Contact Hypersensitivity in MHC Class II-Deficient Mice Depends on CD8 T Lymphocytes Primed by Immunostimulating Langerhans Cells

Anne Bouloc,[1] Andrea Cavani, and Stephen I. Katz

Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.

Studies to determine if $CD4^+$ and/or $CD8^+$ T cells are critical for the initiation and propagation of contact hypersensitivity (CHS) reactions have yielded conflicting results regarding their roles. We studied the induction and expression of CHS to trinitrochlorobenzene (TNCB) using major histocompatibility complex class II-deficient mice that display normal numbers of $CD8^+$ T cells but lack $CD4^+$ T cells. CHS to TNCB, detected as an increase in ear thickness 24 h after epicutaneous challenge, was significantly enhanced in major histocompatibility complex class II-deficient mice compared with wild-type controls. Ear swelling responses in major histocompatibility complex class II-deficient mice were decreased by treatment with anti-CD8 antibody or by injection of wild-type $CD4^+$ T cells. To further characterize mechanisms involved in the initiation of CHS responses, phenotypical and functional characteristics of both freshly isolated and cultured Langerhans cells were studied. Like Langerhans cells from wild-type controls, Langerhans cells from major histocompatibility complex class II-deficient mice upregulated B7–1 and B7–2 costimulatory molecules and enhanced major histocompatibility complex class I expression upon short-term culture. Cultured Langerhans cells induced a 3.5-fold increase in the stimulation of autologous hapten-specific $CD8^+$ T cell proliferation compared with fresh Langerhans cells. Finally, TNP-coupled Langerhans cells from major histocompatibility complex class II-deficient mice primed naïve mice to TNCB after transfer. These results demonstrate that hapten-specific $CD8^+$ T cells are sufficient for the expression of CHS and that CD8 priming does not require the presence of $CD4^+$ T cells or major histocompatibility complex class II antigen. ***Key words: CD8 lymphocytes/ MHC class II deficiency. J Invest Dermatol 111:44–49, 1998***

Contact hypersensitivity (CHS) describes the responses elicited by epicutaneous sensitization and challenge with chemically reactive haptens, small chemically defined molecules capable of binding directly to soluble and cell associated proteins (Polak, 1980). Since Landsteiner and Jacobs (1935) first reported the phenomenon of CHS in guinea pigs, many investigators have elucidated various mechanisms involved in the generation and expression of CHS to various haptens (Knight *et al*, 1985; Macatonia *et al*, 1987; Kripke *et al*, 1990; Enk and Katz, 1992, 1995). According to the current paradigm, during the afferent or sensitization phase, hapten-specific T cells are primed by Langerhans cells and/or dermal dendritic cells that migrate from the area of antigen exposure to the skin-draining lymph nodes and present hapten-major histocompatibility complexes (MHC) to naïve T cells. Subsequently, the reapplication of the same hapten to skin results in the influx of hapten-specific T cells that react to the hapten locally, release cytokines, and attract other inflammatory cells. The resultant swelling can be measured and reflects the ongoing immune response. CHS is a T cell-mediated immune response; studies to define the cellular elements that are responsible have yielded conflicting results. Both $CD4^+$ T cells and $CD8^+$ T cells can recognize haptenated peptides (Martin *et al*, 1993; Cavani *et al*, 1995). *In vivo* and *in vitro* cell depletion studies from several laboratories have indicated the importance of $CD4^+$ T cells as the effector cells (Miller and Jenkins, 1985; Gautam *et al*, 1991), and others have demonstrated the ability of $CD8^+$ T cells to mediate CHS (Gocinski and Tigelaar, 1990; Bour *et al*, 1995; Xu *et al*, 1996).

