CD4⁺ T Cell Subsets during Virus Infection: Protective Capacity Depends on Effector Cytokine Secretion and on Migratory Capability

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Abstract
To analyze the antiviral protective capacities of CD4⁺ T helper (Th) cell subsets, we used transgenic T cells expressing an I-Aᵇ-restricted T cell receptor specific for an epitope of vesicular stomatitis virus glycoprotein (VSV-G). After polarization into Th1 or Th2 effectors and adoptive transfer into T–cell-deficient recipients, protective capacities were assessed after infection with different types of viruses expressing the VSV-G. Both Th1 and Th2 CD4⁺ T cells could transfer protection against systemic VSV infection, by stimulating the production of neutralizing immunoglobulin G antibodies. However, only Th1 CD4⁺ T cells were able to mediate protection against infection with recombinant vaccinia virus expressing the VSV-G (VaccIND-G). Similarly, only Th1 CD4⁺ T cells were able to rapidly eradicate VaccIND-G from peripheral organs, to mediate delayed-type hypersensitivity responses against VSV-G, and to protect against lethal intranasal infection with VSV. Protective capacity correlated with the ability of Th1 CD4⁺ T cells to rapidly migrate to peripheral inflammatory sites in vivo and to respond to inflammatory chemokines that were induced after virus infection of peripheral tissues. Therefore, the antiviral protective capacity of a given CD4⁺ T cell is governed by the effector cytokines it produces and by its migratory capability.

Key words: Th1/Th2 cells • vesicular stomatitis virus • vaccinia • chemokines • migration

Introduction
In the case of cytopathic viruses, T cell–dependent cytokines together with neutralizing antibodies are usually essential for viral eradication and protection against reinfection (1, 2). CD4⁺ T cells play a crucial role in many antiviral immune responses. As well as their direct antiviral effects via the production of cytokines such as IFN-γ and TNF-α (3, 4), they provide the cognate signals that induce the production of protective virus-neutralizing IgG by specific B cells (5) and enhance the magnitude of antiviral CTL responses (6–8). Several experimental models of viral infection have emphasized the important role CD4⁺ T cells may play in the eradication of viruses by both humoral and cell-mediated mechanisms. Mice deficient in CD8⁺ CTLs can clear influenza A virus, and adoptively transferred CD4⁺ T cell clones have been shown to be able to promote recovery from lethal infection (9–11). In addition, CD8⁺ CTL-deficient mice can also effectively control vaccinia virus infection (12, 13). Furthermore, polyvirus-specific CD4⁺ T cell clones can confer protection against lethal infection by stimulating neutralizing antibody production (14).

Effector CD4⁺ T cells can be subdivided, on the basis of the cytokines that they secrete, into distinct populations that direct different types of immune response. Two main subsets of effector CD4⁺ T cells have been described: Th1 cells produce inflammatory cytokines such as IFN-γ and TNF and participate in cell-mediated immune responses, whereas Th2 cells secreting IL-4, IL-5, IL-6, IL-10, and IL-13 primarily help B cells produce antibodies and also mediate immunity against intestinal nematodes (15–17).
Although the roles of Th1 and Th2 CD4\(^+\) T cells in protection against some parasitic infections such as Leishmania and Nipponstrongylus have been well described (18–20), thus far little is known about the ability of these cells to protect against different types of virus infection. Vesicular stomatitis virus (VSV)\(^{1}\) infection of immunocompetent mice induces a rapid T cell–independent neutralizing IgM response, followed by production of neutralizing IgG antibodies that are strictly dependent on CD4\(^+\) T cell help (5). CD4\(^+\) T cells seem to be crucial for recovery from primary infections and for eliciting neutralizing IgG antibodies required for protection against reinfection (21, 22). To analyze the antiviral protective capacities of CD4\(^+\) Th cell subsets, we used transgenic mice (designated tg7) expressing an MHC class II (I-A\(^d\))–restricted TCR specific for a peptide derived from the glycoprotein of VSV (VSV-G) on 50% of CD4\(^+\) T cells (23). Naïve tg7 transgenic CD4\(^+\) T cells facilitated protective VSV-neutralizing IgG production after adoptive transfer into T cell–deficient recipients, but were unable to confer cell-mediated antiviral protection against recombinant vaccinia virus expressing the VSV-G (23). In contrast, in vitro–primed tg7 CD4\(^+\) T cells rapidly eliminated recombinant vaccinia virus from peripheral tissues (23). Here, we analyzed the protective capacities of distinct Th1 and Th2 effector populations of CD4\(^+\) T cells in different types of antiviral responses, namely, the induction of VSV-neutralizing IgG antibodies and the cell-mediated clearance of recombinant vaccinia virus expressing VSV-G.

