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Antiviral Immune Responses in the Absence of Organized Lymphoid T Cell Zones in plt/plt Mice

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The paucity of lymph node (LN) T cells (plt) mutation in mice results in strongly reduced T cell numbers in LNs and homing defects of both dendritic cells (DCs) and naive T cells. In this study, we investigated the functional significance of the plt phenotype for the generation of antiviral immune responses against cytopathic and noncytopathic viruses. We found that DC-CD8+ T cell contacts and the initial priming of virus-specific T cells in plt/plt mice occurred mainly in the marginal zone of the spleen and in the superficial cortex of LNs. The magnitude of the initial response and the maintenance of protective memory responses in plt/plt mice was only slightly reduced compared with plt/+ controls. Furthermore, plt/plt mice mounted rapid neutralizing antiviral B cell responses and displayed normal Ig class switch. Our data indicate that the defective homing of DCs and naive T cells resulting from the plt/plt mutation results in a small, but not significant, effect on the induction of protective antiviral T and B cell immunity. Overall, we conclude that the spatial organization of secondary lymphoid T cell zones via the CCR7-CC chemokine ligand 19/CC chemokine ligand 21 pathway is not an absolute requirement for the initial priming and the maintenance of protective antiviral T and B cell responses.  


Secondary lymphoid organs, such as spleen, lymph nodes (LN),1 Peyer’s patches, and the mucosa-associated lymphoid tissue, sample Ag through drainage of the afferent lymph and provide the microenvironment for optimal Ag presentation to naive lymphocytes. The importance of LNs for the generation of protective antiviral T and B cell responses and for the induction of antitumor immunity has been shown, for example, in alymphoblastic (aly/aly) mice which lack LN and Peyer’s patches (1, 2). Absence of the spleen, which filters Ags from the blood, is less dramatic but may lead to deficient induction of Igs against nonreplicating agents (3, 4). In humans, splenectomy can pose the risk of overwhelming bacterial infection (5), although the induction of antiviral immune responses may be unimpaired (6), or even enhanced, if the spleen is a site of extensive viral replication (7, 8).

Within lymphoid organs, induction and maintenance of antiviral immune responses depend on correctly formed lymphoid compartments. Early trapping of virus in the marginal zone leads to the initial extrafollicular induction of antiviral B cells against T cell-independent Ags. Marginal zone macrophages have also been shown to be involved in induction of antiviral CTL responses, as it has been shown that only weak, nonprotective antiviral CTL responses could be initiated after depletion of marginal zone macrophages (9). The environment of germinal centers is required for the maintenance of antiviral B cell memory in mice (10), and patients with X-linked hyperIgM-syndrome lacking germinal centers due to an impaired CD40-CD40 ligand interaction show hypogammaglobulinemia and insufficient B cell and CTL activation leading to increased susceptibility to infections (11, 12).

The localization of lymphocytes within secondary lymphoid organs is controlled by constitutive chemokines differentially expressed in the B and T cell zones (13). Primary B cell follicles, most probably follicular dendritic cells (DCs), produce CXC chemokine ligand 13 (B lymphocyte chemoattractant; Ref. 14), which attracts mature resting B cells and a T cell subpopulation recently described as follicular Th cells via CXCR5 (15). T cell zones stromal cells express both the serine isoform of CC chemokine ligand (CCL)21-Ser/secondary lymphoid organ chemokine-Ser and CCL19/EBI1-ligand chemokine (16, 17). These chemokines act via the CCR7 receptor and are capable of attracting naive T cells and mature DCs, and thus coordinate their interaction within the T cell zone (18, 19). T cells down-regulate CCR7 after TCR triggering and concomitant to their evasion to the periphery (20), suggesting that the CCR7-CCL19/CCL21 interaction may be involved in T cell priming (21, 22). However, only little is known about the role of constitutive chemokines in the modulation of antiviral immune responses.

