**CD8⁺ T Cells Mediate CD40-independent Maturation of Dendritic Cells In Vivo**

By Christiane Ruedl, Manfred Kopf, and Martin F. Bachmann

From the Basel Institute for Immunology, CH-4005 Basel, Switzerland

**Summary**

Induction of cytotoxic T lymphocyte (CTL) responses against minor histocompatibility antigens is dependent upon the presence of Th cell help and requires the interaction of CD40 on dendritic cells (DCs) with CD40 ligand on activated T helper cells (Th). This study demonstrates that CD40 is neither involved in Th-dependent nor Th-independent antiviral CTL responses. Moreover, the data show that DC maturation occurs in vivo after viral infection in the absence of CD40 and Th. This maturation did not require viral infection of DCs but was mediated by peptide-specific CD8⁺ T cells. Surprisingly, naive CD8⁺ T cells were able to trigger DC maturation within 24 h after activation in vivo and in vitro. Moreover, peptide-activated CD8⁺ T cells were able to induce maturation in trans, as DCs that failed to present the relevant antigen in vivo also underwent maturation. Upon isolation, the in vivo–stimulated DCs were able to convert a classically Th-dependent CTL response (anti-HY) into a Th-independent response in vitro. Thus, antiviral CD8⁺ T cells are sufficient for the maturation of DCs in the absence of CD40.

Key words: virus • infection • cytotoxic T lymphocyte • dendritic cell

Induction of CTL responses after immunization with minor histocompatibility antigens, such as the HY antigen, requires the presence of Th (1, 2). CTL responses induced by cross-priming, i.e., by priming with exogenous antigens that reached the class I pathway, have also been shown to require the presence of Th (3). These observations have important consequences for tumor therapy, where Th may be critical for the induction of protective CTL responses by tumor cells (4). An important effector molecule on activated Th is the CD40 ligand (L) interacting with CD40 on B cells, macrophages, and dendritic cells (DCs) (5). Triggering of CD40 on B cells is essential for isotype switching and the generation of B cell memory (5). More recently, it was shown that stimulation of CD40 on macrophages and DCs leads to their activation and maturation (6, 7). Specifically, DCs upregulate costimulatory molecules and produce cytokines, such as IL-12, upon activation. Interestingly, this CD40L-mediated maturation of DCs seems to be responsible for the helper effect on CTL responses. In fact, it has recently been shown that CD40 triggering by Th renders DCs able to initiate a CTL response (8–10). This is consistent with the earlier observation that Th have to recognize their ligands on the same APC as the CTLs, indicating that a cognate interaction is required (3). Thus, CD40L-mediated stimulation by Th leads to the activation of DCs, which subsequently are able to prime CTL responses.

In contrast to these Th-dependent CTL responses, viruses are often able to induce protective CTL responses in the absence of Th (for review, see reference 11). Specifically, lymphocytic choriomeningitis virus (LCMV) (12–16), vesicular stomatitis virus (VSV; reference 17), influenza virus (18), vaccinia virus (19), and ectromelia virus (20) were able to prime CTL responses in mice depleted of CD4⁺ T cells or deficient for the expression of class II or CD40. The mechanism for this Th-independent CTL priming by viruses is not presently understood. Moreover, most viruses do not completely stimulate Th-independent CTL responses, but virus-specific CTL activity is reduced in Th-deficient mice. Thus, Th may enhance antiviral CTL responses, but the mechanism of this help is not yet fully understood. DCs have recently been shown to present influenza-derived antigens by cross-priming (21). It is therefore possible that, similar to the mechanism shown for minor histocompatibility antigens and tumor antigens (8–10), Th may assist induction of CTLs via CD40 triggering on DCs.

