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Evaluation of Immunological Paradigms in a Virus Model: Are Dendritic Cells Critical for Antiviral Immunity and Viral Clearance?1

Richard P. Ciavarra,2* Amber Stephens,* Sandra Nagy,* Margaret Sekellick,† and Christina Steel*

We have examined the role of dendritic cells (DCs) in the antiviral immune response and viral clearance using a transgenic mouse model (CD11c-diphtheria toxin (DT) receptor GFP) that allows for their conditional ablation in vivo. DT administration systemically ablated conventional and IFN-producing plasmacytoid DCs (pDCs) in transgenic, but not nontransgenic littermates, without elimination of splenic macrophages. Unexpectedly, early (12 and 48 h postinfection) viral clearance of vesicular stomatitis virus was normal in DC-depleted mice despite markedly reduced serum titers of type I IFN. DC-depleted mice remained virus-free with the exception of a subset (~30%) that developed overwhelming and fatal brain infections 6 days postinfection. However, DT treatment profoundly inhibited clonal expansion of naïve CD8+ vesicular stomatitis virus-specific T cells without altering the primary Th1 and Th2 cytokine response. Optimal clonal expansion required pDCs because selective elimination of these cells in vivo with a depleting Ab also suppressed expansion of tetramer+ cells, although Th1/Th2 cytokine production remained unaltered. Collectively, these data indicate that conventional DCs and to a lesser extent pDCs are critical for proliferation of naïve antiviral T cells. However, other components of the primary adaptive immune response (Th1/Th2 cytokines) are essentially normal in the absence of DCs, which may account for the efficient viral clearance seen in DC-depleted mice. Thus, sufficient redundancy exists in the immune system to sustain efficient viral clearance despite loss of an APC considered essential for induction of a primary antiviral immune response. The Journal of Immunology, 2006, 177: 492–500.

The induction of an adaptive CTL-mediated immune response requires a complex interaction among professional APCs, CTL precursors, and CD4+ Th cells. This response is initiated when immature phagocytic cells capture Ag, migrate to draining lymph nodes, and engage migrating T cells (1, 2). Within this ménage a trois, inductive signals are delivered for clonal expansion of naïve T cells. However, subsequent studies revealed that DCs are indispensable for processing and presenting these types of Ags in situ (7, 14–17). While this belief is especially apparent for peptide Ag, the host is generally confronted with complex (e.g., virus) or particulate (e.g., bacteria) Ags, and much controversy still exists as to which APC subsets are critical for processing and presenting these types of Ags in situ (7, 14–17). Prior studies using a liposome-mediated “suicide” technique to deplete macrophages demonstrated that cell-mediated immune responses to particulate Ags (cells, bacteria) were dependent on macrophages (18–20). However, subsequent studies revealed that DCs in the splenic marginal sinus were phagocytic and were also depleted by this treatment thus confounding interpretation of these studies (21).

To examine how macrophages and DCs regulate the cell-mediated immune response to complex viral Ags, we depleted these cells in vivo and then examined the immune response to vesicular stomatitis virus (VSV), a simple enveloped RNA virus related to rhabdovirus that has been well characterized biochemically. Mice infected with this rhabdovirus produce IgM and neutralizing IgG Abs and mount a CD4-dependent CTL response (22–24). Murine VSV-specific CTL recognize a single immunodominant epitope in the nucleocapsid protein (VSV-N52–59) in the H-2b haplotype, whereas epitopes derived from both the viral envelope glycoprotein (VSV-G) and VSV-N are recognized by L5-restricted CTL in the H-2d haplotype (25–28). Studies with gene-targeted mice have demonstrated a critical role for type I IFN in early viral clearance, whereas T and B cells cooperate for long-term survival (29, 30). Both the VSV-induced Th1/Th2 cytokine response and priming for the secondary VSV CTL response are critically dependent on pDCs because depletion of these cells by liposome-encapsulated clodronate before virus infection markedly reduces these responses (31). In addition, VSV disseminates to multiple organs and replicates to high titers in these mice, whereas control mice rapidly clear this virus 48 h postinfection (p.i.) (32).