The paucity of LN T cells (plt) mutation, which arose as a spontaneous recessive mutation in mice (23), was recently mapped to the chemokine locus chromosome 4 and results in loss of both the only functional CCL19/ELC and the CCL21-Ser/SLC-Ser genes and in an aberrantly formed lymphoid T cell zone (24–26). Therefore, these mice are an excellent model to investigate the induction and maintenance of immune responses in a situation where the recruitment of naive T cells and DC to the T cell zone is defective (27).

Infection of mice with the lymphocytic choriomeningitis virus (LCMV) is a well-characterized model system for the investigation of antiviral T and B cell responses in vivo. LCMV can infect various cells of the immune system, such as B cells, macrophages,
and DC. In particular, the tropism of different LCMV strains to DC determines whether the virus infection results in induction of protective CTL responses and clearance, or leads to exhaustion of virus-specific CTL and establishment of persistent infection (28, 29). In this study, we used the LCMV system and other well-established virus infection models, vesicular stomatitis virus (VSV) and vaccinia virus (VV), to investigate the impact of defective DC and T cell homing on the induction and maintenance of antiviral T and B cell responses in plt/plt mice. We found that T and B cell priming in plt/plt mice occurred mainly in the marginal zone of the spleen and in superficial cortical areas of LNs. Furthermore, both antiviral T and B cell responses were comparable in plt/plt virus infections than seems necessary for nonreplicating Ag.

**Materials and Methods**

**Mice**

B6-plt/plt mice were bred at the Institut für Labortierkunde (University of Zürich, Zürich, Switzerland). They were back-crossed to C57BL/6 5–6 times and used for experiments in sex-matched groups with heterogeneous littersmates at the age of 8–12 wk. plt/plt mice were typed by PCR using the D4 Mit286 and D4 Mit237 primer pairs as described (30). B6PL-Thy1.1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred to the LCMV-gp33-specific TCR transgenic line 318 (31). Heterozygous F1 animals (318 × Thy1.1) served as donors of CD8+ T cells in adoptive transfer experiments.

**Viruses and peptides**

LCMV, WE strain, originally obtained from Dr. F. Lehmann-Grube (Heinrich-Pette Institut, Hamburg, Germany), was propagated on L929 cells at a low multiplicity of infection and was plated as previously described (32). VSV Indiana strain (VSV-IND, Mudd-Summers isolate), was originally obtained from D. Kolakofsky (Department of Genetics and Microbiology, University of Geneva School of Medicine, Geneva, Switzerland); VSV-IND was propagated on baby hamster kidney-21 cells and plated on Vero cells. For some experiments, UV-irradiated VSV-IND was obtained using UV irradiation (TUV 15W; Philips, Eindhoven, The Netherlands) for 5 min in a thin layer of liquid in a 60-mm petri dish. The VV recombinant for the VSV-recombinant LCMV-WE were injected in a volume of 50 μl in balanced salt solution into both hind footpads in experimental groups of three mice. The footpad thickness was measured at the indicated time points with a spring-loaded caliper (37).

**Construction of tetrameric class I-peptide complexes and flow cytometry**

MHC class I (H-2Dd) tetramers complexed with gp33 were produced as previously described (38). Briefly, H2-Dd and human β2-microglobulin molecules were recombinantly expressed in Escherichia coli (the plasmids were kindly provided by J. Altman, Emory University, Atlanta, GA). Biotinylated H2-Dd peptide complexes were purified using an Abx Explorer 10 chromatography system (Pharmacia, Uppsala, Sweden) and labeled by addition of streptavidin-PE (Molecular Probes, Eugene, OR). At the indicated time points after immunization, animals were bled and single-cell suspensions were prepared from spleen and LNs. Aliquots of 5 × 106 cells or three drops of blood were stained using 50 μl of a solution containing tetrameric class I-peptide complexes at 37°C for 10 min followed by staining with anti-CD8-PE (BD PharMingen) Ab in PBS 1% PBS for 30 min, LCMV-NP was detected using BD Biosciences, Mountain View, CA), and the cells were analyzed on a FACScan flow cytometer (BD Biosciences) after gating on viable leukocytes. For analysis of absolute cell counts, the number of total viable leukocytes was assessed in an improved Neubauer chamber.