This study analyzes the mechanism of Th-independent versus Th-dependent CTL priming triggered by viruses. We found that CD40 is not measurably involved in the induction of CTLs specific for LCMV or VSV. Thus, although VSV-specific CTL responses are partly dependent upon the presence of CD4⁺ T cells (17), this helper effect was not mediated by CD40L. However, the virus-specific
Materials and Methods

Mice and Viruses. Mice deficient for the expression of CD40 (22), MHC class II (23), β2-microglobulin (24), and RAG-2 (25) have been described previously. Mice expressing a TCR receptor specific for the HY antigen have been immunized intravenously with LCMV (200 PFU) or VSV (2 × 10^6 PFU). 6 or 5 d later, respectively, spleens of uninfected and virus-infected mice were dissected, cut into pieces, and digested twice with collagenase D (Boehringer Mannheim) for 30 min at 37°C in a shaking water bath. Cells were recovered by centrifugation and resuspended in an Optiprep™ (Nycomed Pharma) gradient as previously described (30). Low density cells were then incubated for 30 min on ice with FITC-labeled anti-CD11c (1:400; Pharmingen) and with PE-labeled anti-B7-1, anti-B7-2, anti-CD40, and isotype control, in the presence of 5% normal mouse serum. Cells were washed and analyzed by a FACSCalibur™ flow cytometer (Becton Dickinson), excluding propidium iodide–positive cells. Percent upregulation of median fluorescence intensity (MFI) was calculated as follows: % upregulation = [(MFI induced − MFI control)/MFI control] × 100.

In Vivo Induced CD M aturation by Peptide. Transgenic mice expressing a TCR specific for LCMV were intravenously injected with 10 μg LCMV-derived peptide p33 (KAVYNFATM), A4Y (KAVYNFATM), V4Y (KAVYNFATM), or S4Y (KAVSNFATM). After 24 h, DCs were isolated from spleens of treated and, as control, untreated mice and monitored for expression of CD40, B7-1, B7-2, and CD40 as described above. Activation of T cells was assessed by measuring CD69 (PE) expression on CD8+ T cells (FITC).

For the T cell stimulation assay, DCs were sorted using a FACSStar™, obtaining a purity >97%. Different numbers of sorted DCs (H-2d) were added to 2 × 10^4 purified T cells obtained from spleens of H-2d BALB/c (MLR) or female HY-transgenic mice (H-2d on a RAG-2 background) cultured in 96-well plates (Falcon; Becton Dickinson) for 3 d. T cell proliferation was assessed by [3H]thymidine (1 μCi/well) uptake in a 16-h pulse after 72 h.

In Vivo M aturation of CD. CD8+ T cells were positively selected from mesenteric LNs of a RAG-2-/- LCMV-specific, TCR-transgenic mouse by anti-CD8-coated magnetic beads (GmbH; Miltenyi Biotec). DCs (10^5 cells/well) obtained from control B6, CD40-/-, and MHC class II-/- mice were used for 1 h with 10 μg/ml peptide p33, A4Y, V4Y, or S4Y, washed, and cocultured with 3 × 10^5 naive CD8+ T cells at 37°C. After 20 h, DCs were double-stained with FITC-labeled anti-CD11c and PE-labeled anti-B7-1, anti-B7-2, and anti-CD40 and analyzed by a FACSCalibur™ flow cytometer, excluding propidium iodide–positive cells.

Generation of Chimeric Mice. RAG-2-/- mice were irradiated (3 Gy) and reconstituted with 5 × 10^6 bone marrow cells from CD45.1 congenic C57BL/6 mice and 5 × 10^6 bone marrow cells derived from CD45.2 TCR-transgenic B6.C–H-2^m/mice. 10 wk later, recipient mice exhibited large numbers of TCR-transgenic T cells and a mixed DC population of both C57BL/6 and CD45.2 alleles. DCs were isolated 24 h after injection of peptide or saline as described above, triple-stained with APC-labeled anti-CD11c, FITC-labeled anti-CD45.2, and PE-labeled B7-2, anti-CD40, and negative control, and analyzed by using a FACSCalibur™ flow cytometer.