As noted previously, the relative importance of macrophages and DCs including plasmacytoid DCs (pDCs), a major type I IFN-α-producing cell, to antiviral immunity and viral clearance could not be assessed by this depletion protocol because this approach depleted both cell populations. Therefore, to more precisely clarify the physiological contribution of conventional DCs and pDCs to these processes, we exploited a recently described transgenic (Tg) mouse model that allows for the selective and presumptive...
systemic depletion of DCs in vivo. This mouse line possesses a hybrid gene composed of the simian diphtheria toxin (DT) receptor (DTR) and GFP under the control of the CD11c promoter (CD11c-DTR/GFP). DTR transgenic (DTRtg) mice injected with DT are rapidly depleted of splenic DCs without altering the number of F4/80+ splenic macrophages (10). Thus, DTRtg mice in combination with VSV presents an excellent virus model to more precisely define the role of DC subsets in viral immunity and pathogenesis. We now report that DT treatment of DTRtg, but not normal littermates, depletes DCs in multiple organs without altering splenic macrophage subpopulations. Unexpectedly, ablation of DCs including pDCs did not inhibit early clearance of VSV from peripheral tissues, although a subset of cohorts developed overwhelming brain infections 6 days p.i. Similarly, depletion of conventional or pDCs did not reduce VSV-induced Th1 and Th2 cytokine responses. In contrast, clonal expansion of tetramer+ VSV-specific T cells was exquisitely sensitive to DC ablation, whereas selective depletion of just pDCs partially inhibited this response. Thus, in this virus model, conventional and pDCs are critical for some but not all components of the primary antiviral immune response and appear to contribute little to early viral clearance.

Materials and Methods

**Mice**

BALB/c female and hemizygous male C.FVB-Tg(Iftgax-DTR/ EGFP)5Lan/J (DTRtg) mice were obtained from The Jackson Laboratory. Mice were bred at Eastern Virginia Medical School and housed in sterile microisolator units containing sterile water, food, and bedding. Male DTRtg mice were bred with female C37BL/6 mice (The Jackson Laboratory) to obtain F1 progeny. Mice were genotyped for transgene expression by multiplex PCR using established primers for DTR (forward 5’-GGG ACC ATG AAG CTG CCG C-3’, reverse 5’-TCA GTG GGA ATT AGT CAT GCC-3’) (10). Control reactions were performed using primers for the TCR-6 chain (forward 5’-CAAT ATG TTG CTT GTC TG-3’, reverse 5’-CGT ATG CTA GTG CAC AGT TT-3’). Primer pairs were purchased from Integrated DNA Technologies. Mice lacking the transgene were used as non-DTRtg controls. Mice were used at 6–10 wk of age following protocols approved by The Institutional Animal Care and Use Committee at the Eastern Virginia Medical School according to federal guidelines.

**Virus and type I IFN**

Wild-type VSV Indiana strain, provided by Dr. P. Marcus (University of Connecticut, Storrs, CT), was grown and assayed as previously described (33). Virus was grown in confluent monolayers of Vero cells and virus titers determined by standard plaque assays (34). Unless indicated otherwise, mice were infected with VSV by a single i.p. injection of 2 × 10⁶ PFU. Serum type I IFN levels were determined 12–15 h p.i. with a standard plaque assay. To determine whether DCs were required for virus clearance and expansion of Ag-specific T cells, mice were treated with 100 μg of an anti-mouse pDC mAb (clone 120G8), provided by Dr. C. Asselin-Paturel (Scherling-Plough Research Institute, DarIdilly, France), 1 day before virus infection. A second injection was administered 24 h p.i. and the number of VSV PFU and tetramer+ cells determined 6 days later.

**ELISPOT assay**

Cytokine-producing cells were estimated by ELISPOT assay following established procedures (37). All matched capture and detection Ab pairs were obtained from eBioscience. Splen cells from either mock- or VSV-infected mice were incubated overnight in ELISPOT plates (Millipore) containing anti-cytokine capture Abs. No exogenous virus was added during this incubation. The following day, ELISPOTs were detected with the appropriate biotin-conjugated detection mAb and then revealed with a HRP-conjugated avidin (Sigma-Aldrich) and the AEC substrate (3-amin-9-ethylcarbazole; Sigma-Aldrich).