In this study, we used MHC class II-deficient mice that were generated by disruption of the MHC class II Ab β gene in embryonic stem cells. The mice lack cell surface expression of class II molecules and are depleted of mature $CD4^+$ T cells (Grusby *et al*, 1991). CHS to TNCB was significantly enhanced in these mice compared with wild-type controls. Our data indicate that Langerhans cells from MHC class II-deficient mice undergo functional activation upon short-term culture, stimulate hapten-specific $CD8^+$ T cells *in vitro*, and can prime T cells *in vivo*.

MATERIALS AND METHODS

Animals MHC class II-deficient mice that have a disruption of the Ab β gene and wild-type controls (C57BL/6) were obtained from the Taconic Laboratory (Germantown, NY). Mice were used at ages ranging from 8 to 12 wk. All animals were used in accordance with institutional guidelines.

Culture media Cell culture and cell proliferation assays were performed in RPMI 1640 supplemented with 10% fetal calf serum (Biofluids, Rockville, MD), 2 mM glutamine, 1% penicillin/streptomycin/fungizone, 10 mM HEPES buffer, 1% nonessential amino acids, 1 mM sodium pyruvate (all from Life Technologies, Chagrin Falls, OH), and 5×10^{-5} M β2-mercapto-ethanol (Sigma, St. Louis, MO). The following monoclonal antibodies were produced by hybridomas obtained from the American Type Culture Collection (ATCC,

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Reprint requests to: Dr. Stephen I. Katz, Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Abbreviation: CHS, contact hypersensitivity.

[1]Dr. A. Bouloc is now affiliated with the Department of Dermatology, Inserm U448, Henri Mondor Hospital AP-HP, 94010 Créteil, France.

Rockville, MD) GK1.5 (anti-CD4), 53–6.72 (anti-CD8). Anti-CD45, B7–1, B7–2, and MHC class I (H-2K^b/H-2D^b) antibodies were purchased from Pharmingen (San Diego, CA).

Chemicals TNCB was obtained from Polysciences (Warrington, PA), TNBS and fluoroscein isothiocyanate (FITC) were obtained from Sigma.

Preparation of Langerhans cell-enriched fresh epidermal cell suspensions Epidermal cell suspensions were obtained from the ears of nontreated mice as previously described (Tamaki *et al*, 1979). Epidermal cell suspensions were applied to Lympholyte M density gradients (Cedarlane, Ontario, Canada) and centrifuged at 800 × *g* for 10 min at room temperature. Interface cells, containing 5–10% Langerhans cells referred to as fresh Langerhans cells (fresh epidermal cells), were washed extensively in complete medium and used as antigen-presenting cells in the proliferation assays.

Preparation of Langerhans cell-enriched cultured epidermal cell suspensions Epidermal cells were suspended at 2 × 10^6 cells per ml in complete medium and cultured for 72 h in 25 ml T flasks. Non-adherent cells were collected by vigorous pipetting and enriched in Langerhans cells by Lympholyte M gradient centrifugation as described. Such suspensions contained 20–30% Langerhans cells and are referred to as cultured epidermal cells.

Hapten coupling of cells TNP-coupled fresh epidermal cells and TNP-coupled cultured epidermal cells were obtained by incubating cell suspensions with 1 mM TNBS for 10 min at 37°C, and were washed extensively in complete medium. FITC-coupled fresh epidermal cells were obtained by incubating cell suspensions with 1 mM FITC for 10 min at 37°C, and were washed extensively in complete medium.

Animal sensitization Mice were sensitized with 100 μl of 3% TNCB solution (4:1, acetone:olive oil) (both from Sigma) painted onto shaved abdominal skin. Mice were challenged on day 6 with 20 μl 1% TNCB solution on the right ear and ear swelling was quantitated with a spring-loaded micrometer (Mitutuyo, Japan) 24 h after challenge. Mice that were ear-challenged without prior sensitization served as negative controls. The results are presented for each group (four mice) as the average difference (±SD) in ear thickness before and after the challenge.

In some experiments, CD4$^+$ T cells or CD8$^+$ T cells were depleted *in vivo* by intraperitoneal injection of 100 μg of anti-CD4 or anti-CD8 antibodies, on three consecutive days (–3, –2, –1) prior to sensitization as described (Cobbold *et al*, 1985). Cell depletion was assessed by staining for CD4 and CD8 bearing cells in peripheral blood.