**Intracellular cytokine staining**

Spleens were removed at the indicated time points after infection with LCMV. Single-cell preparations of 1 × 106 splenocytes were incubated for 5 h at 37°C in 96-well round bottom plates in 200 μl medium culture containing 25 U/ml IL-2 and 2 μg/ml brefeldin A (Sigma-Aldrich, St. Louis, MO). Splenocytes were stimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) as a positive control or left untreated as a negative control. For analysis of peptide-specific responses, 106 splenocytes were stimulated by adding 10−8 M gp33. After stimulation, splenocytes were surface stained with anti-CD8-PE (53-5.8; BD PharMingen) Ab in PBS 1% FCS and incubated for 1 h at 4°C. Spleens were washed once with FACS buffer, fixed with 100 μl 4% parformaldehyde in PBS for 5 min at 4°C, and permeabilized with 2 ml of permeabilization buffer (FACS buffer + 0.1% saponin) for 5 min at 4°C. Cells were then stained intracellularly with anti-IFN-γ-PE (AN18; Ref. 39) in permeabilization buffer for 30 min at 4°C. Cells were washed twice with permeabilization buffer, and the percentage of IFN-γ-producing cells was determined after gating on CD8+ cells using a FACScan flow cytometer.

**Fluorescence microscopy and immunohistochemistry**

Spleens and LNs of infected or noninfected animals were removed at the indicated time points, immersed in HBSS, and snap-frozen in liquid nitrogen. Six-micrometer cryostat sections were fixed in acetone for 10 min and dried for 30 min. After blocking of FcRs with 10% normal horse serum, each sample for 2 h. For immunohistochemistry, sections were counterstained with 0.1% crystal violet. The highest dilution of the serum that reduced the percentage of positive cells was used as a positive control.

**DC- and T cell homing on the induction and maintenance of antiviral T and B cell responses in plt/plt mice:**

In this study, we used the LCMV system and other well-established virus infection models, vesicular stomatitis virus (VSV) and vaccinia virus (VV), to investigate the impact of defective DC and T cell homing on the induction and maintenance of antiviral T and B cell responses in plt/plt mice. We found that T and B cell priming in plt/plt mice occurred mainly in the marginal zone of the spleen and in superficial cortical areas of LNs. Furthermore, both antiviral T and B cell responses were comparable in plt/plt virus infections than seems necessary for nonreplicating Ag.

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for 1 h with each Ab and washed extensively with PBS between incubations. The fluorescence was monitored on a Zeiss Axioshot microscope (Carl Zeiss, Feldbach, Switzerland) with a JVC KYF70 camera (Spitzer Electronic, Oberwil, Switzerland), using the Analysis software (Soft Imaging System, Münster, Germany).

For immunohistochemistry, cryostat sections were fixed in acetone for 10 min and subsequently incubated with anti-mouse N220 (RA3-3A1/6.1; American Type Culture Collection, Rockville, MD) or VL-4. Alkaline-phosphatase-labeled, species-specific goat-anti-donkey Abs (Tago Scientific, Burlingame, CA) were used as secondary reagents. The substrate for the red color reaction was AS-BI phosphate/New Fuchsin. Sections were counterstained with hemalum.