Results

CD40 Is Not Involved In Virus-specific T H-dependent and -independent CTL Responses. To compare the role of CD4+ T cells versus the presence of CD40 for the induction of LCMV-specific CTL responses, control, CD40-/-, and MHC class II-/- mice that lacked Th were immunized
with LCMV. At the peak of the response, i.e., 8 d after infection, spleen cells were harvested and tested in a $^{51}$Cr-release assay. To be able to quantify the relative numbers of CTL effector cells in the different mice, lytic units were calculated (Fig. 1). Absence of MHC class II or CD40 did not affect the frequency of CTL precursor after infection with LCMV.

The Th dependence of the VSV-specific CTL response was analyzed next. Control, CD40$^{-/-}$, or MHC class II$^{-/-}$ mice were injected intravenously with VSV, and the presence of CTLs was assessed 6 d later in a $^{51}$Cr-release assay. In contrast to LCMV-specific CTL responses, VSV-specific CTL responses were reduced in the absence of Th in the different mice, 75–80% (Fig. 1). Similar results were obtained in mice depleted of CD4$^+$ Th (data not shown). However, CTL responses were completely normal in CD4$^{-/-}$ mice. Thus surprisingly, CD40 did not mediate the Th-dependent component of the VSV-specific CTL response (Fig. 1).

CD40 and MHC class II have been implicated in thymic selection (23, 31). We therefore wanted to exclude the possibility that alterations in the CTL populations in CD40$^{-/-}$ or MHC class II$^{-/-}$ mice were responsible for the Th independence of the CTL response. Thus, T cells from transgenic mice expressing a TCR specific for LCMV (26) were adoptively transferred into the different mice before viral infection. It has been shown previously that this leads to a dramatic expansion of transgenic T cells (32). 8 d after infection, the peak of the antiviral response, presence of T cells expressing the transgene-encoded TCR (V$\alpha$2) was assessed in the spleens of the mice. The absolute number and the frequency of transgenic T cells was comparable in control, CD40$^{-/-}$, and MHC class II$^{-/-}$ mice (Fig. 2, A and B). Moreover, a comparable lytic activity of the transgenic cells was observed in a $^{51}$Cr-release assay on peptide p33-pulsed target cells (Fig. 2). Similar results were obtained with peptide MB6, which is selectively recognized by the transgene-encoded TCR (data not shown). These results demonstrate that a defined population of CTLs expressing a single TCR can be stimulated by LCMV in the absence of both CD40 molecules and CD4$^+$ Th.

LCMV and VSV Induce Maturation of DCs In Vivo in the Absence of Th and CD40. DCs have been shown to be activated in vitro upon stimulation with various inflammatory cytokines and, in particular, after stimulation with CD40L (33, 34). This activation step leads to a maturation of DCs that is thought to be essential for the generation of immune responses (35). The upregulation of costimulatory molecules on DCs is a hallmark of this maturation step. To analyze whether viral infection may induce maturation of DCs in vivo, mice were infected with LCMV, and DCs were isolated 6 d later from the spleen and directly analyzed by flow cytometry (Fig. 3). LCMV infection induced the upregulation of B7-1, B7-2, and CD40 on splenic DCs. Surprisingly, LCMV infection induced generalized activation of DCs, as the great majority displayed upregulated costimulatory molecules. To analyze the role of Th and CD40 in the activation of DCs, mice deficient for the expression of MHC class II (and therefore lacking Th) and CD40$^{-/-}$ mice were infected with LCMV, and DCs were

![Figure 1](image1.png)

Figure 1. CD40 does not mediate Th-dependent antiviral CTL responses. CD40$^{-/-}$, MHC class II$^{-/-}$, and respective control mice were injected intravenously with LCMV (200 PFU) or VSV (2 $\times$ 10$^6$ PFU), and spleen cells were isolated 8 or 6 d later, respectively. Spleen cell suspensions were prepared and tested directly in a $^{51}$Cr-release assay on peptide-pulsed target cells. As a quantitative measure, lytic units were calculated per spleen. Lytic units in control mice were: LCMV, 1,020 in C57BL/6 and 910 in CD40$^{-/-}$; VSV, 930 in C57BL/6 and 210 in CD40$^{-/-}$. Three mice were used per group. One representative experiment of three is shown.