In some experiments, 2 × 10^6 CD4$^+$ T cells from sensitized mice were injected intravenously 6 d after sensitization immediately prior to challenge.

In others experiments, Langerhans cell-enriched epidermal cell suspensions (2 × 10^6) modified with TNBS (1 mM) or with FITC or unmodified control cells were injected in the footpads (day 0) as a procedure of sensitization.

CD4$^+$ and CD8$^+$ T cell purification Inguinal, axillary, and brachial lymph nodes were obtained 1 wk after *in vivo* sensitization. T cells were enriched by nylon wool column filtration (Polysciences). CD4$^+$ and CD8$^+$ T cells were isolated by negative selection using either rat monoclonal antibodies to CD8 or rat monoclonal antibodies to CD4 followed by incubation with sheep anti-rat Ig-coated magnetic beads (Dynal, Great Neck, NY) as described (Levin *et al*, 1993). Purity of the recovered CD4$^+$ or CD8$^+$ T cells was ⩾95% as determined by staining with anti-CD4 and anti-CD8 antibodies.

Flow cytometry Cells were washed in flow cytometry buffer (PBS, 5% fetal calf serum, 0.02% sodium azide) and were stained for 30 min at 4°C with the following antibodies: FITC-anti-CD45, PE-anti-CD45, FITC-anti-B7–1, FITC-anti-B7–2, PE-anti-MHC class I (H-2K^b/H-2D^b) (Pharmingen, San Diego, CA). The cells were then washed three times with flow cytometry buffer and analyzed on a Becton Dickinson FACScan.

T cell proliferation assays T cells were plated at 2 × 10^5 cells per well in 96 flat-bottomed microplates (Costar, Cambridge, MA) and cultured in the presence of different antigen-presenting cells for 5 d. T cell proliferation was determined by the incorporation of [^{3}H]thymidine during the last 16 h of culture using a gas ionization counter (Packard, Meriden, CN). Results are presented as the mean (±SD) of assays performed in triplicate.

Statistical analyses The significance of differences among groups was examined by the Student's t test; $p < 0.05$ was considered significant.

