Crucial Role of Tumor Necrosis Factor Receptor 1 Expression on Nonhematopoietic Cells for B Cell Localization within the Splenic White Pulp

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Summary

During immune responses the initial activation of B cells takes place in T cell zones of periarteriolar lymphoid sheaths (PALS) of the splenic white pulp. After initial activation, B cells migrate into primary follicles and, in association with follicular dendritic cells (FDCs), undergo clonal expansion and differentiation giving rise to germinal centers (GCs). Peanut agglutinin binding (PNA⁺) cells of the GC differentiate further into memory or plasma cells. Here we report that in tumor necrosis factor receptor 1-deficient mice (TNFR1⁻/⁻), the location of B cells was altered and that plasma cells were abnormally distributed in the splenic PALS. In contrast to lymphotoxin α-deficient mice (LTα⁻/⁻), bone marrow or fetal liver transplantation did not correct the abnormal organization of the spleen, location of B cells, the lack of an FDC network, nor the antibody response in TNFR1⁻/⁻ mice. These results argue for a crucial role of TNFR1 expression on nonhematopoietic cells for the maintenance of the splenic architecture and proper B cell location. In addition, the lack in development of an FDC network after adoptive transfer suggests that either FDCs are not of bone marrow origin or that they depend on signals from nonhematopoietic cells for maturation.

The immune system often requires the cognate interactions of T cells, B cells, and antigen-presenting cells to respond to invading antigens/pathogens (1). A primary B cell follicle contains surface (s)IgM⁺ resting recirculating B cells and follicular dendritic cells (FDCs). A secondary B cell follicle is composed of a follicular mantle containing sIgM⁺IgD⁺ resting B cells and a germinal center (GC) composed of centroblasts, centrocytes, activated CD4⁺ memory T cells, and FDCs (2, 3). In addition, a third compartment, the marginal zone, observed in spleen, contains a subset of nonrecirculating sIgM highIgD low B cells (4, 5), marginal zone macrophages, as well as marginal metallophilic macrophages (6–8).

GCs are sites of B cell activation in secondary lymphoid tissues (9) and FDCs represent the major nonlymphoid cellular component of a GC, retaining the antigen as an immune complex and providing a variety of costimulatory signals. Within the GC, the B cells closely interact with FDCs and T cells, providing both stimuli to the B cells that prevent their entry into apoptosis and promote their differentiation into memory cells or plasma cells (10). FDCs are thought to be required to support formation and maturation of GCs (3, 11, 12). In support of this concept, both FDC clusters and GCs are absent from the spleens of immunized lymphotoxin α-deficient (LTα⁻/⁻), TNF-α⁻/⁻, and TNFR1⁻/⁻ mice (13–15).

TNF-α and LT-α bind to the same receptors TNFR1 (P55/CD120a) and TNFR2 (P75/CD120b) (16). In addition, when a LT-α monomer trimerizes with two identical LT-β subunits, the heterotrimers bind to a third type of receptor, LT-βR (17). Mice with targeted disruption of the LT-α gene manifest congenital absence of LN s and Peyer’s patches (18, 19). The splenic white pulp is reduced and lacks clearly defined B and T cell compartments. Mice deficient for another TNFR1 ligand, TNF-α, also lack GCs and FDCs (15). TNFR1⁻/⁻ mice retain a normal layer of

Abbreviations used in this paper: BM, bone marrow; FDC, follicular dendritic cell; FL, fetal liver; GC, germinal center; LT-α, lymphotoxin α-deficient; PALS, periarteriolar lymphoid sheaths; PNA, peanut agglutinin; RT, room temperature; s, surface; SRBC, sheep red blood cell; WT, wild type.

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marginal metallophilic macrophages, yet they cannot form an organized FDC network and GCs (14). Since no such defect can be observed in TNFR2−/− mice (13, 20), the activity to form GC and FDC networks in response to TNF-α homotrimers is most probably signaled exclusively through the TNFR1.

Distinct signals regulate the formation of discrete B and T cell zones in the splenic white pulp and LN s (21). T and B cell segregation in the splenic white pulp requires expression of LT-α and is independent of TNFR1 (21). Furthermore, activation of B cells to form GC-like structures in the mesenteric LN of LT-α−/− and TNFR1−/− mice, but not in their spleens (21). But, strikingly, both LT-α−/− and TNFR1−/− mice lack FDCs in both LN s and spleen (21).