![Figure 2](image2.png)

Figure 2. Normal activation and expansion of LCMV-specific TCR-transgenic T cells in the absence of CD40 or MHC class II. 10$^6$ transgenic T cells expressing a TCR specific for LCMV-derived peptide p33 were transferred into normal, nonirradiated C57BL/6 mice, which were infected intravenously with 200 PFU LCMV 2 h later. Presence of TCR-transgenic T cells in the spleen were calculated for the different mice (B). CTL activity was assessed in a $^{51}$Cr-release assay on p33-pulsed EL-4 target cells (C). Frequencies of CD8$^+$V$\alpha$2$^+$ T cells were <2% in the absence of infection. Three mice were used per group. One representative experiment of three is shown. ■, control; ○, CD40$^{-/-}$; ▲, class II$^{-/-}$. 

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Figure 3.  LCMV–specific CD8+ and CD4+ T cells can induce maturation of DCs in vivo. C57BL/6 control mice (WT) or CD40−/−, MHC class II−/−, and β2M−/− were infected with LCMV (200 PFU, i.v.). DCs were isolated from spleens 6 d later, and the expression of B7-1, B7-2, and CD40 was analyzed by flow cytometry. Results are shown for CD11c+ DCs. Mean expression values are indicated in the histograms. One representative experiment of three is shown.

| Table I.  LCMV Triggers Stronger Upregulation of Costimulatory Molecules on DCs than VSV |
|---------------------------------------------------------------|
| Percent upregulation* | LCMV 1 | VSV 1 |
|-----------------------|--------|-------|
| B7-1                  | 310    | 28    |
| B7-2                  | 395    | 47    |
| CD40                  | 121    | 58    |

*Percent upregulation of MFI was calculated as follows: % upregulation = (MFI induced − MFI control)/MFI control × 100.

1 Mice were infected with LCMV (200 PFU), and CD11c+ DCs were analyzed 6 d later by flow cytometry. As controls, saline-injected transgenic mice were used.

Table 1.  LCMV Triggers Stronger Upregulation of Costimulatory Molecules on DCs than VSV.
peptide p33 exhibited an activated and matured phenotype after injection of specific peptide (Table II). In contrast, DCs derived from TCR-transgenic H-2bm13 mice injected with peptide p33 did not exhibit activated DCs, confirming that peptide p33 was not presented by H-2bm13 (data not shown).

CD8^+ T Cells Can Trigger Maturation of DCs In Vitro. To analyze CD8^+ T cell–induced maturation of DCs in a two-cell coculture system, purified CD8^+ T cells derived from TCR-transgenic mice on a RAG-2^−/− background were mixed with freshly isolated, peptide-pulsed splenic DCs and incubated at 37°C for 20 h. Analysis of the expression of B7-1, B7-2, and CD40 demonstrated efficient maturation of DCs triggered by peptide-specific CD8^+ T cells (Fig. 5). Moreover, as seen in vivo, weak, altered peptide ligands were inefficient at inducing the maturation program in DCs. As expected, MHC class II^−/− and CD40^−/− DCs matured comparably to control DCs, indicating that the CD8^+ T cell–mediated maturation of DCs occurred in the absence of CD40 and Th.

Activated DCs Exhibit an Enhanced Immunogenicity and Stimulate HY-specific CTLs in the Absence of Th. We next analyzed the immunogenicity of DCs after activation by peptide p33 exhibited an activated and matured phenotype after injection of specific peptide (Table II). In contrast, DCs derived from TCR-transgenic H-2bm13 mice injected with peptide p33 did not exhibit activated DCs, confirming that peptide p33 was not presented by H-2bm13 (data not shown).

