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

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Summary

Induction of cytotoxic T lymphocyte (CTL) responses against minor histocompatibility antigens is dependent upon the presence of T 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

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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)[1] 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

[1]*Abbreviations used in this paper:* DCs, dendritic cells; L, ligand; LCMV, lymphocytic choriomeningitis virus; MFI, median fluorescence intensity; VSV, vesicular stomatitis virus.

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CTLs were observed to solicit their own help in vivo by themselves triggering a maturation process in DCs. This maturation of DCs occurred in complete absence of viral infection upon injection of specific peptide in a TCR-transgenic mouse model. The degree of Th independence of the CTL responses induced by the two different viruses was paralleled by a correspondingly efficient maturation of DCs. Interestingly, $CD8^+$ T cells not only mediated activation of the DCs they were interacting with but also triggered DC maturation in trans, leading to widespread activation of DCs in lymphoid organs. Thus, in contrast to minor histocompatibility antigens that only induce abortive CTL responses in the absence of Th, viruses nucleate a sufficiently strong CTL response that leads to generalized activation of DCs, rendering the response independent of Th.

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 exhibiting a mutation in their H-$2D^b$ molecule (B6.C–H-2^{bm13}) were provided by Dr. P. Ohashi, Ontario Cancer Institute, Toronto, Canada. Transgenic mice expressing a TCR specific for peptide p33 derived from LCMV presented in association with H-$2D^b$ and transgenic mice expressing a TCR receptor specific for the HY antigen have been described previously (26, 27). Mice were bred under specific pathogen-free conditions according to Swiss federal law. Class $II^{-/-}$ mice were back-crossed on a C57BL/6 background, and C57BL/6 mice were used as controls. $CD40^{+/-}$ mice were used as controls for $CD40^{-/-}$ mice.

The LCMV isolate WE was provided by Dr. P. Ohashi, Toronto, Canada and grown on L cells at a low multiplicity of infection. VSV serotype Indiana was originally provided by R.M. Zinkernagel (Institute of Experimental Immunology, Zürich, Switzerland) and grown on BHK cells at a low multiplicity of infection.

Detection of LCMV- and VSV-specific CTL In Vitro. Mice were immunized intravenously with LCMV (200 PFU) or VSV (2×10^6 PFU), and 8 or 6 d later, respectively, spleen cell suspensions were prepared and tested directly in a ^{51}Cr-release assay, using LCMV-derived peptide p33 (KAVYNFATM)- or VSV-derived peptide (SDLRGYVYQGLKSG)-pulsed EL-4 cells as target cells (28). Lytic units were calculated for a 50% level of cell lysis. As different control mice were used for $CD40^{-/-}$ versus class $II^{-/-}$ mice, results are expressed as percent lytic units of controls.

In Vivo Expansion and Effector Cell Induction. Spleen cells from TCR-transgenic control mice (10^6 cells) were adoptively transferred into normal C57BL/6 recipient mice. 2 h later, mice were challenged with LCMV. After 8 d, spleen cells were harvested and stained for CD8 (PE; PharMingen) and transgenic Vα2 expression (FITC; PharMingen), and the presence of TCR-transgenic cells was assessed by flow cytometric analysis.

To assess cytolytic effector function, spleen cells were tested in ^{51}Cr-release assays using peptide p33 (KAVYNFATM)-pulsed EL-4 target cells. To distinguish endogenous CTL activity of the C57BL/6 recipient mice from CTL activity of the transferred TCR-transgenic T cells, EL-4 cells pulsed with peptide MB6 (KAVVNIATM) were also used as target cells. MB6 is recognized by the TCR-transgenic T cells but not by the polyclonal C57BL/6 T cells (29).

Isolation and Characterization of DC. Mice were 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 − 1) × 100.

In Vivo–induced DC Maturation by Peptide. Transgenic mice expressing a TCR specific for LCMV were intravenously injected with 10 μg LCMV-derived peptide p33 (KAVYNFATM), A4Y (KAVANFATM), V4Y (KAVVNFATM), or S4Y (KAVSNFATM). After 24 h, DCs were isolated from spleens of treated and, as control, untreated mice and monitored for expression of 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 FACStar$^{PLUS™}$, obtaining a purity >97%. Different numbers of sorted DCs (H-2^b) were added to 2×10^4 purified T cells obtained from spleens of H-2^d BALB/c (MLR) or female HY-transgenic mice (H-2^b on a RAG-2 background) and cultured in 96-well plates (Falcon; Becton Dickinson) for 3 d. T cell proliferation was assessed by [^{3}H]thymidine (1 μCi/well) uptake in a 16-h pulse after 72 h.