Here we report that a significant number of plasma cells were abnormally located in the periarteriolar lymphoid sheaths (PALS) of the TNFR1−/− mice. Neither wild-type bone marrow (WT-BM) nor wild-type fetal liver (WT-FL) transplantation could normalize the distribution pattern of plasma cells in TNFR1−/− spleen. In contrast to LT-α−/− mice, the spleen architecture of TNFR1−/− mice, including GC and FDC networks, also could not be rescued by transplantation of wild-type hematopoietic precursors. Taken together, our findings illustrate that TNFR1 expressed by donor marrow cells from Ly 5.2-C57BL/6 mice or 8×10⁶ fetal liver cells from 14-d-old Ly 5.2-C57BL/6 embryos.

**Materials and Methods**

Mice. C57BL/6 (Ly 5.1 and Ly 5.2 strains) and hybrid 129 Sv × C57BL/6 mice were bred and maintained under specific-pathogen-free conditions in the animal facility of the Basel Institute for Immunology (Basel, Switzerland) or in conventional animal facilities of the Cantonal Hospital Research Department and the Swiss Tropical Institute (Basel, Switzerland). LT-α−/− mice (19) and TNFR1−/− mice (14) were maintained in Ly 5.1 genetic background. Fetal liver cells were obtained from 14-d pregnant mice. The day of the vaginal plug was counted as day 1 of pregnancy.

Reagents. Primary antibodies for FACScan® (Becton Dickinson, San Jose, CA), immunofluorescence, and immunohistochemical analysis included rat anti-mouse CD19, Bcl-2, and anti-mouse Ly 5.1/2 (clone 104-2), and Ly 5.2 (clone 145-2; these antibodies were a gift from A. Rolink, Basel Institute for Immunology). Secondary antibodies included streptavidin-PerCP, -FITC, -Texas red, and biotin-conjugated mouse anti-rat IgG (Becton Dickinson), FITC-conjugated and biotin-conjugated mouse anti-rat IgG (both from Jackson Immunoresearch Laboratories, West Grove, PA), and biotinylated PNA (Vector Labs, Burlingame, CA.). Immunohistochemistry was developed with ABC kit followed by incubation with 0.5 mg/ml solution of diamobenzidine substrate (both from Vector Labs).

Bone marrow and Fetal Liver Transplantation. BM was harvested and recipients were prepared as previously described (31). Recipient mice received a lethal total-body irradiation (900 rad) before the intravenous injection of 2–9×10⁶ freshly collected bone marrow cells from Ly 5.2-C57BL/6 or 8×10⁵ fetal liver cells from 14-d-old Ly 5.2-C57BL/6 embryos.

**FACScan® Analysis.** The pattern of donor-recipient-derived cells was analyzed by a double labeling technique. To block Fc receptor-mediated binding of antibodies, cells were preincubated for 15 min at 4°C with mouse IgG (Sigma Chemical Co., St. Louis, MO) in PBS containing 1% BSA and 0.1% sodium azide. Afterwards, cells were incubated for 20 min at 4°C with the following primary antibodies: anti-Ly 5.1-biotin, anti-Ly 5.1-FITC, anti-Ly 5.2-biotin, anti-Ly 5.2-FITC, anti-CD3e-FITC, or B220-PE, washed, and sequentially incubated, when necessary, with streptavidin-PerCP for 20 min at 4°C. FITC and PerCP fluorescence of the cells was measured with a FACScan® (Becton Dickinson) using excitation light from an argon laser at 488 nm. Electronic gates were set so that ~85% of the observed cells belonged to the lymphocyte population. Samples were analyzed by comparison with negative and positive controls to determine the cut off for positively labeled cells.

Immunization. 8–12-wk-old mice of each strain were injected intraperitoneally at day 0 with either 2×10⁶ sterile sheep red blood cells (SRBC), 100 μg of ovalbumin (Miles Inc., Elkhart, IN), or with 100 μg of KHL (Chemicon International Inc., Temecula, CA). The protein antigens were mixed with incomplete Freund’s adjuvant (Difco Laboratories, Detroit, MI). KHL administration was repeated 12 d after the priming. 16 d after immunization with ovalbumin, spleens were obtained for histological processing. The spleens from KHL immune mice were obtained 30 d after the priming.