**Immunofluorescence staining and multiparameter flow cytometry**

Spleens were incubated with collagenase to promote release of DCs and macrophages from splenic stroma. Following addition of unconjugated anti-mouse macrophage Abs or directly conjugated Abs and a second wash step, unconjugated Abs were detected using a biotin-conjugated anti-rat IgG followed by fluorescein (FITC)-conjugated streptavidin. Splenic DCs and macrophage subpopulations were identified using Abs to DCs, CD11c+ N418 (BD Pharmingen), red pulp macrophages (RPM) F4/80, marginal metallophic macrophages (MMM) MOMA-1, SER-4 (38), and marginal zone macrophages (MZM) ERTR9 (39). Abs to macrophage subpopulations were also obtained either from G. Kraal (Vrije Universiteit, Amsterdam, The Netherlands) (ERTR9 and MOMA-1), P. Crocker (Sir William Dunn School of Pathology, Oxford, U.K.) (SER-4) (40), or commercial sources (F4/80; Serotec). Clonal expansion of VSV-N T cells in vivo was evaluated by gating on CD8+ cells and then determining using the percentage of cells that bound tetramers and up-regulated the activation Ag CD11a. No significant tetramer staining was detected on gated CD8+ cells (data not shown). Flow cytometric analysis of 20–200,000 events per sample was performed on a FACSscan (BD Biosciences) using CellQuest software (BD Biosciences). Gates were set with the use of appropriate isotype controls.

**Results**

**DT depletes selectively conventional and pDCs in Tg mice**

We first determined whether DT treatment selectively depletes DCs and pDCs without altering splenic macrophages. Fig. 1A demonstrates that DT did not diminish the number of splenic DCs (CD11c+ I-A<sup>4+</sup>) or pDCs (CD11c+ pDC Ag (PDCA)-1<sup>+</sup>) in non-DTRtg mice. In contrast, DCs and pDCs were markedly depleted in DTRtg mice given DT. In this and subsequent studies pDCs are defined as CD11c+PDCA-1<sup>+</sup> cells. Cells that stain with this Ab also coexpress Gr-1 and CD45R (B220) confirming the specificity of this Ab (Fig. 1C). It is also apparent from these data that the number of splenic F4/80+ (RPM), SER-4<sup>+</sup> MMM, and ERTR9<sup>+</sup> (MZM) remained unchanged relative to normal and non-DTRtg mice treated with an equivalent dose of toxin (Fig. 1B). Thus, DT treatment in this model selectively ablates splenic DC subpopulations without altering splenic macrophages.

**DT treatment ablates DCs in multiple cellular compartments**

To evaluate the contribution of DCs to the systemic antiviral immune response and viral clearance, it was necessary first to verify that DT depletes DCs in different organs/tissues. Therefore, mice were treated with a single injection of DT and the number of DCs in different organs assessed by multicolor flow cytometry. Administration of this toxin to non-DTRtg mice did not alter the frequency of DCs (CD11c+ I-A<sup>4+</sup>) relative to normal mice given PBS (Fig. 2, A, D, and G). In contrast, DT treatment of DTRtg mice consistently diminished the frequency of DCs (CD11c+ I-A<sup>4+</sup>) in the spleen, bone marrow, blood (Fig. 2, A, D, and G), and thymus (data not shown). Frequencies of pDC were also markedly reduced in these compartments whether defined as
DC depletion does not inhibit early viral clearance

These data indicate that systemic depletion of DCs is achieved with DT treatment of Tg mice. To determine whether early viral clearance is dependent on conventional and pDCs, mice were treated with DT and the following day infected with VSV. VSV titers were determined either at 12 or 48 h p.i. At the earliest time point (12 h), ablation of DCs resulted in a slight delay in viral clearance relative to non-DTRTg mice treated with DT (Fig. 3A). However, by 48 h, VSV was either at undetectable or at barely detectable levels in every organ tested (Fig. 3B).