Results

Clonal expansion of LCMV-GP-specific CD8⁺ T cells in plt/plt mice

After immunization with LCMV-WE, CD8⁺ T cells directed against the immunodominant epitope gp33 undergo a vigorous systemic clonal expansion. To test the effect of defective DC and T cell homing on the initiation of LCMV-specific T cell responses, we used MHC class I tetramers complexed with the immunodominant CTL epitope gp33 derived from the LCMV-GP (tetramer (tet)-gp33) (38, 41). At different time points after i.v. infection with 200 PFU LCMV-WE, the proportion of tet-gp33-positive cells in the CD8⁺ T cell pool (Fig. 1, A–C), total cell numbers of CD8⁺ T cells (Fig. 1, D–F), and total cell numbers of gp33-specific CTL (CD8⁺tet-gp33⁺, Fig. 1, G–I) were assessed from blood, spleen, and inguinal LNs. As expected, naive plt/plt mice showed a significant paucity of total CD8⁺ T cell numbers in LNs (Fig. 1F). Following LCMV infection, the expansion of CD8⁺ T cells and the gp33-specific CTL response in both plt/plt and plt/+ mice peaked on day 8. The kinetics of the relative numbers of tet-gp33⁺ CTL revealed marked differences in the anti-LCMV response between plt/plt and plt/+ mice, particularly in peripheral blood (Fig. 1, A–C). However, the total numbers of tet-gp33⁺CD8⁺ CTL were comparable in plt/plt and plt/+ mice (Fig. 1, G–I). This discrepancy may be explained by the fact that the expansion of CD8⁺ T cells in the respective compartments of plt/plt mice was slightly faster than in plt/+ mice (Fig. 1, D–F), thus counterbalancing the differences in relative numbers.

Different LCMV strains induce CTL responses of different magnitude. The slowly replicating strain Armstrong (ARM) elicits weaker CTL responses and is rapidly cleared, whereas fast replicating strains such as Docile (DOC) induce exhaustive immune responses and tend to persist in the host. It has been suggested that the α-dystroglycan-dependent DC-tropism of LCMV is critical for the strength of the CTL response; and therefore, determines whether the virus is controlled by the immune system or can persist (28, 29). To assess whether the distinct DC-tropism of different LCMV strains affects the induction of CTL responses when DC homing to secondary lymphoid organs is impaired, we compared gp33-specific CTL responses in spleens of plt/plt and plt/+ control mice after infection with ARM, WE, and DOC (Fig. 2). The peak response on day 8 after infection with 200 PFU of ARM or DOC was comparable in plt/plt and plt/+ mice although in percentages of tet-gp33⁺ CTL within the CD8⁺ pool (Fig. 2A) and in total numbers of CD8⁺tet-gp33⁺ CTL (Fig. 2B). The proportion of tet-gp33⁺ CTL in the CD8⁺ T cell pool of plt/+ mice after LCMV-WE infection was slightly higher than in plt/+ mice (Fig. 2A, middle panel); however, like in the previous experiments, the total numbers of tet-gp33⁺ CTL were comparable in plt/plt and plt/+ mice (Fig. 2B). Taken together, these data show that the clonal expansion of gp33-specific CD8⁺ T cells in plt/plt mice after infection with LCMV mice is not impaired.

LCMV-specific CTL activity and antiviral protection against systemic and peripheral challenge infection

To examine the functional status of LCMV-specific CTL in plt/plt mice in more detail, the cytolytic activity of CTL was assessed in a ⁵¹Cr release assay using cells from spleens and mesenteric LNs on day 8 after LCMV infection. CTL responses against the LCMV-NP-derived peptide np396 and the LCMV-GP-derived peptide gp33 were comparable in both compartments (Fig. 3A). Virus dissemination and clearance in different organs were also determined on days 4 and 8 after infection. As shown in Fig. 3B, virus titers in various organs of plt/plt and plt/+ mice showed no significant differences at either time point. Similar results were obtained for LCMV-ARM and LCMV-DOC (data not shown).