Materials and Methods

Mice: C57BL/6 (H-2\(^b\)) mice, TCR transgenic tg7 mice (23), and SMAR TA mice (24) were obtained from the breeding colonies of the Institut für Zuchthygiene, Zürich, Switzerland. T cell–deficient mice (TCR\(^{-}\)–/–, CD4\(^{-}\)–/–) (25) on a C57BL/6 (H-2\(^b\)) background were obtained from The Jackson Laboratory.

Cytofluorimetric Analysis of Intracellular Cytokines. The following mAbs were used: FITC–conjugated anti–IFN-\(\gamma\), PE–conjugated anti–IL-4, FITC–conjugated anti–TNF-\(\alpha\), PE–conjugated anti–IFN-\(\gamma\), and FITC–conjugated IL-10 (all from BD PharMingen). Staining was performed as described previously (26). In brief, aliquots of 5 \(\times\) 10\(^6\) CD4\(^+\) T cells were stimulated in vitro at 37\(^\circ\)C for 4 h in RPMI 1640/10% FCS containing PMA (10 ng/ml), ionomycin (100 ng/ml), and monensin (2 mM; all from Sigma–Aldrich). Samples were then stained for 30 min at 4\(^\circ\)C with Tricolor–anti–CD4 (Caltag). Surface staining was fixed by incubation in 100 \(\mu\)l of PBS/4% paraformaldehyde for 10 min, and the cells were permeabilized by addition of 2 ml permeabilization buffer (PB: PBS/1% saponin/0.05% sodium azide, both from Sigma–Aldrich) for 10 min. Samples were stained for 30 min at 4\(^\circ\)C in PB containing the appropriate anticytokine antibodies. After washing twice with PB, samples were resuspended in FACS buffer and analyzed using a FACSscan™. To control for nonspecific intracellular staining, parallel samples of stimulated and permeabilized CD4\(^+\) T cells were stained with PE–conjugated isotype–matched mAbs of irrelevant specificity, which did not result in any staining signal.

Viruses and Immune Response Assays. VSV was grown on C6/36 cells. VSV serotype Indiana (VSV–IND), VSV serotype Indiana (VSV–IND, Mudd-Summers isolate) and VSV serotype New Jersey (VSV–NJ, Pringle isolate) were grown on BHK 21 cells andplaqued on Vero cells (27). UV light inactivation of VSV was performed under a 15 W UV lamp for 4 min and verified by plaqueing on Vero cells (28). Recombinant vaccinia virus expressing the VSV–IND glycoprotein (Vacc–IND–D–G) (29) or lymphocytic choriomeningitis virus nucleoprotein (LCMV–NP) (Vacc–LCMV–NP) [30] were grown and plaqued on BSC 40 cells. LCMV isolate WE (31) was grown on L929 cells. Recombinant baculoviruses expressing VSV–IND–D–G or VSV–IND–N–P were obtained and grown as described previously (32).

Immune Response Assays. Mice were immunized intravenously or intranasally with 2 \(\times\) 10\(^6\) PFU of live or inactivated VSV–IND or VSV–NJ. Sera were collected by bleeding from the retroorbital plexus at different time points after immunization for determination of VSV–specific neutralizing antibody titers using a plaque assay as described previously (33). To determine IgG titers, undiluted serum was pretreated with an equal volume of 0.1 M 2-ME in saline (34). Alternatively, mice were infected intraperitoneally with 5 \(\times\) 10\(^6\) PFU of Vacc–IND–D–G or Vacc–LCMV–NP. Organs were harvested at the indicated time points, and the vaccinia titers were determined on BSC 40 monolayers as described previously (35).

For footpad delayed–type hypersensitivity (DTH) responses, mice received 10 \(\mu\)g of baculovirus–derived recombinant VSV–G (Bac–G) or recombinant VSV–NP (Bac–NP), or 500 PFU LCMV–WE, in a total of 50 \(\mu\)l into the right hind footpad. Footpad swelling was monitored daily using calipers (Kroeplin). T cell Proliferative Assays and Cytokine Production. Proliferative responses and cytokine secretion by tg7 Th1 and Th2 effector cells were assayed after in vitro restimulation with irradiated C57BL/6 spleen cells and VSV–G peptide p8 (36; VSV–G amino acids 415–433), using \(^{3}H\) thymidine incorporation and ELISA, respectively, as described previously (23).