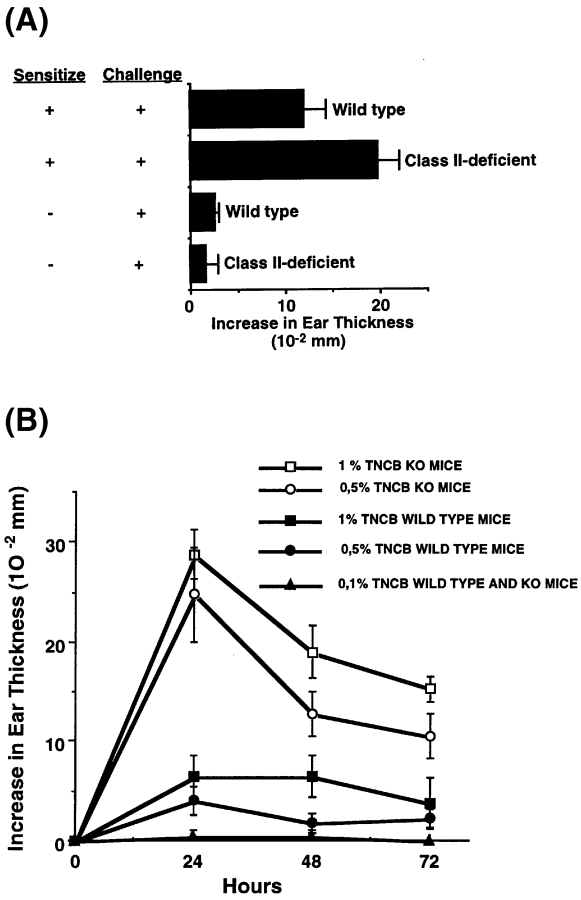

Figure 1. MHC class II-deficient mice have enhanced CHS reactions to TNCB *in vivo* compared with wild-type littermate controls. (*A*) Wild-type and MHC class II-deficient mice were sensitized with 100 μl 3% TNCB on the abdomen and challenged 6 d later on the right ear with 20 μl 1% TNCB (four mice per group). The results are expressed as the mean of increase of ear swelling (±SD) 24 h after challenge. The data are representative of two separate experiments. (*B*) Wild-type and MHC class II-deficient mice were sensitized with 100 μl 3% TNCB on the abdomen and challenged 6 d later on the right ear with 20 μl of different concentrations of TNCB: 1%, 0.5%, 0.1% (four mice per group). The results are expressed as the mean of increase of ear swelling (±SD) 24, 48, and 72 h after challenge.

RESULTS

MHC class II-deficient mice have enhanced CHS reactions to TNCB compared with wild-type littermate controls To test the role of MHC class II and CD4$^+$ T cells in CHS responsiveness, we examined CHS reactions in wild-type and MHC class II-deficient mice after sensitization with TNCB. Sensitization of wild-type mice with 100 μl 3% TNCB led to antigen-specific swelling at the site of challenge 24 h after painting the ear with 1% TNCB. Mice deficient in MHC class II regularly showed significantly increased ear swelling responses (**Fig 1*A***). The kinetics of CHS responses with different challenging doses are shown in **Fig 1(*B*)**.

***In vivo* treatment with anti-CD8 antibody inhibits CHS to TNCB in MHC class II-deficient mice** To confirm that CHS in MHC class II-deficient mice is due to hapten-specific CD8$^+$ T cells, we injected anti-CD8 monoclonal antibody on three consecutive days prior to sensitization. This treatment with anti-CD8 antibody resulted in depletion of CD8$^+$ T cells and abrogated CHS responses to TNCB, indicating that the effector cells in these MHC class II-deficient mice are the CD8$^+$ T cells (**Fig 2**).

Injection of anti-CD8 antibodies into wild-type controls results in inhibition of CHS to TNCB and the injection of anti-CD4 antibodies results in enhancement of CHS To investigate the

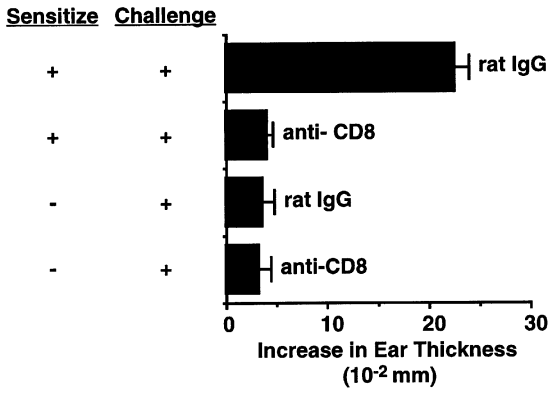

Figure 2. *In vivo* depletion with anti-CD8 monoclonal antibody inhibits CHS to TNCB in MHC class II-deficient mice. MHC class II-deficient mice were injected intraperitoneally with 100 μg anti-CD8 monoclonal antibody (53.6.72) or with rat IgG on three consecutive days (four mice per group). One day later the animals were sensitized with 100 μl 3% TNCB on the abdomen. They were then challenged after 6 d on the right ear with 20 μl 1% TNCB. The results are expressed as the mean of increase of ear swelling (±SD) 24 h after challenge. The data are representative of two separate experiments.