An important function of antiviral CTL is their potential to migrate through peripheral tissue. To further characterize the functional activity of antiviral CTL in plt/plt mice, we used the intra footpad route of infection and monitored the CTL-mediated footpad swelling reaction. The kinetics and degree of the swelling reaction directly correlate with the vigour and the kinetics of the

**FIGURE 1.** Expansion of LCMV-gp33 specific CD8⁺ T cells in plt/plt and plt/+ mice. Plt/plt (▲) and plt/+ mice (□) were infected i.v. with 200 PFU LCMV-WE. The proportion of tet-gp33-positive cells in the CD8⁺ T cell pool (A–C), total cell numbers of CD8⁺ T cells (D–F), and total cell numbers of CD8⁺tet-gp33⁺ CTL were assessed for blood, spleen, and inguinal LNs at the indicated time points by FACS analysis. Given values represent mean ± SD (n = 6–8 per data point).
antiviral CTL response (33). Interestingly, plt/plt mice responded with a slightly accelerated, but overall weaker footpad swelling reaction (Fig. 4A), also when LCMV-ARM or LCMV-DOC were used (data not shown).

To distinguish between a peripheral immune reaction initiated and maintained in draining LNs and a generalized immune response with contribution of the spleen, plt/plt and plt/+ mice were splenectomized and infected with 50 PFU LCMV-WE into both hind footpads. The overall footpad swelling after splenectomy was reduced in both plt/plt and plt/+ mice (Fig. 4B); however, the overall kinetics were similar to nonsplenectomized mice, suggesting that the aberrantly structured draining LNs of plt/plt mice alone were capable to initiate and maintain an efficient antiviral immune response. Although the T cell numbers of the LNs of plt/plt mice are dramatically reduced, splenectomized plt/plt mice were also capable of mounting a good systemic LCMV-specific CTL response after peripheral infection. This is shown by the fact that the expansion kinetics of gp33-specific CTL in blood and inguinal LNs were equivalent in splenectomized plt/plt and plt/+ mice (Fig. 4C). Overall, these results indicate that the functional activity of antiviral CTL in plt/plt mice is normal and that CTL responses mediating rapid protection against systemic and peripheral LCMV infection can be generated in plt/plt mice, even in the absence of the spleen.

Antiviral CTL responses against cytopathic VV

To evaluate whether plt/plt mice are able to mount protective antiviral CTL responses against a cytopathic virus, we infected plt/plt and plt/+ mice with $2 \times 10^6$ PFU of LCMV-GP rVV (Vacc-G2). Seven days later, spleens were harvested and cytotoxicity was determined after restimulation for 5 days with irradiated, gp33-pulsed splenocytes. As shown in Fig. 5, plt/plt mice mounted a normal CTL response against the cytopathic Vacc-G2. Furthermore, Vacc-G2 was cleared from ovaries in plt/plt and plt/+ mice with similar kinetics (Fig. 5B), indicating that the plt defect has no major impact on the CTL-mediated control of a cytopathic virus.

Antiviral Ab responses in plt/plt mice

The rapid induction of neutralizing antiviral Abs is crucial for protection against infection with cytopathic viruses. It appears that viral pathogens have the common characteristic to be T cell-independent for IgM production, whereas isotype switching to IgG is mainly dependent on cognate help delivered by the CD4+ T cell subset (42). In the following set of experiments, the induction of neutralizing IgM and IgG Abs was assessed in plt/plt mice and plt/+ controls after i.v. infection with either $2 \times 10^6$ PFU of live VSV-IND (Fig. 6A), or with $1 \times 10^6$ PFU UV-inactivated VSV-IND (Fig. 6B). Both the replication competent (Fig. 6A) and the inactivated VSV-IND (Fig. 6B) elicited comparable neutralizing Ab responses in both groups of mice. Similarly, after i.v. infection with 200 PFU LCMV-WE, anti-np Abs of the IgM (Fig. 6C) and IgG class (Fig. 6D) were induced in plt/plt mice and plt/+ controls to a similar extent.