Elimination of DCs suppresses the type I IFN response

Because pDCs are reported to be the primary producers of type I IFN in mice infected with VSV (41) and type I IFN is crucial for early viral clearance (30), DC ablation should have impaired viral clearance. We therefore examined serum type I IFN levels in Tg mice treated with DT. Fig. 4 demonstrates that ablation of conventional and pDCs markedly diminished serum type I IFN levels as measured by virus neutralization.

DCs are crucial for expansion of naive T cells in vivo

We next tested the paradigm that DCs are the only APC capable of activating naive T cells. To track clonal expansion of VSV T cells, BALB/c × C57BL/6 F1 DTRTg mice carrying the transgene were given multiple treatments of DT to deplete DCs during virus priming. The frequency of virus-specific T cells was then evaluated using VSV-N52–59/H-2Kb tetramers and flow cytometry. Fig. 5 illustrates that 3 days after DT treatment of Tg mice, the frequency of conventional and pDCs was still diminished in most organs (Fig. 5A). Although non-DTRTg mice given multiple injections of DT underwent some weight loss, VSV-specific T cells expanded normally in these mice relative to VSV-infected mice not treated with DT (Fig. 5B and data not shown). In contrast, the same treatment schedule administered to DTRTg mice profoundly suppressed clonal expansion of tetramer+ T cells in these same cellular locations (Fig. 5B). The inability of mice depleted of DCs to generate a normal tetramer response did not prevent efficient viral clearance from spleen, lung, and peritoneum. However, in this experiment, ~50% (two of four) of the Tg mice subsequently developed fatal brain infections (Fig. 5C).

DCs are not crucial for a primary antiviral cytokine response in vivo

Because mice rendered DC-deficient failed to expand virus-specific CD8+ T cells, we next sought to determine whether Th1/Th2 cytokine production, a CD4-mediated response, was also driven by DCs (24, 42). Naive mice infected with VSV produce a protracted Th1 (IL-2, IFN-γ) and Th2 (IL-4) cytokine response that peaks around day 6. Mice were therefore treated with either DT or mAb 120G8 to deplete pDCs selectively, and then infected with VSV. Six days p.i., the frequency of splenic cytokine-producing cells then assessed by single-color flow cytometry. Values in quadrants refer to the percentage of spleen cells that stained positive for the indicated macrophage mAb. CD11c+Gr-1+B220+ (Fig. 2, B, E, and H) or CD11c+PDCA-1+ cells (Fig. 2, C, F, and I).
FIGURE 2. DT depletes DC subpopulations in multiple organs and tissues. Control non-DTR Tg mice were administered either PBS (○) or DT (■). Tg mice received a similar DT injection (■). Two days after DT injection, single-cell suspensions were isolated from the spleen, blood, and bone marrow, stained with the indicated mAbs, and subjected to multiparameter flow cytometry. Conventional DCs were defined as CD11c+I-A$^+$ cells (A, D, and G), whereas pDCs were defined either as CD11c+Gr-1$^+$CD45R (B220)$^+$ (B, E, and H) or CD11c$^+$PDCA-1$^+$ (C, F, and I). Similar results have been obtained in two additional independent experiments.

was estimated by ELISPOT assay without further in vitro restimulation. Unexpectedly, ablation of conventional and pDCs did not diminish the frequency or total number of VSV-induced Th1 or Th2 cytokine-secreting cells (Fig. 6, A and B). Indeed, depletion of these cells resulted in an increase in the number of cytokine-producing cells, although this increase was not statistically significant except for the IL-2 response. For this cytokine, there was a significant increase in the frequency as well as the total number of splenic IL-2-secreting cells that occurred without changes in the cellularity of the spleen (Fig. 6C). Depletion of just pDCs did not decrease the frequency or total number of Th1 and Th2 cytokine-producing cells (Fig. 6, A and B). As demonstrated previously, normal viral clearance was observed for non-DTR Tg mice treated with DT (Fig. 6D). Although most DT-treated Tg mice efficiently cleared virus from peripheral organs, a subset (33%) of mice (three of nine) developed high titers of virus in the brain. Virus clearance in mice treated with mAb 120G8 was indistinguishable from control mice (Fig. 6D).