Polarization of Transgenic CD4\(^+\) T Cells into Th1 and Th2 Cells. Effector Th1 and Th2 cells were generated by in vitro polarization of naive transgenic tg7 CD4\(^+\) cells as described previously (37). Naïve tg7 CD4\(^+\) spleen cells were obtained at a purity of 98% by magnetic cell sorting (MACS) with anti-CD4 microbeads (Miltenyi Biotec). Aliquots of 5 \(\times\) 10\(^6\) CD4\(^+\) T cells were cultured in 24–well tissue culture plates (TPP) in 2 ml RPMI 1640 medium containing 10% FCS, penicillin, streptomycin, 1–glutamine, and 5 \(\times\) 10\(^{–5}\) M 2-ME (polarization medium), in the presence of 5 \(\times\) 10\(^5\) irradiated C57BL/6 spleen cells plus p8 (1 \(\mu\)g/ml). Th1 cultures were supplemented with 50 U/ml recombinant murine IL-2 (rmIL-2; BDI PharmaMingen), whereas Th2 cultures contained rmIL-2 plus rmIL-4 (500 U/ml; BD PharmaMingen) and polyclonal sheep anti–IFN–\(\gamma\) antibodies (1:100 [38]). After 4 d of culture at 37\(^\circ\)C in 5% CO\(_2\), cells were washed.
and expanded for a further 4-5 d in 6-well tissue culture plates (TPP) with 5 ml proliferation medium containing rmlL-2 (Th1 cultures) or rmlL-2 plus rmlL-4 (Th2 cultures). To obtain irreversibly polarized effector populations, three identical rounds of the above in vitro stimulation were performed (39). Effector cells were washed twice in HBSS before use.

A doptive Transfer of Antiviral Immunity. TCR-β⁻/⁻/⁻ or C57BL/6 mice were adoptively transduced intravenously with the indicated numbers of transgenic Th1 or Th2 CD4⁺ T cells and challenged 24 h later with virus or recombinant viral proteins as described in the figure legends.

Immunohistochemistry. Immunohistochemical analysis was performed as described previously (40). In brief, organs were immersed in HBS, snap frozen in liquid N₂, and 5-μm-thick cryostat sections were cut and fixed in acetone. Sections were stained with rat antibodies against CD4 or CD8, followed by alkaline phosphatase–labeled goat anti-rat Ig (TAGO) and then alkaline phosphatase–labeled donkey anti–goat Ig (Jackson Immunoresearch Laboratories). Alkaline phosphatase was detected with naphthol A-BI phosphate and New Fuchsin (Fluka), resulting in a red color reaction. Sections were counterstained with Mayer’s hemalum.

The above in vitro stimulation were performed (39). Effector cells (cultures) or rmIL-2 plus rmIL-4 (Th2 cultures). To obtain irreversibly polarized Th1 effectors secreted high amounts of IFN-γ and no IL-4, whereas Th2 effectors produced high levels of IL-4 and no IFN-γ (Fig. 1 A). Intracellular cytokine staining showed that some heterogeneity existed within these effector populations in that Th1 effectors contained cells producing TNF-α and/or IFN-γ, whereas Th2 effectors contained cells producing IL-4 and/or IL-10 (Fig. 1 B). Th1 and Th2 effector cells mounted similar proliferative responses when cultured with p8 in vitro (Fig. 1 C).

Both Th1 and Th2 C cells Provide Help for VSV-neutralizing Protective IgG Responses. Although T cell-deficient TCR-β⁻/⁻/⁻ mice mount normal T cell-independent neutralizing IgM responses to VSV, they do not produce CD4⁺ T cell-dependent neutralizing IgG antibodies (5, 41). Indeed, despite producing neutralizing IgM antibodies, VSV-infected TCR-β⁻/⁻/⁻ mice succumbed to infection on around day 14 after infection (Fig. 2 A), confirming previous findings with nude mice (22). In contrast, TCR-β⁻/⁻/⁻ mice adoptively transfused with 10⁶ VSV-specific Th1 or Th2 CD4⁺ T cells produced high titers of VSV-neutralizing IgG and were completely protected against disease (Fig. 2).

To evaluate the sensitivity of Th1 and Th2 cells to facilitate neutralizing antibody responses, graded numbers of VSV-specific Th1 or Th2 cells were adoptively transferred into naive C57BL/6 mice and the recipients were subsequently challenged with UV-inactivated VSV (UV-VSV). Noreplicating UV-VSV efficiently triggers T cell-independent IgM responses, but does not induce isotype switching to IgG unless VSV-specific Th cells are present (23, 28). C57BL/6 mice adoptively transferred with Th1 or Th2 CD4⁺ T cells produced high titers of VSV-neutralizing IgG after challenge with UV-VSV, and in both cases at least 10⁶ effector CD4⁺ T cells were required to support isotype switching (Fig. 3 A). Th1 cells induced the production of IgG2a and IgG2b, whereas Th2 cells promoted IgG1 and IgG2b secretion (Fig. 3 B).