Using a peripheral route of infection for VSV ($2 \times 10^6$ PFU VSV-IND into both hind footpads) did not reveal significant differences between plt/plt and plt/+ mice in the kinetics of Ab formation or class switch (data not shown). Similarly, induction of anti-LCMV-NP responses was also not dependent on the route of infection; also i.p. and intrafootpad infection of plt/plt mice with LCMV-WE elicited anti-NP IgG titers comparable to those found in plt/+ controls (data not shown). Thus, the generation of T cell-dependent and -independent antiviral B cell responses in plt/plt appears not to be affected by the homing defect of DC and naive Th cells.
Maintenance of functional CTL memory responses in plt/plt mice

The notion has been put forward that memory T cell population cells can be distinguished by their level of CCR7 expression (43). In this study, we tested whether a disturbance of the CCR7/CCL19/CCL21 system by the absence of CCL19 and CCL21-Ser has an influence on the recirculation of memory CTL through secondary lymphoid organs and their activity. Fig. 7 shows that LCMV-specific CTL memory in plt/plt mice was not impaired even >300 days after infection with 200 PFU LCMV-WE. The total numbers of CD8^+ tet-gp33^+ CTL (Fig. 7A, upper panel) and gp33-specific IFN-γ-producing CTL (Fig. 7A, lower panel) in different lymphoid compartments were comparable in plt/plt and plt/+ controls. These results indicate that the numbers of virus-specific memory CTL, their distribution in lymphoid compartments and peripheral blood, and their ability to differentiate rapidly into IFN-γ-producing effector cells is not affected by the plt mutation.

Furthermore, we tested whether the kinetics of a recall memory response would be affected in plt/plt mice. To this end, groups of plt/plt and plt/+ mice were i.v. infected with 2 × 10^6 PFU Vacc-G2 to generate gp33-specific memory CTL. The acute activity of these gp33-specific cells was comparable in plt/plt mice and plt/+ mice (see above). Fourteen, 40, or 140 days after the initial immunization with Vacc-G2, mice were challenged i.v. with 200 PFU LCMV-WE, and 4 days after the challenge infection, virus titers were measured in spleen, liver, kidney, inguinal LNs, and thymus. LCMV could not be detected in plt/plt and plt/+ mice in any of the organs in these experiments (data not shown), indicating that the Vacc-G2-induced GP-specific CTL memory population was capable of protecting the mice against a challenge infection. In addition, total counts of CD8^+ tet-gp33^+ CTL were
the positioning of LCMV-infected cells in the marginal zone (Fig. 8, E and F, arrows), and for plt/+ mice, also in the T cell zone. Similarly, an immunohistological analysis of mesenteric LNs of LCMV-infected plt/plt mice and plt/+ mice revealed that, in both groups of mice, LCMV-Ag-positive DC and gp33-specific antiviral CTL were in close contact in superficial cortical areas (data not shown). Taken together, these data suggest that the contact between virus-infected DC and naive virus-specific T cells in the splenic marginal zone and in superficial cortical areas of LNs of plt/plt mice was sufficient to generate potent antiviral immune responses.

Discussion

Chemokines and lymphokines coordinate the development of secondary lymphoid organs and the cellular interactions that generate their distinct functional compartmentalization. In the present study, we have analyzed the role of the coordinate chemokine-driven interaction of DC and naive T cells in the T cell zone of secondary lymphoid organs for the induction of protective antiviral immune responses. Our experiments with plt/plt mice indicate that normal antiviral T and B cell responses can be generated even in the absence of the T cell zone organized by the constitutive chemokines CCL21-Ser/SLC-Ser and CCL19/ELC. Because the genomic locus affected by the plt mutation is rather large (23), it cannot be ruled out that the absence of genes other than CCL21a and CCL19 contribute to the phenotype observed in plt/plt mice. However, the morphological similarity of this mouse to the CCR7 knockout mouse (21) strongly suggests that the absence of these chemokines has a major impact on the phenotype of plt/plt mice.