**Immunohistological Analysis of Spleen Sections.** Spleens were embedded in Tissue-Tec (Miles Inc.) and frozen on dry ice. 7-μm-thick cryosections were fixed in acetone for 10 min and incubated with the primary antibody for 30 min at room temperature (RT). Immunofluorescent labeling was performed with fluorescein or Texas red secondary antibodies by incubation for 30 min at RT. The immunohistochemical procedure was followed by incubation with biotin-conjugated secondary antibodies for 30 min at RT, and revealed with ABC kit and diamobenzidine substrate (as described in manufacturer’s protocol, Vector Labs). All incubations were terminated by washing with PBS. To demonstrate the histology of the spleen, the sections were counterstained with 0.5% solution of methyl green zinc chloride (Merck, Darmstadt, Germany).

A say of Immunoglobulins. Specific anti-SR BC IgM and IgG1 antibodies in serum were quantitated using a sandwich ELISA as described previously (14, 21). In brief, maxisorp microtiter plates (Nunc, Roskilde, Denmark) were coated overnight with 50μg of KLH (Chemicon International Inc., Temecula, CA) and blocked with 5% dry milk for 2 h. The plates were blocked with 2% BSA in PBS for 2 h at 37°C. Serial dilutions of the serum were added and incubation was performed overnight at RT. Bound antibodies were detected by incubation with biotinylated goat anti-mouse IgM and IgG1-specific antibodies (both from Southern Biotechnology, Birmingham, AL) for 4 h at RT. Plates were developed by a streptavidin-AP conjugate (Amersham, Buckinghamshire, U.K.) followed by Sigma 104 phosphatase substrate (Sigma Chemical Co.) for 45 min each. The reaction was stopped with 1.5 M NaOH. Absorbance was read at 405 nm.

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**Table 1. FACScan® A analysis of Host- Versus Donor-derived Hematopoietic Cells in the Spleen of LT-α and TNFR1-deficient Mice Reconstituted with WT-FL or WT-BM**

<table>
<thead>
<tr>
<th>Cell surface markers</th>
<th>Percentage of marker-positive cells in splenocyte population</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TNFR1−/− WT-FL rec (n=5)</td>
</tr>
<tr>
<td>Ly 5.1+</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Ly 5.2+</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>B220+/Ly 5.1+</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>B220+/Ly 5.2+</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>CD3ε+/Ly 5.1+</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>CD3ε+/Ly 5.2+</td>
<td>13 ± 2</td>
</tr>
</tbody>
</table>

*Total fetal liver or bone marrow cells (C57BL1/6, Ly 5.2) were injected intravenously into lethally irradiated TNFR1−/− and LT-α−/− deficient mice (129 × C57BL6, Ly 5.1). 2 mo after transplantation, splenocytes were analyzed by flow cytometry for the presence of Ly 5.1−/− versus donor (Ly 5.2)−/− derived lymphocytes. Data are presented as percentage of cells positive for the surface markers within the population of 10^6 splenocytes mean ± SD.

**Results**

Reconstitution of the Hematopoietic System in TNFR1−/− and LT-α−/− Mice by Transplantation of Wild-type Hematopoietic Progenitor Cells. In an attempt to restore the immune system in LT-α−/− and TNFR1−/− mice, deficient animals were lethally irradiated (900 rad) and reconstituted with an intravenous injection of 10^7 FL cells or 2 × 10^6 BM cells from B6-Ly 5.2 congenic donors 1 mo after FL or BM transplantation, the majority of spleen and peripheral blood lymphocytes from LT-α−/− and TNFR1−/− reconstituted mice already were of donor origin (data not shown). Antibodies against Ly 5.1 and Ly 5.2 allotypic markers demonstrated that within a period of 2–10 mo after transplantation, the ratio between donor- and recipient-derived lymphocytes remained stable in spleen as well as in peripheral blood (data not shown). The majority of lymphocytes in both spleen (Table 1) and peripheral blood (data not shown) of reconstituted animals expressed the Ly 5.2 surface marker and therefore were of donor origin. More than 95% of T and B cells had been derived from donor-type precursors. Moreover, immunohistochemical labeling of spleen cryosections also revealed the dominant presence of Ly 5.2-positive donor-derived cells (data not shown). Taken together, these data show that transplantation of bone marrow or fetal liver cells efficiently repopulated the lymphocyte compartment of TNFR1−/− or LT-α−/− mice with wild-type hematopoietic cells.

