Dual Role of Dendritic Cells in the Induction and Down-Regulation of Antigen-Specific Cutaneous Inflammation

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Dual Role of Dendritic Cells in the Induction and Down-Regulation of Antigen-Specific Cutaneous Inflammation

Maya Krasteva,* Jeanne Kehren,* Françoise Horand,† Hitoshi Akiba,* Geneviève Choquet,* Marie-Thérèse Ducluzeau,* Rosine Tédone,* Jean-Luc Garrigue,† Dominique Kaiserlian,‡ and Jean-François Nicolas2*

We have previously reported that contact sensitivity (CS) to dinitrofluorobenzene (DNFB) in C57BL/6 mice was mediated by MHC class I-restricted CD8+ T cells and down-regulated by MHC class II-restricted CD4+ T cells. In this study, we analyzed the contribution of dendritic cells (DC) in the induction of these two T cell subsets endowed with opposite functions. Hapten-pulsed skin- and bone marrow-derived DC, obtained from either normal C57BL/6 mice or from MHC class II (Ii+II-) and MHC class I (Ii-II-) deficient mice, were tested for their ability to prime normal mice for CS to dinitrofluorobenzene. Expression of MHC class I molecules by transferred DC was mandatory both for the induction of CS and for the generation of hapten-specific CD8+ T cells in lymphoid organs. Ii-II- DC were as potent as Ii+II+ DC in priming for CS, demonstrating that activation of effector CD8+ T cells can occur independently of CD4+ T cell help. Ii+II+ DC could not immunize for CS, although they could sensitize for a delayed-type hypersensitivity reaction to protein Ags. Moreover, Ii-II- DC injected simultaneously with cutaneous sensitization down-regulated the inflammatory response, suggesting that hapten presentation by MHC class II molecules could prime regulatory CD4+ T cells. These results indicate that DC can present haptenated peptides by both MHC class I and class II molecules and activate Ag-specific CD8+ effector and CD4+ regulatory T cell subsets, concurrently and independently. The Journal of Immunology, 1998, 160: 1181–1190.

Dendritic cells (DC)3 are a subset of bone marrow-derived APC that have a unique capacity to prime naïve T cells and to initiate Ag-specific adaptive immune responses. DC capture and process Ags into peptides, which can be presented by MHC class I and MHC class II molecules to specific CD8+ or CD4+ T cells, respectively. A large body of evidence from in vivo and in vitro experiments indicates that DC are the only natural APC shown to prime naïve T cells by delivering costimulatory signals for T cell activation that cannot be provided by most other cell types (reviewed in Ref. 1). Because of their efficacy in inducing T cell responses in vivo without other adjuvants, it has been hypothesized that DC could be used to immunize patients with clinically relevant Ags to either induce protective immunity against pathogens and tumor Ags (2, 3) or to generate an Ag-specific tolerance in allergic and autoimmune diseases (4, 5). Recent experimental data have clearly shown that peptide-pulsed DC induce Ag-specific CTLs mediating tumor (6–9) and viral (10, 11) immunity, suggesting that vaccination protocols using Ag-pulsed DC may be valuable. Alternatively, little is known on the ability of DC to induce Ag-specific tolerance.

Contact sensitivity (CS) is a T cell-mediated immune reaction occurring after epicutaneous immunization and challenge with low m.w. chemicals, i.e., hapten, which covalently bind to discrete amino acid residues on self or exogenous proteins. Hapten-modified proteins could then be processed by APC into antigenic peptides, which are transported at the cell surface in association with class I or class II MHC molecules. Epidermal dendritic cells, i.e., Langerhans cells (LC) play a crucial role in the induction of CS. They capture the hapten (or haptenated proteins) in the skin and migrate to draining lymph nodes where they mature into functional APC endowed with the capacity to prime naïve T cells (12). Hapten-specific presursor T cells recognize a conformational complex formed by the hapten-modified peptide within the groove of both MHC class I and class II molecules of the DC (13–16).

Unlike classical delayed-type hypersensitivity (DTH) to protein or cellular Ags, which is mediated primarily by MHC class II-restricted CD4+ T cells (17), the T cell response to hapten appears more complex and is still not well understood. Previous studies reported that CS responses may involve CD4+ and/or CD8+ T cells depending on the hapten and the mouse strain used (18). We have recently demonstrated, using MHC class I- and class II-deficient mice, that the CS reaction to dinitrofluorobenzene (DNFB) in C57BL/6 mice was mediated by MHC class I-restricted CD8+ effector T cells and down-regulated by MHC class II-restricted CD4+ T cells (19). This suggested that the hapten presentation by MHC class I molecules is required for the induction of effector cells of CS, while presentation by MHC class II molecules results in the generation of regulatory cells. Several questions were raised by these data: 1) Do DC represent the APC type responsible for the priming of both CD8+ effector and CD4+ regulatory T cells? 2) Are DC able to present haptons on MHC class I and class II molecules simultaneously and independently? 3) Are DC involved in...
the induction of tolerance through hapten presentation by MHC class II/CD4 pathway?