In Vitro Maturation of DC. $CD8^+$ 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 pulsed 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^{bm13} mice. 10 wk later, recipient mice exhibited large numbers of TCR-transgenic T cells and a mixed DC population of both CD45.1 and CD45.2 allotypes. 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, or negative control, and analyzed by using a FACSCalibur™ flow cytometer.

Results

CD40 Is Not Involved in Virus-specific Th-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

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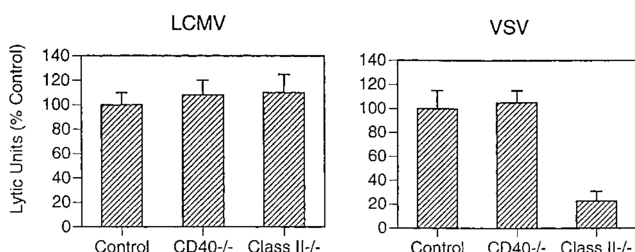

Figure 1. CD40 does not mediate Th-dependent antiviral CTL responses. $CD40^{-/-}$, MHC class $II^{-/-}$, and respective control mice were injected intravenously with LCMV (200 PFU) or VSV (2×10^6 PFU), and spleen cells were isolated 8 or 6 d later, respectively. Spleen cell suspensions were prepared 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.

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 MHC class $II^{-/-}$ mice by 75–80%. Similar results were obtained in mice depleted of $CD4^+$ Th (data not shown). However, CTL responses were completely normal in $CD40^{-/-}$ 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, at the peak of the antiviral response, presence of T cells expressing the transgene-encoded TCR (Vα2) 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 C). 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

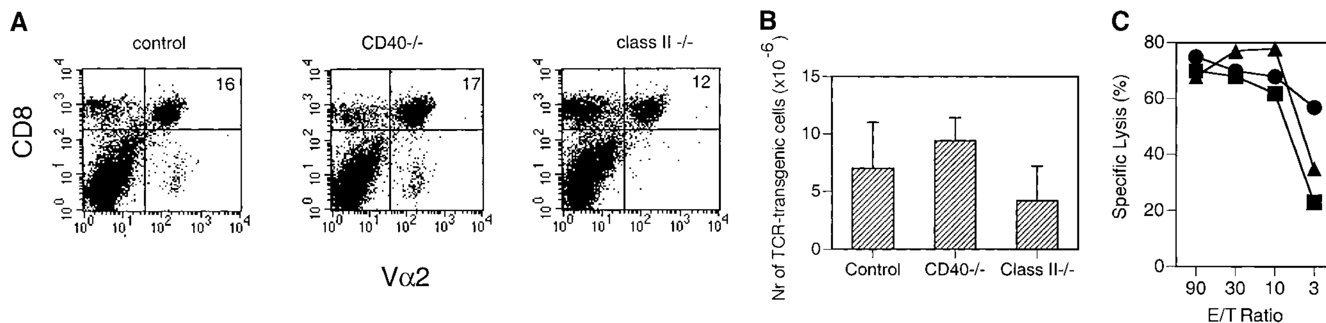

Figure 2. Normal activation and expansion of LCMV-specific TCR-transgenic T cells in the absence of CD40 or MHC class II. 10^6 transgenic T cells expressing a TCR specific for LCMV-derived peptide p33 were transferred into normal, nonirradiated C57BL/6 mice, which were infected intravenously with 200 PFU LCMV 2 h later. Presence of TCR-transgenic mice was assessed 8 d later by flow cytometry (A). Numbers 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 $CD8^+V\alpha2^+$ 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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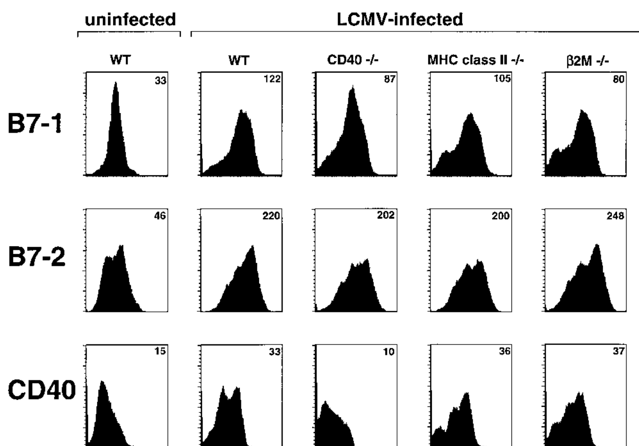