Virus-specific CD8$^+$ T cell expansion in mice depleted of pDCs

Because depletion of pDCs did not suppress CD4-mediated Th1/Th2 cytokine production in vivo, we next tested the extent to which clonal expansion of CD8$^+$ T cells is dependent on pDCs. As demonstrated previously, treatment of non-DTR Tg mice with DT did not prevent normal expansion of VSV-N52–59 T cells located in lymphoid (spleen) and nonlymphoid tissues (lung, peritoneum), whereas the same regimen given to Tg mice profoundly inhibited this response (Fig. 7A). Administration of anti-pDCs mAb 1 day before and after virus infection resulted in a partial but significant inhibition of the frequency of tetramer$^+$ cells. Control non-DTR Tg mice given DT or treated with 120G8 to deplete pDCs efficiently cleared VSV from all organs. As noted previously, depletion of both conventional and pDCs resulted in virus dissemination only to the brain in a subset of Tg mice (Fig. 7B).

**DT is not toxic to activated T cells**

A potential complication in the interpretation of our data is expression of CD11c on activated T cells (10, 43, 44). Thus, inhibition of T cell clonal expansion by DT may reflect ablation of activated CD11c$^+$ T cells and not DCs. To assess this possibility, DT was administered 4 days p.i., a time point when VSV-N T cells are activated as evidenced by up-regulation of CD11a and VLA-4, a very late activation Ag (see Figs. 5 and 7; and data not shown). When DT was administered 1 day before and after virus infection, both the 2 and 0.5 ng/g dose abolished clonal expansion of VSV-N T cells in the spleen, lung (Fig. 8A), and peritoneum (data not presented). However, when Tg mice were treated with 0.5 ng/g (10

FIGURE 3. Efficient viral clearance in mice acutely depleted of DCs. Non-DTR Tg littermates (○) and DTR Tg mice (■) were treated with DT and 2 days later infected with VSV. At 12 (A) or 48 h (B) later, the indicated organs were removed and the number of VSV PFU determined by a standard plaque assay. Note different scale in B. This experiment is representative of three independent experiments with two to four mice per group.

FIGURE 4. DC ablation inhibits the type I IFN (IFN-I) response following virus infection. Tg mice were given a single injection i.p. of either PBS (○) or DT (■) and 2 days later infected with VSV. Twelve hours p.i., serum samples were harvested and type I IFN levels determined by virus neutralization assay. Values represent the mean ± SEM of pooled data from three independent experiments with two to three mice per group.
ng/20 g of mouse) DT 4 days after virus infection, a robust proliferative response was detected in all cellular compartments (Fig. 8A, d and h). These data are consistent with kinetic studies on T cell expression of CD11c in virus-infected mice. Low levels of CD11c were present on a small fraction (~5%) of normal T cells, and this level of expression did not increase significantly 2 and 4 days p.i. (Fig. 8B, a–d). We did not detect tetramer$^+$ (VSV-N) 2 days after VSV infection but observed a small population (1.2%) of CD8$^+$ tetramer$^+$ cells 4 days after virus infection that remained CD11c$^-$ at this time point (Fig. 8B, d). However, essentially all CD8$^+$ VSV-N T cells in the spleen, lung, and peritoneum became CD11c$^+$ by 7 days p.i. (Fig. 8B, e–g, respectively). In contrast, the vast majority of activated CD4$^+$ T cells remained CD11c$^-$ (Fig. 8B, h–j). Thus, these studies demonstrate that DT treatment 1 day before and after VSV infection inhibits T cell clonal expansion because the critical APC for this response has been depleted by DT treatment.