In this study, we used hapten-pulsed DC from MHC class I- and class II-deficient mice to determine the relative contribution of the MHC molecules to the CS reaction. We also took advantage of the fact that hapten presentation through MHC class II molecules can generate cells able to down-regulate CS to test for the possibility of inducing hapten-specific tolerance in naive recipients. Our results demonstrate that DC can very efficiently immunize for CS reactions to DNFB but are not able to induce a long-lasting state of tolerance.

Materials and Methods

Mice

Mice with a mutation in the \( \beta_2 \)-microglobulin (\( \beta_2 \)) (20) or \( \beta_2 \) gene (\( \beta_2 \)) (21) have been created by gene-targeting techniques. Heterozygous (\( I^b/II^2 \) and \( II^2/II^2 \)) knockout mice were backcrossed for more than eight generations to C57BL/6 mice and used to generate the offspring of the present study. These mice were obtained from C. Benoist and D. Mathis (BIBM, IN- SEED, Unité 184, Stassfurt, France) and bred in specific pathogen-free conditions in Iffa Credo/Transgenic Alliance, L’Arbesle, France. C57BL/6 (H-2b) mice were obtained from Iffa-Credo. Mice were used between 2 and 4 mo of age.

Allergens

The hapten, 2,4-dinitro-fluorobenzene (DNFB; Sigma, St. Louis, MO) and picryl chloride (TNP; BDH Chemicals Ltd., Poole, England) were freshly prepared before CS assays. 2,4-Dinitrobenzenesulfonic acid (DNBS; Aldrich, Milwaukee, WI) and 2,4,6-trinitrobenzenesulfonic acid (TNBS; Sigma) were used for in vitro pulsing of dendritic cells. BSA and OVA, both from Sigma, were used in DTH assays.

Culture medium

The culture medium was RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 10% heat-inactivated FCS (Boehringer-Mannheim, Meylan, France), 100 U/ml penicillin, 100 \( \mu \)g/ml streptomycin, 2 mM L-glutamine, and 5.10\(^{-3}\) M 2-ME (all from Sigma); this was referred to as complete medium.

LC-enriched epidermal cell suspensions

Single epidermal (EC) suspensions were prepared from ear skin. Epidermal sheets were obtained by 1-h incubation of the ear in 0.05% trypsin (Type Culture Collection, Rockville, MD). Rat IgG2a anti-mouse CD80 (N418) and polyclonal hamster IgG Abs were purchased from American Type Culture Collection (Rockville, MD). Rat IgG2b anti-mouse CD80 (B7.1, clone 16-10A1) and CD86 (B7.2, clone GL1) were from PharMingen (San Diego, CA). Rat IgG2b anti-mouse Ia (clone CD11) and rat

Immunization with DC

\( 10^7 \) DNBS- or TNBS-derivatized epidermal DC or BM-derived DC from either \( I^b/II^2 \), \( I^b/II^2 \), or \( I^b/I^b \) were injected s.c. in 200 \( \mu \)l of saline into naive C57BL/6 (\( I^b/I^b \)) recipients, mice to prime for CS. 10^6 BSA-pulsed \( I^b/I^2 \), \( I^b/I^2 \), and \( I^b/I^b \) BM-DC were similarly injected s.c. into naive C57BL/6 mice (\( I^b/I^2 \)) to sensitize for DTH.

Assay for CS: the mouse ear swelling test

The procedure for the mouse ear swelling test has been described in details elsewhere (24). For sensitization to DNFB, mice were painted once (day −5) on the shaved dorsal skin with 25 \( \mu \)l of 0.5% DNFB in acetone:olive oil (4:1, v:v) or with 7% TNP in acetone. Five days later (day 0), mice were challenged by the application of either DNFB (0.15% in acetone:olive oil, 4:1) or TNP (1% in olive oil) as control, on each side of the right ear, while the left ear received the vehicle alone. Ear thickness was assessed before and at various intervals after challenge using a spring-loaded micrometer (J15, Blet SA, Lyon, France). Ear swelling was calculated by subtracting the initial value from the values recorded on the corresponding day and further subtracting any swelling recorded for the vehicle-treated ear from the swelling recorded for the hapten-challenged ear. In the present study, mice were injected s.c. with hapten-derivatized DC, and the responses were compared with those elicited in mice sensitized by direct epicutaneous hapten application. A group of mice injected with the same number of unmodified BM-DC served as a negative control. We tested for down-regulation of the inflammatory response by injecting hapten-derivatized BM-DC into mice sensitized via the epicutaneous route.