Characterization of Polarized Transgenic Th1 and Th2 Cells. Transgenic (tg7) CD4⁺ T cells expressing a TCR that recognizes a peptide (p8) derived from the VSV-G in association with I-Aβ (23) were polarized by three rounds of in vitro stimulation with p8-bearing APCs (37). Polarized Th1 effectors secreted high amounts of IFN-γ and no IL-4, whereas Th2 effectors produced high levels of IL-4 and no IFN-γ (Fig. 1 A). Intracellular cytokine staining showed that some heterogeneity existed within these effector populations in that Th1 effectors contained cells producing TNF-α and/or IFN-γ, whereas Th2 effectors contained cells producing IL-4 and/or IL-10 (Fig. 1 B). Th1 and Th2 effector cells mounted similar proliferative responses when cultured with p8 in vitro (Fig. 1 C).

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Th1 CD4⁺ T Cells, but Not Th2 CD4⁺ T Cells, Can Adoptively Transfer Cell-mediated Protection against Recombinant Vaccinia Virus. Previous work has shown that C57BL/6 mice primed with VSV-IND were resistant against challenge with a recombinant vaccinia virus expressing the VSV-IND glycoprotein (Vacc-IND-G) and that this protection was mediated by CD4⁺ T cells (42). Therefore, we examined whether the antivaccinia protection conferred by VSV-G-specific CD4⁺ T cells was dependent on their effector phenotype. T cell-deficient TCR-β⁻/⁻ δ⁻/⁻ mice were unable to control infection with Vacc-IND-G, developing a progressive wasting disease that culminated in death ~3–4 wk after infection (Fig. 4 A). Adoptive transfer of 10⁷ Th1 tg7 CD4⁺ T cells completely protected the TCR-β⁻/⁻ δ⁻/⁻ mice, whereas transfer of 10⁷ Th2 tg7 CD4⁺ T cells failed to protect TCR-β⁻/⁻ δ⁻/⁻ mice against Vacc-IND-G (Fig. 4 A). Death correlated with the presence of vaccinia virus in peripheral organs in mice that had received Th2 tg7 CD4⁺ T cells, whereas those re-
receiving Th1 tg7 CD4+ T cells remained healthy and had no
detectable vaccinia virus (Fig. 4 B). The inability of Th2
cells to protect against lethal vaccinia virus infection was not
due to inefficient inactivation of these cells, since the TCR-β−δ− mice that received Th2 cells mounted strong
VSV-G–specific neutralizing antibody responses (Fig. 4 C).
However, as vaccinia virus may spread from cell to cell by
using actin filaments to form enveloped particles which are
resistant to antibody (43, 44) and because the VSV-G is not
expressed on the surface of the Vacc-IND-G (29), neutral-
izing antibodies are unable to clear the infection.

Protection against Vaccinia Correlates with Enhanced Migrat-
ion of Th1 CD4+ T Cells to Peripheral Tissues. In a second
approach, we examined the ability of Th1 and Th2 cells to
rapidly eradicate Vacc-IND-G from peripheral solid or-
gans. Thus, normal C57BL/6 mice were adoptively trans-
fused with VSV-G–specific CD4+ T cells and challenged
with Vacc-IND-G. In naive female C57BL/6 mice, the
Vacc-IND-G grew well in ovaries, with titers detectable
on day 2 and peaking around day 5 (Fig. 5 A). Adoptive
transfer of 107 naive tg7 CD4+ or Th2 tg7 CD4+ T cells
had no influence on the vaccinia virus titers in ovaries,
whereas mice receiving 106 Th1 tg7 CD4+ T cells rap-
idly eradicated the virus (Fig. 5 A). Histological analysis of
ovaries showed that the transferred Th1 cells were present
in the ovaries on day 2 after infection (Fig. 5, B and D). By day 5 after
infection, extensive inflammatory infiltrates were present in

Figure 4. Th1, but not Th2, cells can protect T cell–deficient
mice from vaccinia virus infection. Groups of 6 TCR−β−δ−
/mice were adoptively trans-
fused with 107 Th1 or Th2 tg7
CD4+ T cells, and 24 h later re-
cipients were infected with 107
PFU of Vacc-IND-G intraperi-
toneally. (A) Mice were moni-
tored daily for signs of disease
and were killed when seriously
ill. (B) Vacc-IND-G titers in or-
gans from sick (Control or Th2
recipients) or healthy (Th1 re-
cipients) mice were determined
by plaqueing. Representative re-
sults from three mice per group
are shown, and symbols repre-
sent individual mice. (C) Sera
from the mice shown in A were
analyzed for the presence of
VSV-IND–neutralizing IgM
(day 4 after infection, white bars)
and IgG (day 20 after infection,
striped bars). Mean titers from
groups of five to six mice are
shown, and intragroup variations
were ±2 titer steps. One of two
similar experiments is shown.