Functional consequences of altered CCR7-SLC/ELC interaction for the induction of antiviral immune responses

It has been speculated (44, 45) that CCR7 and its ligand chemokines CCL19/ELC and CCL21/SLC not only influence T cell and DC homeostasis, but also have a fundamental impact on priming and maintenance of immune reactions. This hypothesis is supported by a number of studies describing the function of the CCR7-ELC/SLC system either in vitro (46) or based on correlations between the in vivo and in vitro migration pattern of T cells (21). In vivo, the ablation of CCR7 expression results not only in severely impaired migration of naive T cells and activated DC, but also in a reduced immune responsiveness such as decreased contact hypersensitivity (22). Furthermore, reduced responsiveness to hapten sensitization after in vivo blockade of CCL21/SLC suggested that selective interference with DC and naive T cell homing may down-modulate adaptive immune responses (47).

An earlier report on the in vivo significance of the CCL19/CCL21-driven DC and naive T cell migration for the induction of antiviral immunity showed an enhanced susceptibility of BALB/c-plt/plt mice to mouse hepatitis virus (27). BALB/c mice express the MHVR1 gene which renders them susceptible to MHV infection (48). However, the divergence in response to MHV among susceptible strains depends mainly on the susceptibility of macrophages (49, 50), which constitute the primary target of the virus. Because the F4/80+ macrophage compartment appears to be less prominent in spleens and LNs of plt/plt mice (T. Junt, unpublished

determined at day 4 after LCMV-WE challenge in spleen and mesenteric LNs. As shown in Fig. 7B, the clonal expansion of gp33-specific CTL was comparable in plt/plt and plt/+ mice. The weaker clonal expansion observed in mesenteric LN shortly after Vacci-G2 infection may be explained by a higher residual activity of the recently primed memory population facilitating the elimination of LCMV; and therefore, eliciting only a minor expansion of gp33-specific CTL.

Localization of initial CTL activation in plt/plt mice

The above experiments established that the chemokine-driven interaction of DC and naive T cells in lymphoid T cell zones in plt/plt mice is not an exclusive prerequisite for the induction of rapid protective antiviral immune responses. To determine the site of primary activation of antiviral T cell responses in plt/plt mice, we visualized the colocalization of LCMV-specific CTL and virus-infected DC in lymphoid organs. To this end, 1.5 × 10⁷ MACS-sorted Thy1.1-positive gp33-specific TCR transgenic CD8+ T cells were adoptively transferred into plt/plt mice. Twenty-four hours later, the mice were i.v. infected with 2 × 10⁸ PFU LCMV-WE, and spleens were removed and processed for immunohistochemistry 3 days postinfection. In the spleens of both plt/plt and control mice, LCMV Ag was largely confined to the marginal zone (Fig. 8, A and B). Double-staining for virus Ag and the DC marker CD11c revealed that a significant proportion of the marginal zone DC in plt/plt mice were infected with LCMV (Fig. 8A, inset, arrowhead), whereas in plt/+ mice, marginal zone DC (Fig. 8B, right inset, arrowhead) as well as T cell zone DC (Fig. 8B, left inset, arrow) harbored LCMV Ag. LCMV-specific 318 × Thy1.1 CD8+ T cells were mainly localized in the red pulp and in the marginal zone of plt/plt mice (Fig. 8C). These virus-specific CTL formed foci in close contact with infected cells exclusively at the marginal zone of plt/plt mice (Fig. 8C, inset, arrowhead). However, in plt/+ mice, virus-specific cells homed preferentially to the T cell zone and had contact with virus-infected cells both from the side of the T cell zone (Fig. 8D, inset, arrow) and from the side of the marginal zone (Fig. 8D, inset, arrowhead).

The preferential accumulation of LCMV Ag in the marginal zone of plt/plt mice was also demonstrated using sequential sections of plt/plt and plt/+ spleens. Staining for LCMV-NP (Fig. 8, E and F) and the B cell marker B220 (Fig. 8, G and H) revealed the positioning of LCMV-infected cells in the marginal zone (Fig. 8, E and F, arrows), and for plt/+ mice, also in the T cell zone.
observation), the increased susceptibility of plt/plt mice to mouse hepatitis virus may be at least partly due to differences in target cell availability. However, the altered lymphoid microenvironment of plt/plt mice did not influence the replication kinetics of LCMV, which is also a virus targeting macrophages.