Assay for DTH response to purified protein Ags

DTH response to protein Ags was measured by a conventional footpad swelling assay. Mice were sensitized by the s.c. injection of 200 \( \mu \)l of either 50 \( \mu \)g of BSA emulsified with CFA (1:1, v:v) or 10^6 BSA-treated or unmodified BM-DC as control. Seven days later, the mice were challenged by the s.c. injection in the left hind footpad of 0.6 mg of BSA or OVA as control, diluted in saline. The right footpad was injected with saline alone. BSA-specific DTH was calculated by subtracting the swelling of the right (control) footpad from the swelling of the left footpad induced by BSA, according to the formula: \( \Delta e (\text{BSA}) = \Delta e (\text{saline}) \), where \( \Delta e (\text{BSA}) \) = footpad thickness after BSA − before BSA, and \( \Delta e (\text{saline}) \) = footpad thickness after saline − before saline. Mice injected with the same number of unmodified BM-DC served as a negative control.

Hapten-specific T lymphocyte proliferation assay

T cells from the spleen and the inguinal and axillary lymph nodes of immunized mice were pooled together and partially purified by negative selection using anti-Ig columns (Biotex, Edmonton, Alberta, Canada). The resulting cell suspensions contained >98% viable Th-1, CD3+ cells. CD8+ cells and CD8+ T cells were enriched by negative selection using anti-Ig columns coated with goat antimouse IgG and either a rat mAb anti-mouse CD8α (clone YTS 169.4) or a rat mAb anti-mouse CD4 (clone YTS 191.1). The residual CD4+ and CD8+ cell populations in the nonadherent effluent cells represented <0.5%. In vivo hapten-primed T cells (2.5 \( \times \) 10^5/well) were cocultured for 3 days at 37°C in 96-well microtiter plates with 10^5 mitomycin-treated syngeneic spleen cells that were either hapten-derivatized with DNBS and TNBS or untreated. T cell proliferation was determined on day 3 of culture by [\(^3\)H]thymidine incorporation (1 \( \mu \)Ci/well) during the last 6 h of culture. The results are expressed as cpm/culture.

Flow cytometric analysis

A panel of mAbs was used to identify and characterize single-cell suspensions obtained from draining lymph nodes and spleens of sensitized animals, as well as the cells in epidermal cell suspensions and in BM-DC cultures. mAbs used included: FITC- and phycoerythrin-conjugated rat anti-mouse CD4 (IgG2a, clone CT-CD4), FITC-conjugated rat anti-mouse CD8α (IgG2a, clone CT-CD8a), FITC-conjugated Thy 1.2 (mouse IgG2b, clone 5a-8), FITC-conjugated anti-Ia\(^a\)-\(^d\) (mouse IgG2a, clone 28−16−85), mouse anti-mouse FITC-conjugated H-2D\(^b\) (IgG2a, clone CD8b), FITC-conjugated rat CD11b (Mac-1, rat IgG2b, clone M1/ 70.15), and FITC-conjugated rat anti-mouse Ly-5 (B220, IgG2a, clone RA3−6B2), all purchased from Caltag Laboratories (Burlingame, CA). Phycoerythrin-conjugated anti-Ia\(^a\)-\(^d\) (rat IgG2b, M5/114) was purchased from Boehringer Mannheim. The hamster anti-mouse CD11c (clone N418) and polyclonal hamster IgG Abs were purchased from American Type Culture Collection (Rockville, MD). Rat IgG2a anti-mouse CD80 (B7.1, clone 16-10A1) and CD86 (B7.2, clone GL1) were from PharMingen (San Diego, CA). Rat IgG2b anti-mouse Ia (clone CD11) and rat
IgG2b anti-mouse NLDC-145 (DEC 205) were kindly provided by A. Glasebrook (25) and George Kraal (Amsterdam, The Netherlands), respectively.

Cells (10^6) were incubated with Abs for 30 min at 4°C and washed twice with PBS containing 1% FCS and 0.1% sodium azide. Cell suspensions were preincubated with 50 μg/ml of anti-mouse CD16/CD32 (rat IgG2b, clone 2.4G2) from PharMingen to block FcγIII/IIR, whenever mouse, hamster, or directly conjugated rat Abs were used. FITC-conjugated donkey anti-rat IgG (H1L) (Jackson Immunoresearch Laboratories, West Grove, PA) and FITC-conjugated goat anti-hamster IgG (H1L) (Caltag) were used as secondary Abs.

The appropriate isotype-matched Abs were used as controls of specific staining. Fluorescence was measured with a FACScan (Becton Dickinson, Mountain View, CA) flowcytometer, and data were analyzed with LYSYS II software (Becton Dickinson). A minimum of 20,000 events were scored.