Figure 5. Th1, but not Th2, cells can protect against peripheral vaccinia virus infection, and this correlates with enhanced migration of Th1 cells to
peripheral organs. (A) Graded numbers of naive or Th1 or Th2 tg7 CD4+ T cells were adoptively transferred intravenously into syngeneic naive C57BL/6
female mice. 1 d later, recipients were challenged with 5 × 106 PFU Vacc-IND-G intraperitoneally. After 5 d, ovaries were removed and Vacc-IND-G
titers were determined. Symbols represent individual mice, and one representative experiment of three is shown. (B–J) Immunohistological analysis of
T cell infiltration into the ovary. C57BL/6 mice that had received 107 naive (B, E, and H), Th1 (C, F, and I), or Th2 (D, G, and H) tg7 CD4+ T cells
were killed 2 d (B–D) or 5 d (E–J) after Vacc-IND-G challenge, and frozen sections of ovaries were analyzed for infiltration by CD4+ (B–G) or CD8+
(H–J) T cells using immunohistochemistry. Positively stained cells exhibit a bright red color. Original magnifications ×125.
ovaries of mice receiving naïve or Th2 tg7 CD4+ T cells, comprising both CD4 and CD8 T cells (Fig. 5, E–J). In contrast, much smaller infiltrates were present in ovaries of mice that received Th1 tg7 CD4+ T cells (Fig. 5, F and I). Thus, earlier migration of Th1 cells into peripheral organs correlated with a rapid elimination of vaccinia virus and a decreased late inflammatory reaction.

Induction of Virus-specific DTH Responses Confirms That Th1 Cells, but Not Th2 Cells, Effectively Respond to Peripheral Antigen Challenge. We next examined Th1 and Th2 cells for their ability to mediate virus-specific DTH responses. Thus, normal C57BL/6 mice were adoptively transfused with VSV-G–specific CD4+ T cells and challenged in the hind footpad with recombinant baculovirus-derived VSV-G (Bac-G). As shown in Fig. 6 A, control C57BL/6 mice exhibited a small transient increase in footpad thickness, peaking 24 h after injection and declining 48–72 h after injection. C57BL/6 mice adoptively transfused with Th2 tg7 CD4+ T cells had identical responses to control mice (Fig. 6 A). In contrast, mice given Th1 tg7 CD4+ T cells mounted strong DTH responses after challenge with Bac-G, which peaked 72–96 h after injection and slowly declined thereafter (Fig. 6 A). These responses were antigen specific, since they were not provoked by challenge with baculovirus-derived VSV-NP (Bac-NP) (Fig. 6 A). Histological analysis of footpads showed that the ability of Th1 cells to mediate DTH responses correlated with enhanced migration and accumulation of CD4+ T cells in the footpad, which was evident as early as 48 h after injection (Fig. 6, B–E).

Figure 6. Th1, but not Th2, cells rapidly migrate to inflammatory sites and mediate DTH responses after peripheral antigen challenge. (A) 10⁷ Th1 or Th2 tg7 CD4+ T cells were adoptively transfused intravenously into syngeneic naïve C57BL/6 mice. 1 d later, recipients were challenged in the right hind footpad with 10 μg Bac-VSV-G or Bac-VSV-NP. Footpad swelling was monitored daily using calipers. Symbols represent mean values from groups of four to six mice, and intragroup variations were ±0.4 mm. One representative experiment of three is shown. (B–E) Immunohistological analysis of CD4+ T cell infiltration into the footpad. Two mice from each group described in A were killed 2 d after antigen challenge, and frozen sections of footpads were analyzed for infiltration by CD4+ T cells using immunohistochemistry. Positively stained cells exhibit a bright red color. Original magnifications ×50.