Figure 3. LCMV-specific CD8+ and CD4+ T cells can induce maturation of DCs in vivo. C57BL/6 control mice (WT) or CD40$^{-/-}$, MHC class II$^{-/-}$, and β2M$^{-/-}$were infected with LCMV (200 PFU, i.v.). DCs were isolated from spleens 6 d later, and the expression of B7-1, B7-2, and CD40 was analyzed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

isolated 6 d later (Fig. 3). Maturation of DCs was induced irrespective of the presence of CD40 or Th, indicating that DC maturation was triggered by CD8+ T cells. However, DCs also matured in class I$^{-/-}$ mice (Fig. 3), indicating that Th are sufficient but not necessary for activation of DCs. Thus, virus-specific CD4+ and CD8+ T cells are able to trigger an activation program in DCs. Taken together, these data demonstrate that (a) both virus-specific CD8+ and CD4+ T cells are able to trigger maturation of DCs in vivo and (b) CD40 is not involved in this process.

LCMV exhibits a high virulence in vivo and is able to activate T cell responses in the absence of a variety of accessory molecules. In contrast, VSV exhibits a low virulence in vivo, and T cell responses depend to a higher degree on costimulation (Fig. 1) (11). To analyze whether the low virulence of VSV may be reflected in inefficient activation of DCs in vivo after infection, mice were inoculated with VSV, and splenic DCs were isolated 5 d later (Table I). Indeed, upregulation of costimulatory molecules on DCs occurred less efficiently after infection with VSV compared with LCMV. Thus, the low virulence of VSV was reflected in inefficient activation of DCs.

Table I. *LCMV Triggers Stronger Upregulation of Costimulatory Molecules on DCs than VSV*

Percent upregulation*	LCMV‡	VSV§
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 − 1) × 100.
‡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.
§Mice were infected with VSV (2 × 10^6 PFU/ml), and CD11c+ DCs were analyzed 5 d later by flow cytometry.

Viral Infection Is Not Required for Maturation of DCs. The majority of splenic DCs exhibited an activated phenotype after infection with LCMV. As, by the time of the analysis, little LCMV was left in the spleen (data not shown), it was unlikely that only virally infected DCs underwent maturation. However, it remained possible that few virus-infected DCs nucleated the CTL response that subsequently activated splenic DCs. To analyze whether viral infection was required for activation of DCs, transgenic mice expressing an MHC class I–restricted TCR specific for LCMV (26) were injected with the LCMV-derived peptide p33, and DCs were isolated 1 d later. The naive CD8+ TCR-transgenic T cells were able to induce maturation of DCs within 24 h after activation in the complete absence of viral infection (Fig. 4). We have recently developed a panel of altered peptide ligands that trigger transgenic T cells with various efficiencies (36). Peptide A4Y behaves as a weak agonist, peptide V4Y behaves as a partial agonist, and peptide S4Y is a strict antagonist. If these altered peptide ligands were injected, the efficiency of the peptides in activating T cells, as assessed by upregulation of CD69 expression (Fig. 4 B), was directly reflected in their ability to trigger maturation of DCs (Fig. 4 A). Interestingly, although peptide A4Y is able to induce proliferation of specific T cells in vitro (36), it fails to efficiently trigger maturation of DCs in vivo and induced only low level expression of CD69. This finding is compatible with the observation that weak antigens generally require the presence of Th to induce CTL responses in vivo, because only CD8+ T cells interacting with strong agonist peptides are apparently able to stimulate maturation of DCs, thereby replacing T help.