In contrast to LT-α−/− mice, B cell follicles and GCs did not be restored by BM or FL transplantation in TNFR1−/− mice. Using immunohistochemical (Fig. 1) and immunofluorescence (Fig. 2) methods, the splenic architecture in TNFR1−/− and LT-α−/− mice after WT-BM adoptive transfer was compared. As shown in these figures, WT-BM transplantation was sufficient to reconstitute most features of the splenic architecture in LT-α−/− mice, as was previously shown (31). Indeed, discrete B cell follicles, absent in LT-α−/− spleen (Figs. 1 a, m, and 2 a and o), appeared after BM transplantation (Figs. 1 q, and 2 q r). WT-BM reconstitution restored also the development of GC (PNA− clusters, Fig. 2 n), absent in LT-α−/− mice (Fig. 2 n). This phenotype developed as soon as 1 mo after the transplantation and was stable for at least 10 mo.

In contrast to reconstituted LT-α−/− mice, these elements in TNFR1−/− mice (Figs. 1 e, and 2 e and g) were not restored after WT-BM cell transfer (Figs. 1 i, and 2 i and j). Even after immunization with ovalbumin, the GCs, which appeared in the spleen of wild-type mice (Fig. 2 b), were absent in the spleen of both TNFR1−/− (Fig. 2 f) and TNFR1−/− reconstituted (Fig. 2 j) mice. Similar results were obtained after immunization with KLH (data not shown). Transplantation of WT-FL cells also failed to rescue the splenic architecture in TNFR1−/− mice examined for over a 10-mo period (data not shown). Our data suggest that the expression of TNFR 1 on nonhematopoietic cells is a prerequisite for the development of B cell follicles and GC in murine spleens.

In contrast to LT-α−/− mice, WT- BM or WT-FL Transplantation cannot repopulate FDC networks in TNFR1−/− mice. Experiments were also performed to determine whether FDC networks, a major functional component of GCs, were restored in the spleen after transfer experiments. Labeling performed with the rat anti–mouse FDC-M2 antibody demonstrated that FDC networks can be rescued by WT-BM transplantation in LT-α−/− mice (arrows, Fig. 2 r), but not in TNFR1−/− mice (Fig. 2 j). No FDC networks were detected in TNFR1−/− reconstituted mice upon immunization with ovalbumin (Fig. 2), KLH, or SR BC (data not shown). Even 10 mo after BM transplantation, no FDC-M2 reactivity was observed in the reconstituted TNFR1−/− spleens (data not shown). This defect was also not corrected by WT-FL transplantation (data not shown). Therefore, neither WT-BM nor WT-FL transplantation were sufficient to restore FDC networks in TNFR1−/− mice.

A normal distribution of Plasma Cells in TNFR1−/− Mice Cannot Be Restored by WT-BM or WT-FL Transplantation. A striking observation of the TNFR1−/− mice was a localization of plasma cells within the T cell area of the white pulp (Figs. 1 and 2 g). In contrast, this type of distribution of antibody-secreting cells was not observed in the spleen of WT, LT-α−/−, or LT-α−/− reconstituted mice. In WT mice, after ovalbumin immunization, plasma cells tended to accumulate in the peripheral area of the PALS, in the marginal zone, and in the red pulp (brown-stained cells, Fig. 2 d). As distinct T and B lymphocyte compartments did not form in the spleen of LT-α−/− mice (21), it was difficult to draw conclusions about the distribution of their plasma cells. However, they...
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appeared to be clustered in an area similar to red pulp (Figs. 1 and 2 o). In the spleen of LT-α−/− mice reconstituted with WT-BM, the plasma cells were located in the red pulp and periphery of the T cell zone (Fig. 1 s, and 2 q and s).