Figure 7. Th1, but not Th2, cells rapidly migrate to inflammatory sites and mediate DTH responses after peripheral challenge with live virus (A) 10⁷ Th1 or Th2 SMARTA CD4+ T cells were adoptively transferred intravenously into syngeneic naïve C57BL/6 mice. 1 d later, recipients were challenged in the right hind footpad with 500 PFU LCMV-WE. Footpad swelling was monitored daily using calipers. Symbols represent mean values from groups of four to six mice, and intragroup variations were ±0.4 mm. One representative experiment of three is shown. (B–D) Immunohistological analysis of CD4+ T cell infiltration into the footpad. Two mice from each group described in A were killed 3 d after LCMV-WE challenge, and frozen sections of footpads were analyzed for infiltration by CD4+ T cells using immunohistochemistry. Positively stained cells exhibit a bright red color. Original magnifications ×50.
To examine whether enhanced responsiveness to peripheral antigen challenge was a general property of Th1 CD4+ T cells, we used SMARTA transgenic CD4+ T cells, which recognize a peptide from LCMV-GP in the context of I-Aβ (24). While control C57BL/6 mice or those that received Th2 SMARTA CD4+ T cells showed no increase in footpad swelling after infection with LCMV-WE, mice that received Th1 SMARTA CD4+ T cells mounted a DTH response 72-96 h after infection (Fig. 7 A). This again correlated with greatly enhanced migration of Th1 CD4+ T cells into the footpad (Fig. 7, B–D).

Th1 Cells Exhibit Enhanced Migratory Capacity toward Chemokines That Are Induced after Virus Infection. Lymphocyte migration involves a complex series of adhesive interactions between T cells and endothelium (45, 46). Both Th1 and Th2 cells had a phenotype characteristic of activated T cells, expressing low levels of CD62L and high levels of CD25, CD69, intracellular adhesion molecule 1 (ICAM-1), and CDS9d (very late antigen 4 [VLA-4]), but there were no readily detectable differences in surface phenotype (Maloy, K., unpublished observations). As evidence is increasing that Th1 and Th2 cells show differential expression of chemokine receptors (47–49), we examined whether chemokines may play a role in the preferential migration of Th1 cells into virus-infected tissues. Using semi-quantitative RT-PCR analysis, we found that Th1 cells expressed higher levels of CCR2, CCR5, and CXCR3 than Th2 cells, whereas Th2 4 was expressed at higher levels by Th2 cells (Fig. 8 A). We then assayed the expression of inflammatory chemokines in the ovaries of normal C57BL/6 mice 48 h after intraperitoneal injection of Vacc-IND-G. As shown in Fig. 8 B, several chemokines were upregulated in the ovaries of vaccinia-infected mice, including MIP-1α, MIP-1β, RANTES, MDC, and SDF-1α. Most striking, however, were our observations that the chemokines JE (MCP-1), Mig, and IP-10, which were undetectable in uninfected ovaries, were strongly induced after vaccinia infection (Fig. 8 B). Using in vitro migration assays, we found that Th1 cells showed strong migratory responses to these newly induced chemokines JE, Mig, and IP-10, as well as to the CCR5-binding chemokine MIP-1β, whereas Th2 cells migrated either poorly (JE) or not at all (Mig, IP-10, and MIP-1β) (Fig. 8 C). These results suggest that virus infection of peripheral tissues leads to the production of inflammatory chemokines that preferentially attract Th1 cells.

Th1 Cells, but Not Th2 Cells, Can Protect against Intranasal Challenge with VSV. Although neutralizing antibodies are sufficient in mediating protection against VSV administered systemically, intranasal infection with VSV facilitates rapid infection of the central nervous system (CNS) via the olfactory nerve, which can be rapidly lethal even in immunocompetent mice (50, 51). Thus, C57BL/6 mice were adoptively transfused with Th1 or Th2 tg7 CD4+ T cells 24 h before intranasal challenge with 2 × 10^6 PFU VSV. As shown in Table I, mice that received Th1 cells were completely protected against intranasal infection with VSV-IND, whereas those that received Th2 cells succumbed to infection with similar kinetics to control C57BL/6 mice. Protection was again antigen specific, since Th1 SMARTA CD4+ T cells were unable to provide protection against intranasal infection with VSV-IND and tg7 CD4+ T cells were unable to protect against VSV-NJ challenge (Table I). Protection by Th1 cells correlated with complete eradication of VSV from the brain, whereas brains from control mice or those that received Th2 cells harbored high titers of VSV (Table I).

