixed with an equal number of partially purified splenic B cells from NP-KLH-immunized wt or LTα−/− mice and transferred to sublethally irradiated (750 rads) naive wt recipients. Following challenge with NP-SRBC, mice that received B cells from the NP-KLH-immunized wt mice showed a robust memory anti-NP IgG response, whereas mice that received B cells from immunized LTα−/− mice showed at least a 50-fold lower anti-NP IgG response (Fig. 4). These data demonstrate a substantial impairment in the formation of memory B cells in LTα−/− mice.

To address whether LTα−/− mice could generate memory T cells, wt mice and LTα−/− mice were primed i.p. with SRBC in PBS to provide a source of potential anti-SRBC memory T cells. Wt and LTα−/− mice were primed i.p. with NP-KLH in PBS to provide a source of potential anti-NP memory B cells. Primed T and B cells (10^7 each) purified from the SRBC-immunized and the NP-KLH-immunized mice were then transferred to irradiated wt recipients. Five days after i.p. challenge with NP-SRBC in PBS, a robust IgG anti-NP response was detected in mice that received primed B cells from wt mice, but not from LTα−/− mice (Fig. 5), confirming that the LTα−/− environment does not support the formation of memory B cells. Of interest, functional memory T cells were recovered from both wt and LTα−/− donors. Thus, the altered microenvironment of LTα−/− mice retains the ability to support maturation of T cells to effective memory function. The methods we have employed may not detect modest or partial impairments of memory T cell formation in LTα−/− mice. Such potential memory T cell defects might be revealed by adoptive transfer of smaller numbers of T cells, or by immunization with more limiting doses of Ag. Nevertheless, our data do show that the tissue requirements for the formation of memory B cells and memory T cells are substantially different.

Wt splenocytes differentiate into nonproductive PNA+ cell clusters in the LTα−/− microenvironment

GC, with their prominent clusters of FDC and scattered Ag-specific T cells, are thought to provide primary venues for the formation of memory B cells (13, 14, 19, 22). The GC represent a dynamic microenvironment in which B cells differentiate first into rapidly proliferating PNA+ cells that then move toward the FDC clusters where further maturation takes place (13, 14, 23). The FDC are thought to play a central role in GC function, with Ag
deposited on their surfaces primarily in the form of complement-coated immune complexes serving to select B cells with high affinity Ag receptors. In the course of this selection process, PNA GC cells are thought to be converted to the precursors of both IgG-producing B cells and long-lived memory B cells. To investigate further the nature of the disturbed B cell memory function in the LTα2−/− mice, we examined the roles of LTα and the lymphoid tissue microenvironment in the formation and function of GC-like clusters of PNA+ cells. Wt or LTα2−/− mice were sublethally irradiated, reconstituted with splenocytes from wt donors, and immediately immunized with an i.v. infusion of 10⁸ SRBC. Ten days after transfer and immunization, sections were stained with PNA (blue) and anti-IgD (brown) in the top panels and by the TUNEL procedure in the lower panels. TUNEL+ cells are brown on the background of methyl green counterstain.

FIGURE 6. Lack of apoptotic activity in PNA+ clusters in LTα−/− mice reconstituted with wt splenocytes. Irradiated wt mice (left panels) and LTα−/− mice (right panels) were reconstituted with splenocytes from naive wt mice together with 10⁸ SRBC. The recipients’ spleens were collected 10 days after transfer and immunization, and sections were stained with PNA (blue) and anti-IgD (brown) in the top panels and by the TUNEL procedure in the lower panels. TUNEL+ cells are brown on the background of methyl green counterstain.

clusters of PNA+ cells that were found in LTα−/− recipients of wt splenocytes showed no association with apoptotic selection (Fig. 6). Wt SRBC-immunized mice that received wt splenocytes showed prevalent clusters of TUNEL+ cells associated with GC. No clusters of TUNEL+ cells were detected in the white pulp of SRBC-immunized LTα−/− mice that had been reconstituted with wt donor splenocytes. Apoptosis of GC cells is thought to represent deletion of B cells that express low affinity Abs. This deletion is thought to correlate with the selection of B cells with high affinity and their conversion into memory cells (14, 15, 19). Our data indicate that the LTα−/− microenvironment characterized by lack of FDC clusters can support the differentiation of Ag-stimulated B cells into PNA+ GC-like cells, but fails to support either affinity-based selection of these cells or their further differentiation into either IgG producing cells or into memory B cells.

The data presented in this study demonstrate that the disturbed lymphoid tissue microenvironment that forms in LTα−/− mice is unable to support the generation of memory B cells from naive progenitors. This LTα−/− microenvironment is also unable to support the conversion of preformed wt memory B cells (produced in a wt environment) into Ag-producing cells that express a mature memory response. At least three characteristics of the normal spleen white pulp fail to form properly in LTα−/− mice (1, 2, 7, 21): 1) the segregated T cell-predominant periarteriolar lymphoid
sheath (PALS), 2) the segregated B cell-predominant marginal sinus structure with MAdCAM-1 (mucosal addressin cell adhesion molecule-1) vascular endothelium and MOMA-1+ metallophilic macrophage components, and 3) primary B cell follicles with clusters of FDC. Cooperative interactions between T and B lymphocytes are required for the formation of functional GC and high affinity isotype-switched Ig responses. Cooperative B/T interactions have also been suggested to be required for the generation of memory B cells (13, 22, 24). It is possible that segregation of T and B cells into separate zones within the splenic white pulp facilitates properly regulated interactions of Ag-specific T and B cells during both primary and memory responses. LTα−/− mice lack normal T and B cell segregation (1, 8). This might be expected to impair effective collaboration between the two cell populations and explain the observed inability to form memory B cells during a primary response or to productively activate memory B cells in a memory response; however, several observations argue against a major role of disorganization of T and B cell zones in the impaired memory responses. First, 6 wk after lethally irradiated LTα−/− mice had been reconstituted with wt bone marrow, segregation of T and B cell compartments remained incomplete but FDC clusters were restored (8). Mice reconstituted in this fashion can generate GC and secondary IgG responses similar in magnitude to wt mice. Second, in preliminary studies, we detect successful generation of memory B cells in LTα−/− mice that have been reconstituted with wt bone marrow in this fashion (data not shown). Thus, normal T cell/B cell segregation appears not to be required for the memory response. Therefore, we speculate, that LTα-dependent disturbances in the primary B cell follicle structure are more likely to underlie the disturbances of the memory response.