Protection against LCMV (51) and VV (33) is mediated primarily by CTL. In view of the fact that CTL also contribute to the control of MHV infection (52, 53) and that plt/plt mice showed increased susceptibility to MHV infection, we expected a significant impairment of the protective immunity in plt/plt mice after infection with the noncytopathic LCMV or the cytopathic VV. However, both the kinetics of virus-specific CTL, i.e., acute and memory responses, and their function were not significantly altered in plt/plt mice. The major difference between plt/plt and plt/+ control mice was found in the relative proportion of virus-specific CTL among CD8 T cells (see Figs. 1 and 2). Nevertheless, these differences were leveled out by a slight overshoot in the CTL expansion rate and differences in the cellular distribution between lymphoid compartments. The reduction in T cell numbers is much more severe in LNs than in the spleen of plt/plt mice (23, 27). Therefore, it is interesting to note that even after splenectomy, plt/plt mice generated equivalent CTL responses compared with plt/+ controls (see Fig. 4). Furthermore, our study describes the quality of humoral responses in plt/plt mice, showing that in addition to the largely intact T cell responses, plt/plt mice also generated normal antiviral B cell responses.

A recently published study by Mori et al. (54) investigated immune responsiveness in plt/plt mice in a model of contact hypersensitivity and described that the plt mutation results in fully functional T cell responses which only differ in their kinetics. Our data corroborate and significantly extend this previous study because we establish that the altered spatial organization of secondary lymphoid T cell zones due to defects in the CCR7-CCL19/CCL21 interaction in plt/plt mice is probably not crucial for initial priming and maintenance of protective T and B cell responses in the course of viral infections.

Where are antiviral immune responses induced when DC and naive T cell cannot meet in the T cell zone?

Stein et al. (55) have demonstrated that peripherally injected CCL21 may accumulate on high endothelial venules of plt/plt
mice, and thus lead to an enhanced activation of lymphocyte trans-
migration. Because plt/plt mice express CCL21b outside lymphoid
organs, it may well be that this peripherally expressed chemokine
has an effect on T cell and DC migration into LNs, accounting for
the small differences observed between plt/plt and plt/+ mice. In
addition, CCL21b is still expressed at very low levels in LNs and
spleen of plt/plt mice (56). Although the mechanism of the mildly
impaired antiviral immune reactions remains elusive, it is surpris-
ing that the absence of a defined T cell zone had only rather mild
effects on the generation of virus-specific immune responses.

Complexes of virus-infected cells and specific T cells were
found in plt/plt mice only in the marginal zone, whereas these
clusters were distributed throughout the white pulp and in the
splenic marginal zone of control mice. This suggests that priming
of antiviral T cells in plt/plt mice occurred in the marginal zone.
Mori et al. (54) have shown that immunization of plt/plt mice with
OVA was followed by a remodeling of lymphoid organs indicating
that T cell responses against nonreplicating proteinaceous Ags
may also be elicited efficiently in the splenic marginal zone.
Furthermore, our data are in line with a recent study by Ciavarra et al.
(57) showing that after selective depletion of phagocytic marginal
zone DCs, the remaining interacting DCs were able to trap Ag
but failed to prime T cell responses against VSV.

It remains to be resolved whether splenic marginal zone DCs
and their LN equivalents alone contribute to the priming of anti-
viral immune responses in plt/plt mice. We found that LCMV Ag
in the marginal zone of the spleen and in the superficial cortex of
the LNs was also associated with CD11c-negative cells. It may
well be that marginal zone macrophages not only function as “Ag
trapping structures” and transmit virus to adjacent DCs, but they
may also contribute directly to the priming of antiviral T cells. This
notion is supported by previous findings showing that functional
marginal zone macrophages are crucial for the induction of anti-
LCMV T cell responses (9). Overall, our study supports the con-
cept that the splenic marginal zone and its LN equivalent are cru-
cial structures for the rapid generation of antiviral immune
responses.

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