Virus Infection Preferentially Induces Differentiation of Th1 Cells In Vivo. To examine whether virus infection preferentially induces the development of Th1 effector cells, naïve tg7 CD4+ T cells were adoptively transferred into TCR-β−/−/− mice that were subsequently challenged with VSV or Vacc-IND-G. In the absence of viral challenge, the adoptively transferred CD4+ T cells remained largely undifferentiated as judged by the low numbers producing IFN-γ after restimulation (Table II). In contrast, in...
Table I. Protection against Lethal Intranasal VSV Infection by Antigen-specific Th1 Cells

| Adoptive transfer | Challenge (2 × 10^6 PFU) | No. of mice surviving | VSV titer in brain |
|-------------------|--------------------------|-----------------------|-------------------|
| N one             | VSV-IND                  | 0/6                   | 6.3 ± 1.3         |
| 10^7 tg7 Th1      | VSV-IND                  | 6/6                   | <1.2              |
| 10^7 tg7 Th2      | VSV-IND                  | 0/6                   | 5.8 ± 1.8         |
| 10^7 SMARTA Th1   | VSV-IND                  | 0/6                   | 5.6 ± 1.5         |
| N one             | VSV-NJ                   | 0/6                   | 4.9 ± 0.8         |
| 10^7 tg7 Th1      | VSV-NJ                   | 0/6                   | 4.5 ± 1.2         |

Naive C57BL/6 mice were adoptively transferred intravenously with transgenic Th1 or Th2 CD4^+ T cells 1 d later, recipients were challenged with 2 × 10^6 PFU VSV intranasally. Mice were monitored daily for hind limb paralysis and killed when seriously ill. Brains were isolated from killed mice or from surviving mice (on day 21 after challenge), and VSV titers were measured by plaqueing on Vero cells. Mean VSV titers are shown. One of two similar experiments is shown.

Infection with VSV-IND, or with Vacc-IND-G, led to the differentiation of high numbers of IFN-γ–secreting CD4^+ T cells, whereas very few IL-4–producing cells could be detected (Table II).

Discussion

Our results show that several factors contribute to whether a given type of CD4^+ effector T cell can efficiently mediate antiviral protection, including viral attributes such as route of infection and protective mechanisms, as well as T cell characteristics such as cytokine production and migratory patterns. Furthermore, our findings clearly demonstrate that Th1 CD4^+ effector T cells possess much greater antiviral protective capacity than their Th2 counterparts. Th1 cells were able to mediate protection against viruses controlled by antibodies (VSV) or cytokines (vaccinia), regardless of the route of virus administration. In contrast, although Th2 cells protected against systemic infection with VSV by inducing neutralizing antibodies, they were unable to eradicate vaccinia virus and were also unable to efficiently respond to peripheral virus challenge in several different infection models.

Th1 and Th2 cells showed similar capabilities in helping B cells produce protective, VSV-neutralizing IgG antibodies, with Th1 cells inducing higher amounts of IgG2a and Th2 cells inducing IgG1 production. Previous studies with VSV have shown that antibody concentration, rather than IgG isotype, is the primary factor in determining protection (52). There were no quantitative differences in the levels of neutralizing antibodies induced by Th1 or Th2 cells, or in the number of effector T cells required to mediate class switching. It is not surprising that Th1 cells are as capable as Th2 cells in this respect, since the antibody response against most viruses is dominated by IgG2a, an isotype that is associated with IFN-γ production and Th1 responses (53, 54).

The successful control of vaccinia infection depends on the production of T cell–derived cytokines, most notably IFN-γ and TNF (2). We found that adoptively transferred Th1, but not Th2, CD4^+ T cell effectors could protect T cell–deficient mice from lethal Vacc-IND-G infection, confirming the important role of Th1-type cytokines in the eradication of vaccinia virus. This difference did not appear to be due to less efficient activation of the adoptively transferred Th2 cells, since the mice that received Th2 cells produced even higher titers of VSV-G-neutralizing IgG antibodies than those receiving Th1 cells. The preferential ability of Th1 cells to mediate antivaccinia protection was confirmed by our experiments examining the clearance of Vacc-IND-G from peripheral tissues of normal mice. We previously found that although naive tg7 CD4^+ T cells were unable to adoptively transfer protection against peripheral Vacc-IND-G challenge, in vitro–primed tg7 CD4^+ T cells quickly eradicated the vaccinia from ovaries (23). Here, we have extended these findings by showing that only Th1 CD4^+ T cells are capable of such antiviral protection and that rapid clearance of vaccinia correlated with enhanced migration of the Th1 cells into ovaries as early as 48 h after infection. In contrast, Th2 CD4^+ effector T cells were unable to clear vaccinia from peripheral tissues and did not efficiently migrate into infected organs. The ability of Th1 cells to efficiently respond to peripheral antigen challenge was confirmed by their induction of footpad DTH responses in response to either nonreplicating virus antigen (tg7 Th1 cells to Bac-VSV-G) or live virus (SMARTA Th1 cells to LCM V). Histological analysis showed that the enhanced responsiveness of Th1 cells again correlated with their rapid migration into peripheral tissues. The observed differences in homing did not appear to be due to preferential survival or expansion of the Th1 cells in vivo, since we observed that similar numbers of Th1 and Th2 cells homed...
to the spleen and peripheral lymph nodes after adoptive transfer (Maloy, K., unpublished observations).