Consistent with this hypothesis, the generation of memory T cells appears to require a microenvironment distinct from that required for the formation of memory B cells. Memory T cells can be formed effectively in LTα−/− mice, with GC and FDC appearing not to be required for the generation of memory T cells. FDC clusters, on the other hand, are thought to play critical roles in the formation of B cell follicles and in the presentation of Ag to activated B cells (13, 14). We suggest that the lack of FDC clusters in LTα−/− mice primarily underlies the failure to generate memory B cells and also to support the response of passively transferred memory B cells to rechallenge. Consistent with this hypothesis, our preliminary data suggest that LTα−/− mice fail to manifest affinity maturation following repeated challenge with hapten in the absence of adjuvant (data not shown). Generally, a lack of somatic mutation correlates tightly with failure to elaborate memory B cells (14, 25).

The generation of the B cell arm of the memory response requires the induction of memory B cells as well as their maintenance. The experiments described here cannot distinguish between failure of the initial differentiation of activated B cells into B cells of the memory phenotype and failure to sustain the survival of these cells after Ag is catabolized and cleared. Additional experiments in which exposure to Ag is sustained continuously will be required to discriminate between these potential mechanisms of the failure to generate transferrable memory B cells in the LTα−/− mice.

In addition, the failure of memory B cells induced in a wt environment to express a memory IgG response after adoptive transfer into sublethally irradiated LTα−/− recipients could be based on several potential mechanisms. Expression of a memory response by adoptive transferred cells is contingent first on their survival in the recipient mice. Although we know that similar numbers of donor B cells are present in the spleen of recipient wt and LTα−/− mice after adoptive transfer (data not shown), the methods used here cannot measure specifically the survival of memory cells. Assuming survival of memory B cells after adoptive transfer, the expression of the memory IgG response requires activation of these cells by Ag, and then their conversion into IgG secreting cells. Our observation that primed wt cells can support the Ag-dependent formation of clusters of PNA+ cells after adoptive transfer into LTα−/− recipients suggests that Ag recognition does occur in this setting, and that at least one key defect in the LTα-deficient microenvironment may be in conversion from activated, proliferating B cells to Ab-secreting cells. Thus, the failure to express B cell memory in this environment may represent a general failure of maturation of proliferating, isotype-switched cells to Ab-secreting B cells and plasma cells.

GC contain significant numbers of Ag-specific T cells, and these cells appear to be essential for GC physiology, supporting somatic mutation and affinity maturation of GC B cells (12, 13, 22, 26). However, it has not been previously determined whether GC are required for the generation of T cell memory. Our data indicate that generation and subsequent activation of memory T cells and memory B cells require different environmental elements. In contrast to memory B cells, memory T cells can be generated in easily detectable numbers without the formation of GC and FDC (Fig. 5).

Our data support the emerging concept that members of the TNF ligand/receptor family play critical roles not only in the establishment of normal lymphoid tissue structure, but also in the cellular interactions that occur characteristically within these tissues. In addition to the LTβ receptor, several other members of the TNF receptor family, including OX40, the nerve growth factor receptor (NGFR), CD40, and type I TNF receptor (5, 27) are required for different steps of the memory IgG response. OX40 and CD40 on B cells interact with their ligands on the surfaces of T cells, with these interactions prominent during the generation of B cell memory responses and crucial for the development of high affinity IgG responses (5, 24). Signaling via the NGFR delivers autocrine signals supporting the survival of memory B cells (28). In these interactions, the B cell expresses the TNF receptor family member and receives signals primarily from T cells to support the Ab response. In contrast, B cells do not express the LTβR. Instead, B cells express LT ligands (LTα and LTα,β2). Via these ligands, B cells signal LTβR-bearing cells to support the development and maintenance of the lymphoid tissue structure that is required for mature B cell responses (11). The data presented here indicate that this LT-dependent microenvironment is required both for the generation of memory B cells and for their proper activation by recall Ag. These results underscore the central role of the NGFR/TNF receptor family in the generation of the memory response.

In summary, this study has demonstrated that LTα−/− mice show defective generation of memory B cells in response to T-dependent Ags. Furthermore, they cannot support LTα-expressing memory B cells to express memory responses to recall Ags. LTα-expressing B cells, 10 days after they have been transferred into LTα−/− mice, can be activated by Ag to form clusters of PNA+ cells; however, these cells do not form IgG-producing cells. Unlike T cells that can develop memory responses in LTα−/− mice, B cell memory requires LTα-dependent structures, with either clusters of FDC or structures closely linked to the expression of FDC being centrally required for the development of this response. Our studies clearly separate the requirements for the formation of memory T and memory B cells and establish lymphotoxin as a member of the growing group of TNF family ligands that are required for the maturation of the B cell response and the development of B cell memory.
References