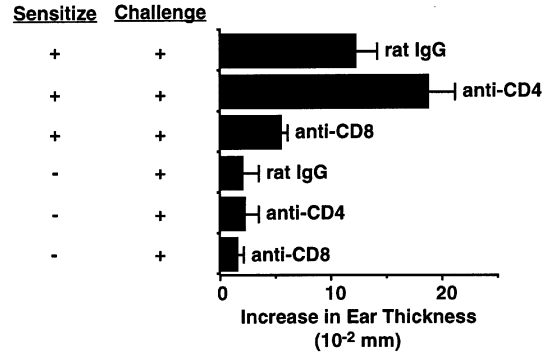

Figure 3. Injection of anti-CD8 monoclonal antibodies into wild-type controls results in inhibition of CHS to TNCB and the injection of anti-CD4 antibodies results in enhancement of CHS. Mice were injected intraperitoneally with 100 μg anti-CD8 (53.6.72) or anti-CD4 monoclonal antibodies (GK1.5) or with rat IgG on three consecutive days (four mice per group). One day later the animals were sensitized with 100 μl 3% TNCB on the abdomen. They were then challenged after 6 d on the right ear with 20 μl 1% TNCB. The results are expressed as the mean of increase of ear swelling (±SD) 24 h after challenge. The data are representative of two separate experiments.

role played by $CD8^+$ T cells in CHS to TNCB in wild-type mice, we injected anti-CD8 monoclonal antibody before sensitization. This resulted in inhibition of CHS to TNCB, showing that $CD8^+$ T cells play a major role in CHS response initiation. The increased CHS to TNCB in MHC class II-deficient mice and the role of effector cells played by $CD8^+$ T cells suggested that $CD4^+$ T cells could be downregulators of the CHS responses. Consistent with this hypothesis, depletion of $CD4^+$ T cells with anti-CD4 antibody resulted in enhancement of CHS to TNCB compared with the non-injected wild-type mice (**Fig 3**).

Transfer of hapten-specific $CD4^+$ T cells from wild-type mice into MHC class II deficient mice results in diminution of CHS to TNCB The increased CHS response in MHC class II-deficient mice and the results of the *in vivo* depletion of $CD4^+$ T cells with anti-CD4 monoclonal antibody on CHS in wild-type mice suggested that $CD4^+$ T cells downregulate CHS responses to TNCB. Hapten-specific $CD4^+$ T cells were therefore obtained from lymph nodes of TNCB-painted mice and were injected immediately before challenge into MHC class II-deficient mice, which had been previously sensitized on the abdomen with TNCB 3%. The mice exhibited a diminution

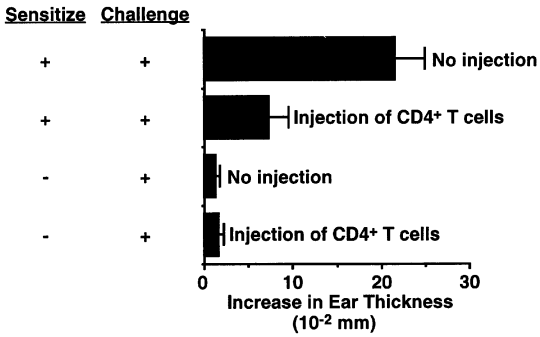

Figure 4. Transfer of hapten-specific $CD4^+$ T cells from wild-type mice into MHC class II-deficient mice results in diminution of CHS to TNCB. MHC class II-deficient mice were sensitized with 100 μl 3% TNCB on the abdomen. They were injected intravenously with 2×10^6 wild-type hapten-specific $CD4^+$ T cells 6 d later and immediately challenged on the right ear with 20 μl 1% TNCB (four mice per group). The results are expressed as the mean of increase of ear swelling (±SD) 24 h after challenge. The data are representative of two separate experiments.

of CHS responses to TNCB (**Fig 4**). These data confirm that the increased CHS observed in MHC class II-deficient mice is due to their lack of $CD4^+$ T cells.

Transfer of hapten-specific $CD8^+$ T cells from wild-type mice or MHC class II-deficient mice in both types of mice did not result in a diminution of CHS to TNCB (data not shown).