Lymphocyte migration into peripheral tissues is a multi-step process, involving adhesion and extravasation via selectins and integrins and chemotactic attraction by chemokines (45, 46). The recirculation patterns of T cells are dependent on their differentiation state; naïve T cells are restricted to the lymphoid tissues, whereas primed T cells may patrol through peripheral tissues (55, 56). Selective recruitment of Th1 cells to sites of peripheral inflammation has previously been observed using protein antigens and involves interactions with P- and E-selectin molecules expressed on vascular endothelium (57). Furthermore, evidence is growing that effector CD8+ T cell subsets differ in terms of chemokine receptor expression, which may also influence their migratory patterns (47–49). We found similar differences in chemokine receptor expression, with Th1 effectors expressing higher levels of CCR2, CCR5, and CXCR3, whereas Th2 cells had higher levels of CCR4. Importantly, we found that several inflammatory chemokines were upregulated in virus-infected tissues, including MIP-1α, MIP-1β, and RANTES (all of which bind CCR5) as well as MDC (a CCR4 ligand). Most significantly, we observed that the chemokines JE (MCP-1), MIP-1α, and IP-10 were newly induced after vaccinia virus infection and these chemokines all bind to receptors which were expressed at higher levels by Th1 cells (CCR2 and CXCR3). An almost identical pattern of chemokine upregulation and induction was observed after LCMV infection of the footpad (Junt, T.M., unpublished observations) or brain (58). In vitro migration assays showed that Th1 cells migrated well in response to these newly induced chemokines, as well as to the upregulated CCR5 ligand MIP-1β, whereas Th2 cells did not. Taken together, our results suggest that virus infection induces the production of chemokines that preferentially attract Th1 cells. Furthermore, they demonstrate positive correlations among protection against peripheral virus infections, migration into virus-infected tissues in vivo, and migration in response to chemokines that are induced after virus infection, all properties exhibited by Th1 effectors but not by Th2 cells. Interestingly, the antiviral capacities of effector CD8+ T cells may similarly correlate with their migratory properties. Effector CD8+ T cells can mediate protection against peripheral vaccinia infection, and this is dependent on recent antigenic stimulation, which enhances extravasation of effector T cells (59, 60). In addition, recent studies with hemagglutinin-specific CD8+ effector T cells found that Tc1 effectors (analogous to Th1 cells) were much more effective than Tc2 effectors (analogous to Th2 cells) in protecting against pulmonary infection with influenza virus, despite the fact that both populations were equally cytolytic (61). The greater protective capacity of Tc1 effectors correlated with more rapid migration of these cells into the airway epithelium and with differences in chemokine receptor expression between the Tc1 and Tc2 cells (61). Together with our findings, these results demonstrate that the antiviral protective capabilities of both CD4+ T cells and CD8+ T cells are governed not only by the effector molecules they produce, but also by their migratory characteristics.

Intranasal inoculation of VSV leads to infection of local olfactory receptor neurons, which is quickly followed by acute infection of the CNS (50). Recovery from CNS infection is T cell dependent and is associated with increased T cell infiltration of the CNS and with enhanced production of cytokines that are characteristically present during Th1 responses (51, 62). We observed that Th1 cells, but not Th2 cells, were able to protect mice against the lethal consequences of intranasal challenge with VSV. This again is likely to have been due to enhanced migration of the Th1 cells and/or increased production of cytokines such as IFN-γ, which may play a crucial role during viral infection of the CNS (63).

The primary role of Th1 responses in antiviral protection was confirmed by our finding that virus infection predominantly promoted differentiation of Th1 effectors from naïve CD4+ T cells in vivo. Our results are consistent with other reports showing that CD4+ T cells with a Th1 cytokine phenotype contribute to protection against a variety of viral infections, including influenza virus (10, 11), poliovirus (14), murine cytomegalovirus (64), herpes simplex virus (65), and measles virus (66). Viral infection is known to stimulate the production of IL-12, which has beneficial effects on many viral infections and is a potent inducer of Th1 responses (62, 67–69), although it is worth noting that viruses can induce Th1 responses in the absence of IL-12 (70). Thus, adjuvants that promote Th1 responses may be the best for vaccinating against cytopathic viruses, which are controlled by neutralizing antibodies and T cell cytokines (1). However, in the case of noncytopathic viruses, CD8+ CTLs play a central role in protection (71); thus, caution must be used, since pathological consequences of excessive virus-specific CD4+ T cell activation have been observed (72).

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