**Immunohistochemical analysis of epidermal sheets**

Epidermis from the ears was peeled off the connective tissue by incubation for 1 h at 37°C in PBS supplemented with 20 mM EDTA (Sigma). The sheets were cut in small pieces, rinsed, and incubated overnight at 4°C with specific Abs or isotype-matched irrelevant Abs. After three washes in PBS/1% BSA, the sheets were incubated for 30 min at room temperature with biotinylated F(ab)2 fragment of goat Abs specific for rat IgG (H + L) (Pierce, Interchim, Montluçon, France), washed, and then further incubated with streptavidin-conjugated to peroxidase (ABC kit, Dako, Capitentia, CA). The reaction was developed using 3-amino-9-ethylcarbazole (AEC) substrate and H2O2 (Dako).

**Statistical analysis**

Panels consisted of five mice each, and all experiments were performed at least twice. The statistical significance of differences between mean values of experimental groups was evaluated using the two-tailed Student’s t test (p < 0.05).

**Results**

**MHC class I expression on DC is required for induction of contact sensitivity to DNFB**

We analyzed the ability of LC from C57BL/6 mice or from syngeneic MHC class I- and MHC class II-deficient mice to immunize for CS to DNFB. Immunohistochemical analysis of epidermal sheets using the DC-specific anti-CD11c Ab (N418) revealed that the network of LC was comparable in all three types of mice. In addition, we verified that N418+ epidermal LC from Iγ/IIγ mice...
lack expression of MHC class II (Fig. 1A). EC suspensions from either C57BL/6 mice or from syngeneic I\(^{+}/II^{-}\) or I\(^{-}/II^{+}\) mice were enriched in LC by Ficoll gradient centrifugation followed by 24-h culture in the presence of GM-CSF. EC suspensions from C57BL/6 (Fig. 1B) and I\(^{-}/II^{+}\) mice (data not shown) contained 20 to 30% LC-expressing MHC class II molecules.

The relative contribution of MHC class I and II molecules of LC in the induction of CS to DNFB was tested in normal non-immune C57BL/6 recipient mice immunized by the s.c. injection of haptenated DC before challenge by skin painting with the hapten. As shown in Figure 2, mice receiving DNBS-derivatized LC from either C57BL/6 or MHC class II-deficient (class I\(^{-}/II^{+}\)) mice, developed a strong hapten-specific CS response (Fig. 2). The intensity and magnitude of the inflammatory reaction was similar to that observed in mice immunized by the epicutaneous application of DNFB (ear swelling of mice sensitized by skin painting shown in Fig. 3A). In contrast, s.c. injection of DNBS-derivatized I\(^{-}/II^{+}\) EC from MHC class I-deficient mice was unable to prime naive mice for CS to DNFB, indicating that the presence of MHC class I but not the presence of MHC class II molecules was mandatory for the induction of CS effector T cells.

**BM-DC are as potent as EC in priming CS effector T cells**

Since EC contain only 20–30% of LC, the contribution of DC in priming for CS effector T cells was addressed more directly using highly enriched DC suspensions obtained by in vitro culture of bone marrow progenitors for 7 days in the presence of GM-CSF. These in vitro-differentiated DC (referred to as BM-DC), were then tested for their ability to induce CS in naive recipient mice. BM-DC, established from either MHC I\(^{-}/II^{+}\), MHC class I-, or MHC class II-deficient mice, comprised 40 to 50% of cells exhibiting a dendritic morphology with slender cytoplasmic processes. FACS analysis revealed that BM-DC cultures from C57BL/6 mice contained 40 to 50% of MHC class II\(^{+}\) cells coexpressing CD11c, DEC 205, B7.1, B7.2, CD11b, and F4/80 and lacking B220, Thy1.2, CD4, and CD8 molecules (data not shown). These data confirmed that BM-DC had a phenotype characteristic of mature interdigitating DC.

DNBS-derivatized I\(^{-}/II^{+}\) BM-DC could prime naive mice for CS as efficiently as I\(^{-}/II^{+}\) BM-DC (Fig. 3A). Unmodified I\(^{-}/II^{+}\) and I\(^{-}/II^{+}\) BM-DC failed to sensitize mice for a DNFB-specific CS. The CS response induced by DNBS-pulsed BM-DC was hapten specific. Indeed, mice immunized with DNBS-pulsed DC and challenged with TNP did not develop a CS reaction. Similarly, mice immunized with TNBS-treated DC did not develop a CS reaction upon challenge with DNFB (Fig. 3B).

Alternatively, mice injected with DNBS-modified I\(^{-}/II^{+}\) BM-DC were unable to develop a CS response upon DNFB challenge (Fig. 3A). This was not due to impaired Ag processing and presentation function, since these cells, when pulsed with native BSA delivered in FCS-containing medium, could induce a specific DTH of a magnitude comparable with that generated by immunization with BSA emulsified in CFA (Fig. 3C). Thus, MHC class I but not class II molecules expressed by BM-DC were necessary and sufficient for priming MHC class I-restricted CS effector T cells in vivo.