**Table II.** CD8^+ T Cells Can Activate DCs in Trans

| Expression | CD11c^+ CD45.1 (H-2^b) | CD11c^+ CD45.2 (H-2bm13) |
|------------|------------------------|--------------------------|
| +p33       | 95 140 114 30          | 31 17 36 19              |
| −p33       | 31 4 3 4               |                          |

Chimeric mice were injected with peptide p33, and expression of B7-2 and CD40 was analyzed for CD45.1^+ (H-2^b) and CD45.2^+ (H-2bm13) DCs.

**Figure 4.** Peptide-specific CD8^+ T cells can induce maturation of DCs in the absence of viral infection. Transgenic mice expressing a TCR specific for LCMV-derived peptide p33 were injected with 10 μg of peptide p33, the altered peptide ligands A4Y, V4Y, or S4Y, or saline. (A) Expression of B7-1, B7-2, and CD40 was analyzed 24 h later by flow cytometry on CD11c^+ splenic DCs. (B) Expression of CD69 was assessed on CD8^+ T cells. One representative experiment of two is shown.

**Figure 5.** CD8^+ T cells mediated maturation of DCs in vitro. Freshly isolated splenic DCs derived from control (open bars), CD40^−/− (hatched bars), or MHC class II^−/− (closed bars) were pulsed with peptide p33, A4Y, V4Y, or S4Y (all at 10 μg/ml) for 1 h at 37°C. DCs were washed and incubated with purified CD8^+ T cells derived from RAG-2^−/−, TCR-transgenic mice. Expression of B7-1 (a), B7-2 (b), and CD40 (c) was analyzed 20 h later. One representative experiment of four is shown.
trated numbers of DCs were used to stimulate purified allo-specific T cells (H-2^d) derived from BALB/c mice, and proliferation was assessed 3 d later by means of [3H]thymidine incorporation. DCs derived from p33-immunized mice were able to induce efficient proliferation of allo-specific T cells, whereas DCs derived from control animals were barely able to stimulate T cells (Fig. 6 A). Alternatively, DCs were used to stimulate T cells derived from transgenic mice expressing a TCR specific for HY in association with class I (27). To avoid the contribution of T cells with endogenous TCR α chains, T cells derived from HY-TCR-transgenic mice on a RAG-2^−/− background were used. As expected for a Th-dependent CTL response, control DCs stimulated only low-level proliferation of specific T cells. In contrast, CTL-activated DCs derived from p33-primed animals triggered much stronger proliferation of specific T cells (Fig. 6 B). Thus, CTL-mediated activation of DCs rendered Th-dependent CD8^+ T cell responses largely Th independent.

Discussion

This study demonstrates that maturation of DCs occurs in vivo after viral infection in the absence of Th and CD40. Surprisingly, this Th-independent maturation was triggered by virus-specific CD8^+ T cells and not by the viral infection per se. Moreover, the matured DCs were able to induce a classically Th-dependent CTL response in the absence of Th, indicating that antiviral CD8^+ T cells can replace Th in vivo. These results help explain why viruses are able to trigger Th-independent CTL responses, and highlight important differences between virus- and tumor-specific CTL responses.

Viruses Trigger Th-independent CTL Responses. It has been known for some time that viruses are able to stimulate protective CTL responses in the absence of CD4^+ T cells (12–20). However, CTL responses are impaired in the absence of Th after infection with poorly replicating viruses such as VSV (17). To date, it has not been analyzed whether CD4^+ T cells assist CTLs via a CD40L-dependent mechanism under these circumstances. This study demonstrates that this is not the case. Thus, even the Th-dependent part of a virus-specific CTL response is not dependent upon the CD40–CD40L interaction. Compared with tumor-specific CTL responses, where Th assist CTL induction by activating DCs via CD40 triggering, the Th-dependent production of cytokines, such as IL-2, seems to be more important during viral infections. This interpretation is consistent with the previous observation that cytokine secretion by Th after viral infection is not impaired in the absence of CD40 (39).