**Discussion**

In this report, we have rendered mice conditionally deficient of conventional and pDCs to determine the extent to which antiviral immunity and viral clearance are dependent on distinct DC subsets. Treatment of non-DTRTg mice with a single injection of 2.0 ng/g DT did not diminish the frequency of splenic CD11c$^+$ I-A$^d$+ DCs, whereas this treatment markedly decreased this cell population in the spleen of DTRTg mice. Because macrophages express low levels of CD11c, the possibility existed that these cells would also be depleted by DT treatment. However, flow cytometric analysis using Abs specific for macrophage subpopulations demonstrated that RPM, MMM, and MZM in DTRTg mice were unaffected by DT. This confirms and extends the initial work by Jung et al. (10) who reported that DCs but not F4/80$^+$ RPM were depleted by DT treatment. In contrast, Probst et al. (45) reported that DT treatment was not specific for DCs but also ablated splenic macrophages. This discrepancy in the specificity of this treatment may reflect their use of a higher dose of DT (4.0 ng/g) and/or variation in the potency and specificity of different DT preparations. In our experience, the 4.0 ng/g dose was toxic and is ≥16 times the dose necessary to deplete DCs in this model (data not shown).

DT treatment of Tg mice appeared to ablate DCs globally because the frequency of CD11c$^+$ I-A$^d$+ cells was diminished in the bone marrow, thymus, lymph node, and blood. Because type I IFN is critical for host resistance against VSV (30), pDCs play a potentially crucial role in host resistance against this virus. We therefore examined the impact of this treatment on pDCs and observed a decrease in the frequency of CD11c$^+$ Gr-1$^+$ B220$^+$ cells in all organs/tissues examined. A similar conclusion was reached using PDCA-1, a new mAb directed against mouse pDCs. The specificity of this Ab was confirmed by demonstrating that CD11c$^+$ PDCA-1$^+$ cells coexpressed Gr-1 and B220. Thus, the DTRTg model allows for the selective and global depletion of DC subsets without altering other cells potentially important for antiviral immunity such as phagocytic macrophages.

Because pDCs are reported to produce copious quantities of type I IFN in response to viruses including VSV (41), we expected that ablation of pDCs by DT would promote virus replication and dissemination. However, early viral clearance in mice depleted of
pDCs was essentially normal at 12 and 48 h despite markedly reduced serum titers of type I IFN. It is possible that the reduced type I IFN levels were sufficient to contribute to viral clearance. In that regard, we detected a CD11c⁺ PDCA-1⁺ population present in the bone marrow of virus infected Tg mice, but not control mice, treated with DT that may represent the cellular source of residual type I IFN activity. In contrast, viral clearance is impaired in mice depleted of both splenic macrophages and DCs even though serum titers of type I IFN are within normal ranges (32). These different outcomes may be due to efficient clearance of VSV by splenic macrophages present in Tg mice rendered deficient of DCs but not macrophages. Consistent with this view, rabies virus accumulates rapidly (≤30 min) in MZM following i.v. administration (46).

According to current dogma, DCs are the only APC capable of inducing functional activation of naive T cells in vivo. To test this paradigm in a virus model, mice were given multiple injections of DT before and after virus infection to deplete DCs during the sensitization phase of the immune response. This regimen had no impact on the weight gain and general health of non-DTRTg mice infected with VSV. However, Tg mice lost weight progressively during DT treatment, although their general health and activity appeared normal (data not shown). Tetramer staining confirmed that VSV induced a robust expansion of CD8⁺ T cells specific for VSV-N52–59 peptide in non-DTRTg mice treated with DT. In contrast, depletion of DC profoundly suppressed clonal expansion of tetramer⁺ cells in both lymphoid and nonlymphoid compartments. Inhibition of the proliferative response reflected ablation of DCs and not toxicity of DT for activated CD11c⁺ T cells (10, 43, 44). Thus, DT treatment administered at a time when DCs were no longer required for T cell activation, failed to inhibit clonal

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Acute depletion of DCs fails to prevent a virus-induced cytokine response in vivo. Wild-type mice were injected i.p. with either DT (□) or mAb 120G8 (■) 1 day before and after infection with VSV. Tg mice were given just DT (2.0 ng/g) following the same DT schedule (■). Six days later, the frequency (A) and total number (B) of splenic cytokine-producing cells as well as splenic cellularity (C) was determined in each treatment group. Individual virus titers were also evaluated in these mice 6 days p.i. (D) where non-DTRTg mice treated with DT (○), non-DTRTg mice treated with mAb 120G8 (△), and DTRTg mice given DT (●) are represented. Data in A–C have been pooled from three independent experiments that included two to three mice per group. Significant difference (*, p = 0.05) was between control non-DTRTg and DTRTg mice treated with DT.