**MHC class I\(^{+}\) DC can prime class I-restricted CD8\(^{+}\) T cells in vivo**

To confirm that class I-restricted CD8\(^{+}\) T cells could be primed by haptenated DC in vivo, we tested the ability of CD8\(^{+}\) T cells isolated from draining lymph nodes to proliferate in response to in vitro restimulation by haptenated spleen cells from either I\(^{-}/II^{+}\), I\(^{-}/II^{+}\), or I\(^{-}/II^{+}\) mice. As shown in Table I, CD8\(^{+}\) T cells, purified from spleen and lymph nodes on day 5 after DC transfer, proliferated in response to restimulation by I\(^{-}/II^{+}\) and I\(^{-}/II^{+}\) but not I\(^{-}/II^{+}\) DNBS-pulsed DC. No proliferation was ever observed in response to TNBS-modified spleen cells. Thus, hapten-specific class I-restricted CD8\(^{+}\) T cells were primed by DC in vivo and were present in secondary lymphoid organs at the time of hapten challenge.

**MHC class I\(^{-}/II^{+}\) BM-DC can prime class II-restricted CD4\(^{+}\) T cells in vivo**

We next examined whether the lack of CS response to DNFB in mice immunized with DNBS-treated I\(^{-}/II^{+}\) BM-DC was due to induction of class II-restricted CD4\(^{+}\) T cells, which down-regulate CS (19). We tested for the presence of hapten-specific CD4\(^{+}\) T cells in lymphoid organs of C57BL/6 mice after s.c. immunization with DNBS-treated I\(^{-}/II^{+}\) BM-DC. We assayed the ability of un-fractionated or purified CD4\(^{+}\) T cells to proliferate in response to in vitro restimulation by DNBS-modified spleen cells from either normal (MHC class I\(^{-}/II^{+}\)) mice or MHC class I- or MHC class II-deficient mice. DNBS-specific T cell proliferation was only found in either un-fractionated or CD4\(^{+}\)-enriched but not in the CD8\(^{+}\)-enriched T cell suspensions, upon in vitro restimulation with DNBS-derivatized spleen cells from either C57BL/6 (class I\(^{-}/II^{+}\)) or MHC class I-deficient (class I\(^{-}/II^{+}\)) mice, both expressing MHC class II molecules (Table II). Proliferation was never observed when the T cells were stimulated with TNBS-haptenated spleen cells. These results demonstrated that immunization with DNBS-modified MHC class I\(^{-}/II^{+}\) BM-DC induced hapten-specific class II-restricted CD4\(^{+}\) T cells.
MHC class I/II" DC inhibit the afferent and efferent limb of the CS response to DNFB

Since we had previously reported that MHC class II-restricted CD4⁺ T cells down-regulate CS to DNFB in C57BL/6 mice (19), we next examined whether DNBS-modified I⁺/II⁺ BM-DC could block the induction (afferent phase) or the expression (efferent phase) of CS. For this experiment, mice were injected s.c. with haptenated I⁺/II⁺ DC either at the time of skin sensitization (day -5) or 5 days later (day 0) by ear painting with DNFB or TNP. The histograms represent ear swelling values (mean ± SD) of five mice per group 24 h after challenge. C. Naive C57BL/6 mice were immunized (day -7) by either s.c. injection of 10⁵ BM-DC pulsed for 24 h with BSA or s.c. injection of BSA emulsified in CFA. Seven days later (day 0), the mice were challenged by intrafootpad injection of BSA or OVA diluted in PBS. DTH was determined by footpad swelling measured 48 h after challenge. The histograms show footpad swelling values (mean ± SD) of five mice per group and are representative of three experiments.