Langerhans cells from MHC class II-deficient mice upregulate B7–1 and B7–2 costimulatory molecules and exhibit enhanced MHC class I expression upon short-term culture Using flow cytometry, we evaluated the ability of MHC class II-deficient Langerhans cells to undergo phenotypic change upon short-term culture. Like MHC class II positive cells from wild-type animals, a subpopulation of $CD45^+$ epidermal cells recovered from MHC class II-deficient mice showed upregulation of the costimulatory molecules B7–1 and B7–2 after 3 d of culture, as well as enhanced MHC class I expression (**Figs 5**, **6**). CD45 is expressed on all cells of hematopoietic lineage and is not expressed on keratinocytes. It is used as a marker of Langerhans cells; however, it could also be a marker of dendritic epidermal T cells (DETC). MHC class II-deficient mice have a distribution of T cells and DETC similar to wild-type controls (data not shown). There was no upregulation of MHC class I molecules on the $\gamma\delta$ TCR^+ population after short-term culture (data not shown), indicating that it was the Langerhans cells that exhibited the enhanced MHC class I expression.

Langerhans cells from MHC class II-deficient mice can stimulate hapten-specific $CD8^+$ T cells To determine whether Langerhans cells from MHC class II-deficient mice can stimulate hapten-specific $CD8^+$ T cells, we cocultured $CD8^+$ T cells from MHC class II-deficient mice with Langerhans cells: either fresh epidermal cells, fresh epidermal cells coupled with TNBS (TNP-fresh epidermal cells), cultured epidermal cells, or cultured epidermal cells coupled with TNBS (TNP-cultured epidermal cells). Both TNP-fresh epidermal cells and TNP-cultured epidermal cells exhibited antigen-presenting cell function in the stimulation of hapten-specific $CD8^+$ T cells (**Fig 7*A***). TNP-cultured epidermal cells induced a 3.5-fold increase in the stimulation of syngenic hapten-specific $CD8^+$ T cell proliferation compared with TNP-fresh epidermal cells, indicating that MHC class II-deficient Langerhans cells within the epidermal cell population undergo functional maturation upon short-term culture. Similar results were obtained in wild-type mice (**Fig 7*B***).

Transfer of TNP-coupled Langerhans cells from MHC class II-deficient mice can induce CHS to TNCB To investigate whether TNP-coupled Langerhans cell-enriched epidermal cells from MHC class II-deficient mice can prime T cells *in vivo*, we injected TNP-coupled epidermal cells from MHC class II-deficient mice into naïve MHC class II-deficient animals. Mice were sensitized with either