In both, TNFR1−/− (Fig. 2 g) and TNFR1−/− reconstituted mice (Fig. 2 k), the IgM+ plasma cells were distributed mainly in the PALS. Similar results were obtained from TNFR1−/− mice reconstituted with WT-BM or WT-FL and immunized with KLH or ovalbumin. Furthermore, labeling with Syndecan-1, a specific marker of plasma cells (22–29), confirmed the localization of plasma cells in the outer periphery of the T cell areas in both WT (brown-stained cells, Fig. 1 d) and LT-α−/− reconstituted mice (Fig. 1 s), and inside the PALS in both TNFR1−/− (Fig. 1 g, and arrowheads, Fig. 2 g) and WT-BM-reconstituted (R1 ec; j and k) mice. Higher magnifications of the areas outlined in g, k, o, and s are shown in h, l, p, and t, respectively, whereas d is a higher magnification of an area in the red pulp not seen in c. The sections shown are from two months after transplantation. Arrows, central arterioles.

**Figure 1.** Reconstitution of TNFR1-deficient mice with WT-BM fails to restore B cell follicles and normalize the distribution of plasma cells. Spleens from immune WT (a–d), TNFR1-deficient (R1−/−; e–h), TNFR1-deficient WT-BM-reconstituted (R1 ec; i–l), LT-α−/− (LT−/−; m–p) and LT-α−/− WT-BM reconstituted (LT ec; q–t) mice were labeled by immunoperoxidase (brown) for B cells with an anti-CD19 mAb, T cells with an anti-Thy-1 mAb, and plasma cells with the Syndecan 1 mAb. Note the lack of primary follicles in the sections of TNFR1-deficient (R1−/−; e), TNFR1-deficient WT-BM-reconstituted (R1 ec; i), and LT-α-deficient (LT−/−; m) mice. When comparing adjacent sections of Thy 1 and Syndecan-1 labeling, plasma cells are abnormally observed in the Thy 1-positive areas of TNFR1-deficient (R1−/−; f and g) and WT-BM-reconstituted (R1 ec; j and k) mice. The sections shown are from two months after transplantation. Arrows, central arterioles.
higher magnification of the areas containing these cells are presented (Figs. 1 and 2, d, h, l, p, and t). Syndecan-1+ cells (Fig. 1, d, h, l, p, and t) and IgM+ plasma cells (Fig. 2, d, h, l, p, and t) showed similar morphology in both the red pulp and PALS in all groups of mice. Together, these data suggest that in the absence of TNFR1 expression by nonhematopoietic cells, plasma cells are located mainly in the PALS.

Failure to Induce a Significant IgG1 Response in TNFR1−/− Mice Reconstituted with WT-BM Cells. The functional potential of WT hematopoietic cells differentiated in the lymphoid microenvironment of LT-α−/− and TNFR1−/− mice was monitored by their ability to respond to T cell-dependent antigens. The titers of antigen-specific IgM and IgG1 antibodies were measured by ELISA after immunization with SRBCs. The level of the IgM response was similar in all animals examined (Fig. 3, A and C). In contrast, the IgG1 response that was deficient in both TNFR1−/− (14, 33) and LT-α−/− mice was restored in LT-α−/− mice (Fig. 3B; reference 31) but not in TNFR1−/− mice (Fig. 3D) after WT-BM transplantation. These data demonstrate a
correlation between the disturbed splenic architecture and incomplete antibody production in mice deficient for TNFR1 expression on nonhematopoietic cells.

**Discussion**

Organized lymphoid tissues provide a critical framework for many aspects of immune responses, but the nature of cellular and molecular interactions responsible for the maintenance of peripheral lymphoid architecture is still poorly understood. It has been demonstrated previously that TNFR1−/− mice are deficient in GC formation (13), as well as development of FDC networks and IgG responses after SRBC immunization (14). The reconstitution of TNFR1−/− mice with WT-BM or WT-FL provided a model to study the function of wild-type hematopoietic cells transferred into a TNFR1−/− deficient environment.

Here, we report that the hematopoietic cell transfer was not sufficient to restore the splenic architecture in TNFR1−/− mice. Secondary follicles, normally containing FDC networks, remained absent in the TNFR1−/− spleen after WT-BM or WT-FL transplantation and immunization. On the functional level, the TNFR1−/− reconstituted mice were still deficient in the production of IgG1 after SRBC immunization. As a control for the efficiency of the transplantation procedure, we demonstrated restored GC formation, rescued FDC networks, and normalized IgG1 production in LT-α−/− mice upon WT-BM transplantation and SRBC immunization, which is in agreement with previous reports (13, 31, 34). We suggest that the expression of TNFR1 on nonhematopoietic cells is an essential requirement for development of correct splenic architecture and full antibody responses.