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<tr>
<th>Sensitization (Day -5)</th>
<th>Challenge (Day 0)</th>
<th>Ear swelling (in mmX10⁻²)</th>
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<td>DNFB</td>
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</tr>
<tr>
<td>TNBS-modified I⁺/II⁺ BM-DC</td>
<td>TNP</td>
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</tr>
<tr>
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<td>DNFB</td>
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<th>Sensitization (Day -7)</th>
<th>Challenge (Day 0)</th>
<th>Footpad swelling (in mmX10⁻²)</th>
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<tr>
<td>Unmodified BM-DC</td>
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**FIGURE 3.** MHC class I⁺/II⁻ and MHC class I⁺/II⁺ BM-DC can prime for CS to DNFB and for DTH to BSA, respectively. A and B. Naive C57BL/6 mice were sensitized (day -5) by the s.c. injection of either 10⁵ DNBS-modified (A), or TNBS-modified (B) BM-DC and challenged 5 days later (day 0) by ear painting with DNFB or TNP. The histograms represent ear swelling values (mean ± SD) of five mice per group 24 h after challenge. C. Naive C57BL/6 mice were immunized (day -7) by either s.c. injection of 10⁵ BM-DC pulsed for 24 h with BSA or s.c. injection of BSA emulsified in CFA. Seven days later (day 0), the mice were challenged by intrafootpad injection of BSA or OVA diluted in PBS. DTH was determined by footpad swelling measured 48 h after challenge. The histograms show footpad swelling values (mean ± SD) of five mice per group and are representative of three experiments.
We next tested whether MHC class I/II DC priming of CS effector T cells in the lymph nodes, only I/II/II DC were able to induce a CS reaction. Alternatively, no CS reaction occurred in mice immunized with hapten-pulsed DC recovered from normal mice and injected s.c. was always equal to or greater than that obtained in mice immunized by the epicutaneous route. This level of response probably relates to the fact that in vitro pulsing of DC with the hapten generates a much higher number of DC carrying the hapten as compared with that resulting from in vivo hapten application onto the skin. Alternatively, the DC preparations may be enriched for APCs responsible for the induction of effector cells and may lack APCs that could induce the down-regulatory cells of CS. BM-DC were as efficient as epidermal LC cultured for 24 h with GM-CSF, confirming that DC can capture the hapten and subsequently prime for effector cells of CS (31, 32).

Expression of MHC class I molecules by DC is mandatory for the induction of the CS reaction in our experimental system and for the activation of hapten-specific CD8+ T cells in the lymph nodes, since only I/II/II and I/II/II DC were able to induce a CS reaction. Alternatively, no CS reaction occurred in mice immunized with DC obtained from MHC class I-deficient mice, although these cells expressed normal levels of MHC class II molecules. This lack of sensitizing properties was not due to a functional blunt in Ag presentation inasmuch as I/II/II DC were functional for in vivo priming of C57BL/6 mice for a CD4+ T cell-mediated DTH response to BSA. Thus, induction of CD8+ effector T cells of CS is dependent upon the presentation of the hapten by MHC class I molecules and does not require the presence of MHC class II molecules, implying that DC can directly prime CD8+ T cells in the absence of CD4+ T cell help. The requirement for CD4+ helper signals during the generation of CD8+ T cell responses remains a matter of debate and depends on several factors among which are the avidity of the TCR for the peptide/class I MHC ligand (33), the nature of the APC responsible for the CD8+ T cell priming (34, 35), and the contribution of the costimulatory molecules CD80 and CD86.

Discussion
CS is a T cell-mediated skin inflammatory reaction initiated by hapten capture by epidermal DC, which migrate to draining lymph nodes where they mature into functional APC endowed with the capacity to prime naive T cells (12, 26, 27). Effector cells of CS could comprise CD4+, CD8+, or both T cell subsets, depending on the mouse strain and the hapten used (18). We have previously reported that CS to DNFB in C57BL/6 mice is mediated by CD8+

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### Table I. Secondary T cell proliferative response to DNBS- and TNBS-derivatized APC

<table>
<thead>
<tr>
<th>Stimulator Cells</th>
<th>CD8+ T cells</th>
<th>Unfractionated T cells</th>
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<tr>
<td>APC I/II DNBS</td>
<td>2.8 ± 0.6</td>
<td>3.5 ± 1.0</td>
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<tr>
<td>APC I/II TNBS</td>
<td>3.7 ± 1.7</td>
<td>4.4 ± 2.1</td>
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<td>7.0 ± 2.2</td>
</tr>
<tr>
<td>APC I/II TNBS</td>
<td>2.3 ± 1.1</td>
<td>5.3 ± 1.6</td>
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<td>APC I/II DNBS</td>
<td>6.3 ± 11.5</td>
<td>51.4 ± 7.8</td>
</tr>
<tr>
<td>APC I/II TNBS</td>
<td>4.9 ± 0.9</td>
<td>4.7 ± 1.5</td>
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</tbody>
</table>

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**Notes:** Naive C57BL/6 mice were immunized with DNBS-derivatized MHC I/II BM-DC. Five days later, CD8+ or unfractionated T cells (2.5 x 10^5) recovered from inguinal and axillary lymph nodes and spleens of recipient mice were cocultured with DNBS- or TNBS-derivatized syngeneic spleen cells (10^6). T cell proliferation was assessed at day 3 by [3H]thymidine incorporation for the last 6 h of culture. Results are expressed as cpm ± SD of triplicate cultures.