DC Maturation Triggered by CD8^+ T Cells. A large proportion of DCs isolated from virally infected mice exhibited a mature phenotype. This maturation of DCs occurred in the absence of Th and CD40. Various stimuli are known to activate DCs. Specifically, microbial components, such as LPS, bacterial DNA, or cell walls; inflammatory cytokines; and CD40 triggering are able to stimulate expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7). Moreover, it has been shown that LPS is able to activate splenic DCs in vivo and trigger the migration of DCs from the red pulp to T cell areas (40). Interestingly, infection of splenic APCs by influenza virus directly induces the expression of costimulatory molecules on DCs and induce their migration to central lymphoid organs (6, 7).

Figure 6. CD8^+ T cell-mediated activation of DCs renders Th-dependent CD8^+ T cell responses Th independent. Male transgenic mice (H-2^d) expressing a TCR specific for LCMV-derived peptide p33 were injected with 1 μg of peptide p33 (or saline), and DCs were isolated 24 h later by FACS® sorting. Titrated numbers of DCs isolated from p33-primed animals (□) or control animals (■) were used to stimulate purified allo-specific T cells from BALB/c mice (A) or HY-specific, TCR-transgenic T cells (B). To avoid contribution of endogenous TCR chains, HY-TCR-transgenic mice on a RAG-2^−/− background were used for the experiments. Proliferation was assessed after 72 h. One representative experiment of two is shown.
mice. These results demonstrated that DC maturation could be mediated by either CD4+ or CD8+ T cells. DCs isolated from transgenic mice expressing a class I-restricted TCR 24 h after injection of specific peptide also exhibited an activated phenotype. This demonstrated that DC maturation triggered by CD8+ T cells could occur in complete absence of viral infection. Interestingly, the maturation stimulus delivered by CD8+ T cells was not dependent upon a direct interaction of CD8+ T cells with DCs. In contrast, CD8+ T cells could activate DCs in trans, and DCs that failed to present the relevant peptide due to a mutation in the class I molecule also underwent maturation in TCR-transgenic mice injected with peptide. Thus, freshly activated CD8+ T cells are able to induce generalized activation of DCs, even in the absence of direct T cell–DC contact.

There are parallels between the activation of DCs by CD8+ T cells described here and the IFN system. Type I IFNs induce an antiviral state in many different cell types. This antiviral state is induced in trans, i.e., virally infected cells are able to stimulate neighboring cells via secretion of type I IFNs. Similarly, CD8+ T cells induce an immunostimulatory state, not only in virally infected cells that present the relevant peptides but also in neighboring APCs. However, the two systems are apparently not identical, because (a) type I IFNs induce an antiviral rather than an immunostimulatory state in DCs and (b) DC activation by CD8+ T cells occurs in the absence of functional type IFN receptors (data not shown).

Using the in vitro system of CD8+ T cell–mediated DC activation and T cells or DCs derived from various gene-deficient mice together with neutralizing antibodies and other blocking molecules, we tried to define the factor responsible for the observed maturation of DCs. So far, we can exclude maturation mediated by CD28, CD40L, TRANCE, TNF, IL-1, IL-4, IL-6, IL-17, IFN-α/β, and IFN-γ, but have not yet identified the critical molecule. Future studies will therefore address the cloning of the as-yet elusive factor.