CD8+ T Cells Can Activate DCs in Trans. Whether a direct interaction of CD8+ T cells with DCs was required for maturation was analyzed next. To this end, chimeric mice were generated, using bone marrow from H-2^{bm13} mice. The TCR of the transgenic mouse line used in our experiments recognizes peptide p33 presented by H-2D^b. H-2^{bm13} mice exhibit a mutation in the D^b molecule that does not allow presentation of peptide p33 (37, 38). Thus, DCs from H-2^{bm13} mice fail to specifically stimulate TCR-transgenic T cells, although the TCR-transgenic cells are efficiently positively selected by H-2^{bm13} and are reactive to peptide p33 (37). RAG-2$^{-/-}$ mice were reconstituted with a mixture of bone marrow derived from CD45.2+ H-2^{bm13} TCR-transgenic mice and bone marrow from CD45.1+ C57BL/6 mice. These chimeric mice therefore exhibit a population of CD45.2+ H-2^{bm13} DCs and a population of CD45.1+ H-2^b DCs and express a transgenic TCR specific for peptide p33 in association with H-2D^b. 10 wk after reconstitution, chimeric mice were injected with peptide p33 or saline, and activation of CD45.1+ and CD45.2+ DCs was analyzed. Both CD45.1+ DCs (H-2^b) that present peptide p33 and CD45.2+ DCs (H-2^{bm13}) that fail to present

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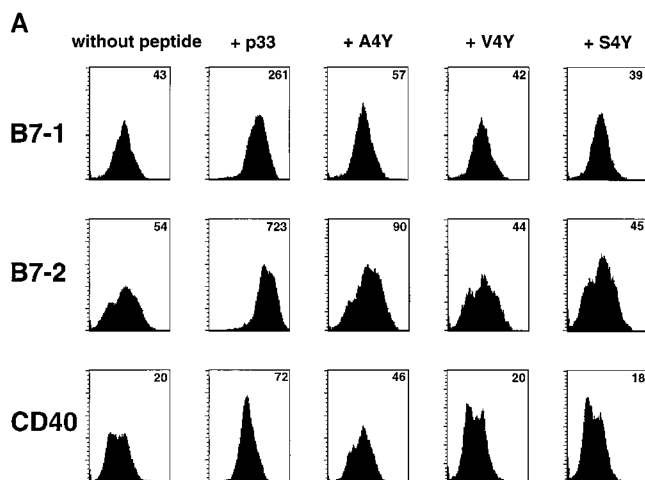

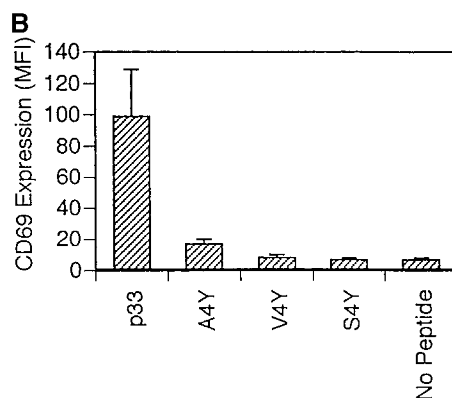

Figure 4. Peptide-specific CD8+ T cells can induce maturation of DCs in the absence of viral infection. Transgenic mice expressing a TCR specific for LCMV-derived peptide p33 were injected with 10 μg of peptide p33, the altered peptide ligands A4Y, V4Y, or S4Y, or saline. (A) Expression of B7-1, B7-2, 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.

peptide p33 exhibited an activated and matured phenotype after injection of specific peptide (Table II). In contrast, DCs derived from TCR-transgenic H-2^{bm13} mice injected with peptide p33 did not exhibit activated DCs, confirming that peptide p33 was not presented by H-2^{bm13} (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 CTLs. Male transgenic mice (H-2^b) expressing a TCR specific for peptide p33 derived from LCMV were injected with peptide p33 or saline, and splenic DCs were isolated 24 h later and purified by cell sorting (purity >97%). Ti-

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

	CD11c+ CD45.1 (H-2^b)		CD11c+ CD45.2 (H-2^{bm13})	
Expression	+p33	−p33	+p33	−p33
		MFI		
B7-2	95	40	114	30
CD40	31	17	36	19
Negative control	5	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-2^{bm13}) DCs.