### Table II. Secondary T cell proliferative response to DNBS- and TNBS-derivatized APC

<table>
<thead>
<tr>
<th>Stimulator Cells</th>
<th>CD8+ T cells</th>
<th>CD8+ T cells</th>
<th>Unfractionated T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC I/II DNBS</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 0.9</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>APC I/II TNBS</td>
<td>27.9 ± 0.5</td>
<td>29.0 ± 0.3</td>
<td>19.9 ± 1.9</td>
</tr>
<tr>
<td>APC I/II DNBS</td>
<td>4.0 ± 1.5</td>
<td>3.4 ± 1.5</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>APC I/II TNBS</td>
<td>21.9 ± 2.5</td>
<td>3.6 ± 0.8</td>
<td>22.4 ± 1.9</td>
</tr>
<tr>
<td>APC I/II DNBS</td>
<td>3.0 ± 0.8</td>
<td>3.2 ± 1.3</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>APC I/II TNBS</td>
<td>2.9 ± 1.6</td>
<td>2.9 ± 1.3</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>APC I/II DNBS</td>
<td>2.8 ± 0.3</td>
<td>3.1 ± 1.2</td>
<td>3.4 ± 1.4</td>
</tr>
</tbody>
</table>

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**Notes:** Naive C57BL/6 mice were immunized with DNBS-derivatized MHC I/II BM-DC. Five days later, CD4+, CD8+, or unfractionated T cells (2.5 x 10^5) of recipient mice were cocultured with DNBS- or TNBS-derivatized syngeneic spleen cells (10^6). T cell proliferation was assessed at day 3 by [3H]thymidine incorporation for the last 6 h of culture. Results are expressed as cpm ± SD of triplicate cultures.
CD86 (36, 37). Since the first observation by Inaba et al. showing that DC were able to prime CD8^+ T cells in the absence of T cell help (38), several studies have confirmed this unique functional property of DC for the development of CD8^+ T cells specific for alloantigens as well as for tumor and viral Ags. MHC class I^+/II^- epidermal LC lines derived from fetal skin, and lacking constitutive and inducible expression of class II proteins and mRNA, can activate naive allogeneic CD8^+ T cells in vitro (37) and are able to prime for transplantation immunity in vivo (39) and to generate cross-priming of class I-restricted CD8^+ CTLs with exogenous viral Ags (40). Our data are in line with these observations and show that hapten presentation by MHC class I molecules on mature DC expressing CD80 and CD86 is necessary and sufficient for the priming of naive CD8^+ T cells in vivo.

An important finding in this study is the observation that DC represent the APC type responsible for priming of hapten-specific class II-restricted CD4^+ T cells, which have been reported to control the magnitude of the inflammatory response to DNFB in C57BL/6 mice (19). These results highlight the differences in the mechanisms involved in CS and DTH, since DTH to BSA was shown to be mediated by MHC class II presentation by DC to CD4^+ T cells. Indeed, transfer into naive mice of haptenated DC, which express MHC class II but not class I molecules, generates peripheral CD4^+ T cells, which proliferate upon hapten restimulation in vitro. Moreover, these hapten-pulsed I^+/II^- DC decrease the magnitude of the inflammatory response in CS when they are injected into naive recipient mice either at the time of epicutaneous DNFB sensitization or 1 day before DNFB challenge. It is unlikely that inhibition of CS results from a nonspecific blockade of the class I/CD8 interaction by an excess of DC inasmuch as injection of equivalent numbers of unmodified DC does not affect the CS response. Alternatively, it may be proposed that DC-induced down-regulation is due to a quantitative increase in the number of CD4^+ T cells, since the down-regulation cannot be detected if the transferred haptenated DC coexpressed class I and class II molecules. In this case, simultaneous priming of CD8^+ and CD4^+ T cells occurs and is induced by hapten presentation on MHC class I and class II present on the transferred DC as well as on the

**FIGURE 4.** MHC class I^+/II^- BM-DC down-regulate CS induced by cutaneous sensitization with DNFB in C57BL/6 mice. Graded numbers: 10^5 (♂), 5.10^5 (♀), 10^6 (●) of DNBS-derivatized or unmodified (■) BM-derived I^+/II^- DC were administered s.c. to syngeneic C57BL/6 mice at the time of epicutaneous DNFB sensitization (day -5) (A) or 24 h before challenge (day -1) (B). The CS response was determined at various time points after ear challenge with DNFB. Controls include normal unsensitized C57BL/6 mice receiving DNFB on the ear only (●). The data represent ear swelling (mean ± SD) of five mice per group and are representative of four experiments.
recipient’s own DC. Such masking of the down-regulatory effect of the CD4\(^+\) T cells is reminiscent of classical studies on suppression and contrasuppression of CS (41) and could be due to a higher proliferation capacity of CD8\(^+\) T cells compared with CD4\(^+\) T cells or to a lower threshold of hapten required to activate CD8\(^+\) vs CD4\(^+\) T cells. This hypothesis is supported by the observation