The fact that FDC networks appeared after hematopoietic cell transfer in LT-α−/−, but not in TNFR1−/− spleen, makes the LT-α/TNFR1 I−/− model suitable to question the origin of FDCs. There are controversial opinions in the literature about the ability to transfer FDC precursors by WT or FL transplantation. FDCs live long, seldom divide, and change their morphology and phenotype during humoral immune responses (35, 36). One FDC derivation theory holds that FDCs are of local origin, probably developing from fibroblastic or primitive reticular cells (37–42) or from mesenchymal cells (pericytes around capillaries reference 43). Another theory suggests BM and FL origin of FDCs (36). The theory is based on the results of cell transfer experiments from normal donors into lethally irradiated recipients (40) or recipients known to bear only FDC precursors, but not fully differentiated FDC networks (SCID mouse; references 44, 45). The nature of the exact cell type in all of these theories has not been elucidated, and such an indisputable determination of FDC origin remains to be obtained.

The rescue of FDC networks in LT-α−/− mice after WT-BM transplantation argues for the presence of FDC precursors either in WT-BM or in the LT-α−/− mice themselves. These results indicate that hematopoietic precursors that repopulate the LT-α− deficient mice provide signals (probably the major signal is LT-α itself) for the maturation of FDC precursor cells. Since the transplantation of WT-BM or WT-FL cells does not restore FDC networks, it is not TNFR1 signaling of B cells that caused the absence of FDC in the spleen of TNFR1−/− mice (46). Instead, it appears that FDC precursors require the expression of TNFR1 for maturation. In addition, our data suggest that FDC precursors either cannot be transferred by BM/FL transplantation or they require the expression of TNFR1 by stromal cells for homing. However, since rat FDCs can develop in SCID mice after transfer of rat BM or rat FL (45), we would favor the latter hypothesis.

We observed that the location of plasma cells within the white pulp was generally disturbed in TNFR1−/− mice. This defect could not be corrected by WT-BM or WT-FL transplantation. However, the nature of these plasma cells remains undefined (i.e., antigen specific versus natural immunity), but it appears that an abnormal distribution by at least some differentiating B cells occurs in TNFR1−/− and TNFR1−/− reconstituted spleens. From these observations, we would like to propose the following model of plasma cell induction in TNFR1−/− and TNFR1−/− reconstituted spleen. After a primary immunization, areas adjacent to the red pulp in the periphery
of PALS are known to develop foci of antigen-specific B cells (30). These B cells differentiate to produce unmutated IgM and IgG eventually undergoing apoptosis and disappearing (30, 47–50). Data at present would suggest that the primary activation of B cells in TNFR1−/− or TNFR1+/− reconstituted spleens is not altered. Later, in wild-type mice, the second foci of B cell activation and differentiation appear, within the B cell primary follicles (30). The cells that seed GCs develop further and form dark zones containing the majority of cells in cycle and the adjacent light zones with an extensive FDC network. Although alternative ideas have been proposed (51), a clonal relationship exists between cells of these two compartments, i.e., the PALS and GCs (49). In TNFR1−/− or TNFR1+/− reconstituted spleens some activated B cells differentiate into plasma cells in the PALS without moving out into the traditional area. Since there are no characteristic FDC networks in PALS, we speculate that interdigitating dendritic cells (IDCs) could be responsible for an antigen delivery to the developing plasma cells. However, other cell types present in PALS, such as stromal elements, may also participate in this process.

In this report we demonstrate that the disrupted expression of TNFR1 on nonhematopoietic cells leads to an abnormal spleen architecture and antibody response. The demonstration of an abnormal distribution of plasma cells within the white pulp of TNFR1−/− mice is a first message that signaling through this receptor may be important to direct B cell traffic. The fact that this phenomenon is not rescued even after wild-type hematopoietic cell transfer strongly argues for the role of TNFR1 expression on nonhematopoietic cells for the direction or promotion of B cell location. Elucidating the role of nonhematopoietic cells in obtaining compartmentalization of the spleen during immune responses is yet another intriguing challenge.

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