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** Ablation of pDCs inhibits T cell clonal expansion but not viral clearance. Non-DTRTg mice were treated with either DT or anti-pDC mAb (120G8), infected with VSV and 6 days p.i. evaluated for the presence of tetramer⁺ cells (A) or VSV plaques (B). DTRTg mice were similarly treated with DT before VSV infection. A. A representative experiment (one of three) in which the percentage of tetramer⁺ vs the percentage of CD11a⁺ cells is displayed on gated CD8⁺ cells. Representative experiment (one of three) in which the percentage of tetramer⁺ vs the percentage of CD11a⁺ cells is displayed on gated CD8⁺ cells. B. Plaques were detected on Vero cells using the indicated tissue extracts from non-DTRTg mice treated with either DT (○) or 120G8 (△). DTRTg mice (●) treated with DT. Data represent virus titers from individual mice pooled from two independent experiments.
expansion. These data are consistent with our kinetic studies demon-
strating delayed expression of CD11c on activated CD8+ T cells. Thus, CD11c was not expressed on CD8+ T cells during DT treatments. It should be noted that phenotypic analysis of DCs and pDCs following DT treatment indicated only partial ablation of both these subsets. This suggests that a signif-
icant fraction of cells that express a phenotype typical of DCs and pDCs lack a functional characteristic of DCs. Finally, during the course of these experiments, some Tg mice developed hindlimb paralysis around days 4–5. Although Tg mice depleted of DCs cleared VSV effectively during the early phase of infection (12 and 48 h), virus disseminated to the brain in ∼30% of these mice. It is currently unclear why these mice failed to control VSV replication.

Studies with knockout mice with specific immune defects have demonstrated that T and B cell-deficient (SCID) mice succumbed from encephalitis within 5–9 days of infection (29, 47). Although passive transfer experiments indicated that Abs protected against encephalitis disease, a role for T cells in long-term survival has also been reported. Thus, CD40L−/− mice were susceptible to VSV and residual resistance was almost completely abrogated by de-
pletion of CD8+ T cells. A role for CD8+ T cells was further supported by the observation that MHC class I/II-deficient, but not just class II-deficient mice, succumbed to VSV infection (6).

Mechanistically, T cell-mediated cytotoxic activity was not critical for viral clearance because perforin-deficient mice efficiently elimi-
nated cytopathic VSV and vaccinia virus, whereas this pathway was crucial for elimination of noncytopathic viruses (48). Perhaps importantly, T cell derived IFN-γ inhibited VSV replication in the CNS, although neither IFN-γ nor IL-4 promoted viral clearance in peripheral tissues (49). With respect to studies presented in this report, virus dissemination may be related to the failure of clonally expanded T cells to undergo functional differentiation. For example, tetramer+ T cells in control, but not Tg mice, up-regulated VLA-4, a very late activation Ag (data not presented). Taken together, studies in the DTRTg model are consistent with the view that DCs are crucial for clonal expansion of naive CD8+ T cells in vivo. A recent report with this model also demonstrated that the generation of a primary CTL response against lymphocytic choriomeningitis virus was dependent on DCs (9).