**FIGURE 5.** Transfer of MHC class I\(^+/\)II\(^+\) BM-DC fails to tolerize C57BL/6 mice for CS to DNFB. A, Naive C57BL/6 mice were injected s.c. on day \(-10\) with \(10^5\) DNBS-modified BM-derived I\(^+/\)II\(^+\) DC. On day \(-5\), the mice were sensitized epicutaneously with DNFB. On day 0, the mice were ear challenged with DNFB or TNP as control. The CS response was determined by ear swelling measured 24 h postchallenge. B, Naive C57BL/6 mice were injected s.c. either once (day \(-10\))(■), twice (day \(-11\) and \(-10\))(○) or three times (day \(-12\), \(-11\) and \(-10\))(▲) with \(10^5\) DNBS-modified BM-derived I\(^+/\)II\(^+\) DC. All mice were sensitized on day 5 and ear challenged on day 10 as described above. In control mice immunized with DNBS-modified I\(^+/\)II\(^+\) BM-DC, epicutaneous sensitization on day 5 was omitted (●). The CS response was determined by ear swelling 24, 48, and 72 h postchallenge. The data represent mean ear swelling ± SD of five mice per group.
that down-regulation of CS by DC transfer is proportional to the number of $\Gamma/\Pi^+1$ DC injected. Thus, CD4$^+$-mediated inhibition of CS seems to depend on a balance between the respective sizes of CD8$^+$ and CD4$^+$ subsets responding to the hapten. The present data confirm our previously reported observation that CD4$^+$ T cells can limit the intensity and duration of the CS response to DNFB in C57BL/6 mice and demonstrate that activation of this down-regulatory pathway is initiated by hapten presentation by MHC class II molecules on the DC.

However, CD4$^+$ T cells appear unable to induce tolerance for CS. First, inhibition of the CS response to skin sensitization with DNFB induced by DC transfer can be overcome by a second DNFB challenge (data not shown). Second, class $\Gamma/\Pi^+1$ DC are unable to inhibit the CS response to DNFB when injected 5 days before skin sensitization. Thus, class $\Gamma/\Pi^+1$ DC can prime or reactivate hapten-specific T cells but are unable to induce memory-tolerogenic T cells. Another possibility is that the CD4$^+$ T cells could dampen the inflammatory reaction only during a transient state of activation, which is the case in the coinjection experiments but which is not the case when CD4$^+$ T cell activation has been achieved 5 days before.

The mechanisms underlying down-regulation of inflammatory reactions are not well understood. Although a role for Th2 cytokines, especially IL-4 and IL-10, has been demonstrated in the dampening of various DTH reactions (42–44) including CS (45–47), Xu et al. recently reported that hapten-specific CD4$^+$ T cells produce IL-4 and IL-10, whereas CD8$^+$ T cells produce IFN-$\gamma$ (48). This is in line with our observation that hapten-primed CD4$^+$ T cells, which proliferate upon in vitro restimulation with the specific hapten, produce IL-4 (data not shown). It is thus tempting to speculate that the regulatory effect of CD4$^+$ T cells is mediated by the synthesis of anti-inflammatory cytokines in the lymph nodes and/or in the skin, upon activation through presentation of the hapten by MHC class II molecules. Alternatively, killing of effector T cells by CD4$^+$ T cells through a Fas-dependent mechanism could contribute to control the induction and/or the expression of CS. In this respect, Fas-L$^+1$ CD4$^+$ T cells were recently reported to induce apoptosis in Fas$^+$ CD8$^+$ T cells in vitro (49). The precise mechanisms involved in the control of both the afferent and the efferent limb of CS are currently under investigation.

Our data provide evidence that the hapten DNFB can be presented independently by both MHC class I and class II molecules, raising the question of the mechanisms by which a foreign molecule could be taken up by DC and processed for T cell priming. Hapten can bind to either extracellular or cell surface proteins that are internalized and processed into peptides in the endosomal/lysosomal compartments where they bind to the MHC class II groove. Several mechanisms may contribute to hapten binding to class I. The hydrophobic liposoluble hapten may penetrate through the cell membranes allowing binding to self proteins in the cytoplasm (50–51), which can then be processed through class I. Alternatively, hapten may bind to extracellular proteins, which may have a particulate conformation adequate for uptake in phagolysosomes and may subsequently be processed through class I (52). This possibility seems unlikely for DNFB, which binds covalently to amino acids residues. Finally, since hapten can covalently bind to discrete AA residues (lysine residues for DNFB), it is also possible that part of the hapten may bind directly to peptides within the grooves of MHC class I and class II molecules (50). Our data showing that DC can present haptenated peptides on both Ag-presenting pathways confirm previous observations in allergic contact dermatitis to urushiol (50) and are in line with recent studies that emphasize the property of mature DC to present exogenous Ags on both MHC class I and MHC class II molecules (53, 54).

In conclusion, our study indicates that DC are instrumental in the priming of both MHC class I-restricted CD8$^+$ effector cells and MHC class II-restricted CD4$^+$ down-regulatory cells, concurrently and independently.

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References