Th-independent CTL responses: a Threshold Phenomenon? It has been observed that viruses are able to generate CTL responses in the absence of Th (12–20). Interestingly, those viruses that induced strong CTL responses in the presence of Th also did so in the absence of Th, suggesting that the Th dependence of the response may simply be determined by the overall strength of the response. Along a similar line, recombinant viral proteins injected in association with insect cell debris as an adjuvant were able to induce very strong CTL responses by cross-priming (42), and this cross-priming occurred in the absence of Th (42). Thus, the failure of model antigens to induce CTL responses in the absence of Th is not absolute but rather seems to be determined by the strength of the response. The results presented here may provide an explanation for these findings by suggesting that generation of CTL responses may occur as a threshold phenomenon due to a positive feedback mechanism. Accordingly, virus-infected DCs are able to activate a few naive CTLs. These activated T cells in turn secrete inflammatory cytokines/chemokines that may lead to the activation of neighboring APCs. The number of DCs that can be activated in such a way determines the efficiency of DC activation. Thus, if only few CD8+ T cells are activated initially, the response may be abortive, as too few DCs undergo maturation. In contrast, if a sufficient number of CTLs is triggered, widespread activation of DCs may occur, and an immunostimulatory program is initiated in lymphoid organs that renders the response independent of Th. Thus, during tumor-specific responses or upon cross-priming, few CD8+ T cells are activated, and the response remains abortive in the absence of Th, whereas during antiviral immune responses, a sufficient number of CD8+ T cells becomes triggered to render the response Th independent. In this model, VSV may represent an intermediate case. It induces only weak upregulation of costimulatory molecules in vivo. Correspondingly, CTL responses partly depend on the presence of Th.

Interestingly, the requirements for activation of DCs were very stringent. Peptide A44, which is a relatively weak agonist that nevertheless stimulates efficient proliferation of specific T cells in vitro, almost completely failed to trigger the activation program in DCs in vivo. Thus, only T cells interacting with strong agonists are able to stimulate maturation of DCs, offering an explanation for why many model antigens that are often weak agonists compared with viral peptides require Th for induction of CTL responses.

This study demonstrates that CD8+ T cells can mediate activation of DCs in complete absence of viral infection. However, it should be noted that a cross-talk between the innate and specific immune systems during viral infection may also facilitate the induction of Th-independent CTL responses. Thus, although CD8+ T cells can solicit their own help by themselves inducing maturation of DCs, other factors are likely to contribute to the efficiency of antiviral CTL responses (11).

Autoimmunity Triggered by Viruses: Are Activated DCs the Missing Link? Viral infections are thought to be an important cause for the induction or exacerbation of autoimmune diseases (43). It has been argued that cross-reactive T cells are responsible for disease, and it has been shown that viral peptides can stimulate autoreactive T cell clones (44, 45). Moreover, there is good evidence that Herpes Simplex virus causes autoimmune stromal keratitis by activating self-specific, cross-reactive T cells (46). However, cross-reactive T cells are not always the critical factor for disease. In the case of Coxsackie virus-induced diabetes, it has been shown that the disease is mediated by T cells recognizing self-antigens that do not cross-react with viral proteins. These self-antigens were apparently released from lysed virus-infected cells and subsequently activated the self-specific T cells (47). However, presence of these antigens alone may not be sufficient for the induction of self-specific T cells, because low amounts of self-antigens also reach lymphoid organs in the absence of infection due to physiological cell turnover (48). Thus, the presentation of self-antigens in lymphoid organs per se does not seem to be responsible for the induction of autoimmunity, likely.
because immature DCs in lymphoid organs that process those antigens most efficiently are inefficient at stimulating naïve T cells. However, the nonspecific maturation of DCs and the concomitant upregulation of costimulatory molecules triggered by the antiviral immune response may be responsible for the activation of self-specific T cells that usually ignore their antigens in vivo. The generalized activation of DCs upon viral infection may therefore not only boost virus-specific T cell responses and render them independent of Th but may also shift the balance from ignorance to autoimmunity.

We would like to thank Barbara Ecabert for excellent technical assistance and K. Karjalainen, J. Pieters, and M. Cell for critically reading the manuscript.

The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche Ltd., Basel, Switzerland.

Address correspondence to Martin F. Bachmann, Basel Institute for Immunology, Grenzacherstr. 487, CH-4005 Basel, Switzerland. Phone: 41-61-605-1228; Fax: 41-61-605-1364; E-mail: bachmann@bii.ch

Received for publication 11 December 1998 and in revised form 3 March 1999.

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