We next considered the possibility that elimination of conven-
tional DCs may also inhibit activation of CD4+ T cells. This would suppress production of CD4-derived cytokines such as IL-2 and IL-4 necessary for T cell proliferation (32). However, the fre-
quency of splenic cytokine-producing Th1 (IL-2, IFN-γ) and Th2 (IL-4) cells detected ex vivo was not diminished in Tg mice treated with DT. It should be noted that unlike most prior reports (6, 50), cytokine-producing cells were revealed without further restimula-
tion in vitro and presumably reflect more accurately the host res-
ponse in situ. The ability of naive CD4+ T cells to become func-
tional effector cells in the absence of conventional DCs is unexpected because it is currently believed that only DCs can in-
duce this function in situ. However, we cannot exclude the possi-
ability that a subset of DCs remains following DT treatment that is sufficient to induce cytokine production but not clonal expansion of T cells. Alternatively, another cell type within the spleen, such as class II-positive macrophages, may function as APCs for cyto-
kine production but not for T cell clonal expansion. That splenic macrophages can function as APCs for VSV-induced cytokine

**FIGURE 8.** DT treatment depletes DCs but not T cells. A, Wild-type mice (a and e) were treated with 2.0 ng/g DT 1 day before and after infection with VSV. Tg mice were given either 2.0 ng/g (b and f) or 0.5 ng/g DT (c and g) following a similar treatment schedule. A final group of DTRTg mice were administrated DT (0.5 ng/g) 4 days after infection with VSV (d and h). Cells were isolated from the spleen, lung, and peritoneal cavity 6 days p.i., stained with the indicated Abs and analyzed by flow cytometry. Values in the upper right quadrant indicate the percentage of cells within a CD8+ gate that were CD11a+tetramer+. B, Wild-type mice were infected with VSV, and 2 days (a and b) and 4 days (c and d) after virus infection, spleen cells were phenotyped for expression of CD11c and tetramer binding on CD4+ (a and c) and CD8+ (b and d) T cells. An additional group of mice was examined on day 7 p.i. on gated CD8+ (e–g) and CD4+ (h–j) spleen cells. This experiment has been repeated two additional times and yielded similar results.
production is consistent with loss of this response following liposome-mediated suicide of phagocytic cells (32). Furthermore, a recent report demonstrated that both Ag-pulsed macrophages and DCs activate and induce effector functions in naïve T cells upon adoptive transfer in vivo. Both populations behaved similarly with respect to migration to draining lymph nodes and potency for T cell activation which was mediated by direct Ag presentation and not by cross-presentation by host APC (51).

Because DT ablates pDCs as well as conventional DC subsets, the role of pDCs in the antiviral immune response and viral clearance could not be assessed by this approach. We therefore used a depleting mAb to selectively eliminate pDCs. We confirmed the specificity of this Ab by demonstrating that CD11c<sup>+</sup>PDCA-1<sup>−</sup> cells were depleted following administration of 120G8 mAb. Mice depleted of pDCs were capable of efficient elimination of VSV in both peripheral and CNS locations. Moreover, normal numbers of cytokine-producing cells were generated following following infection with VSV. In contrast, the ability of VSV-N<sub>52−69</sub> T cells to undergo optimal expansion following infection with VSV was significantly inhibited in pDC-depleted mice. It is presently unclear why elimination of this subset diminished clonal expansion. T cell exhaustion cannot be invoked because pDC-deficient mice cleared VSV at rates similar to VSV-infected non-DTR<sup>Tg</sup> mice; thus, viral loads in these mice were similar (52). However, we have not excluded the possibility that conventional DCs require an undefined soluble or membrane-derived signal provided by pDCs necessary for maturation of conventional DCs into effective APC as recently described by Yoneyama et al. (53). Current studies are designed to more precisely define why optimal T cell expansion is dependent on pDCs.

Collectively, these data demonstrate that acute depletion of pDCs does not inhibit the generation of CD4<sup>+</sup>Th1/Th2 cytokine-producing cells or viral clearance. Indeed, ablation of both conventional and pDCs suppresses expansion only of VSV-specific T cells without altering Th1/Th2 cytokine production and viral clearance in the majority of depleted animals. Thus, these data indicate that sufficient redundancy exists in the innate and adaptive immune response to sustain antiviral immunity and host resistance despite loss of a purportedly crucial APC.

**Disclosures**

The authors have no financial conflict of interest.

**References**