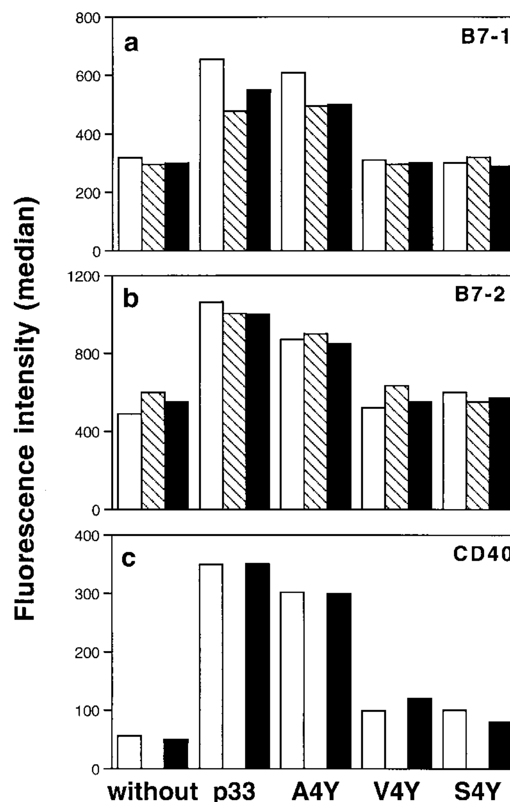

Figure 5. CD8+ T cells mediated maturation of DCs in vitro. Freshly isolated splenic DCs derived from control (open bars), $CD40^{-/-}$ (hatched bars), or MHC class $II^{-/-}$ (closed bars) were pulsed with peptide p33, A4Y, V4Y, or S4Y (all at 10 μg/ml) for 1 h at 37°C. DCs were washed and incubated 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.

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trated numbers of DCs were used to stimulate purified allospecific T cells (H-2[d]) derived from BALB/c mice, and proliferation was assessed 3 d later by means of [^{3}H]thymidine incorporation. DCs derived from p33-immunized mice were able to induce efficient proliferation of allospecific 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.

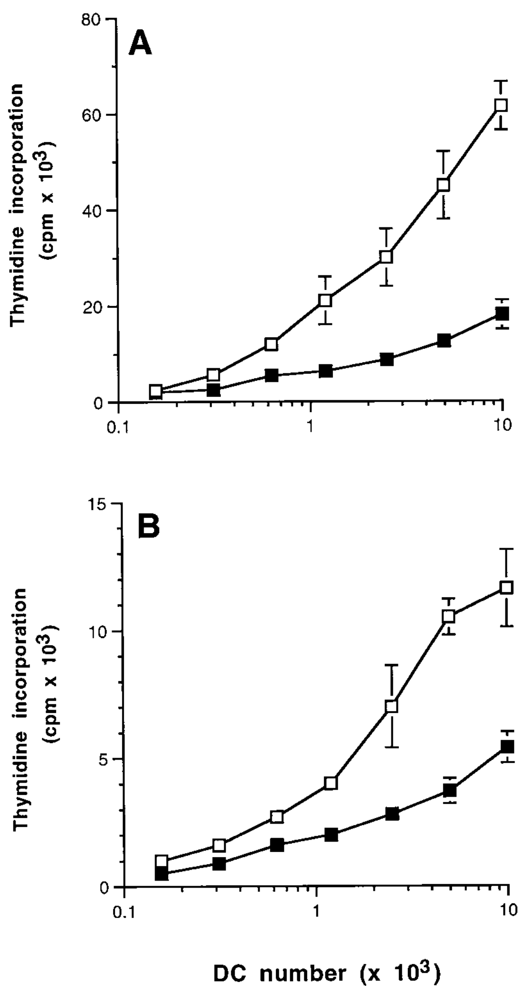

Figure 6. $CD8^+$ T cell–mediated activation of DCs renders Th-dependent $CD8^+$ T cell responses Th independent. Male transgenic mice (H-2[b]) expressing a TCR specific for LCMV-derived peptide p33 were injected with 1 μg of peptide p33 (or saline), and DCs were isolated 24 h later by FACS® sorting. Titrated numbers of DCs isolated from p33-primed animals (□) or control animals (■) were used to stimulate purified allospecific 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.

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 and enhances the immunogenicity of DCs (8, 41). Thus, it was possible that the observed Th-independent maturation of DCs was (a) directly mediated by viral components, (b) mediated by non-T cells such as NK cells, or (c) mediated by $CD8^+$ T cells. To address this question, various gene-deficient mice were infected with LCMV, and the maturation of DCs was assessed. Maturation of DCs occurred in class $II^{-/-}$ as well as class $I^{-/-}$

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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 DCs 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 $CD8^+$ T cells 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 A4Y, 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 most 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

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because immature DCs in lymphoid organs that process those antigens most efficiently are inefficient at stimulating naive 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.

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