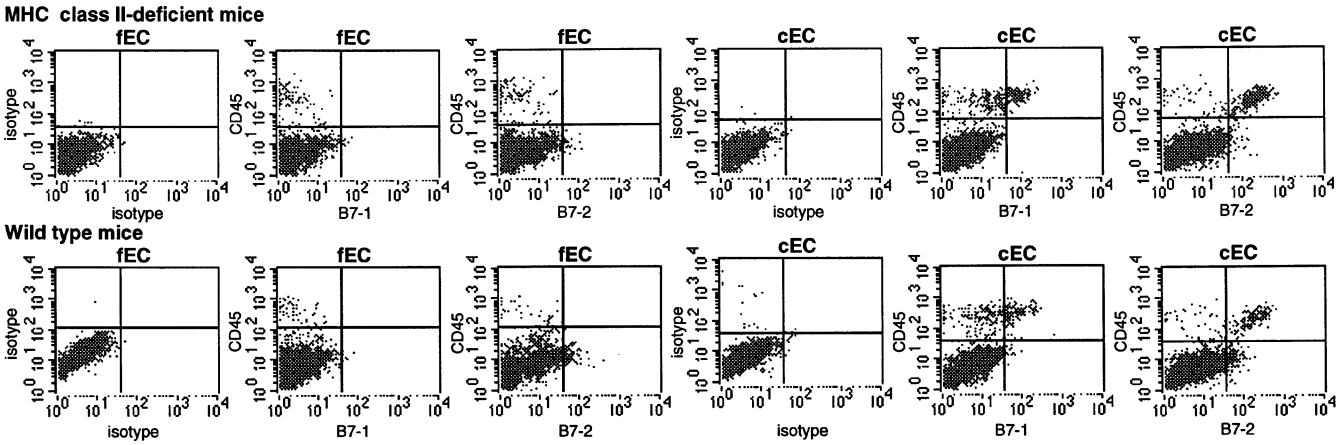

Figure 5. Epidermal cell from MHC class II-deficient mice upregulate B7–1 and B7–2 costimulatory molecules upon short-term culture. Detection of costimulatory molecules B7–1, B7–2, and CD45 on MHC class II-deficient and wild-type epidermal cell suspensions by two color flow cytometry (data are plotted as intensities of FITC fluorescence on the *x* axis *versus* phycoerythrin fluorescence on the *y* axis). Epidermal cells were studied in freshly prepared suspensions (fEC) and after 3 d of culture (cEC).

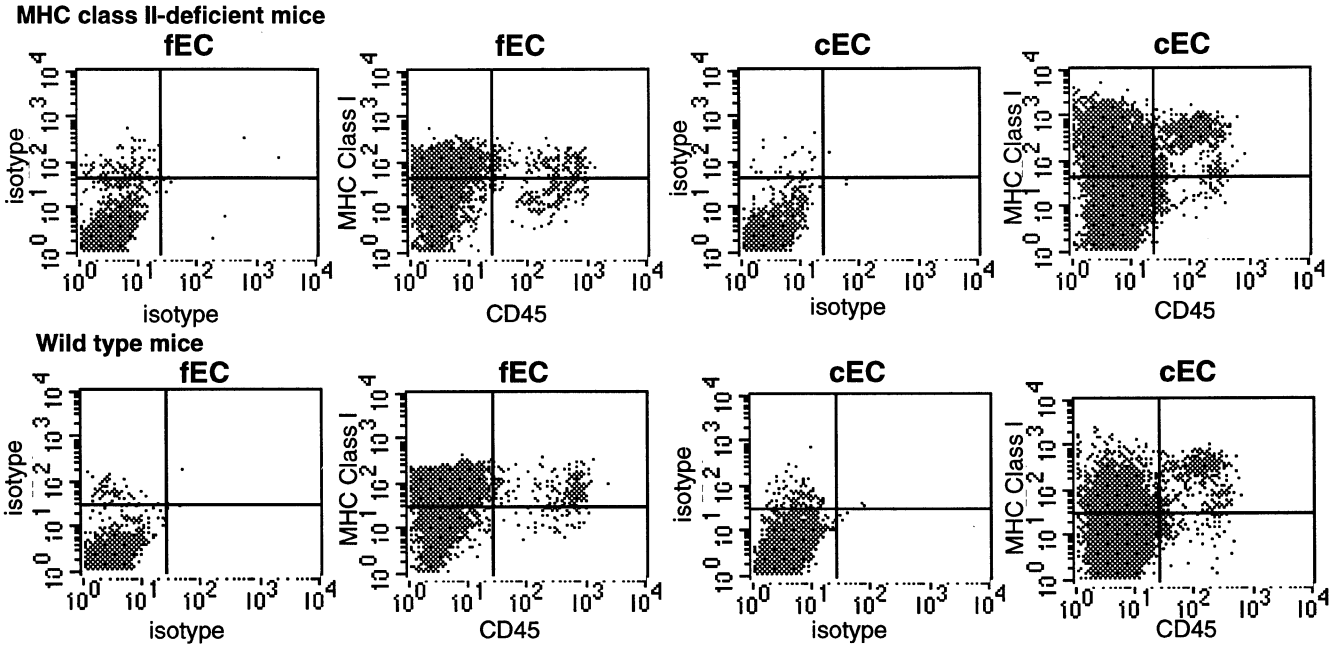

Figure 6. Epidermal cells from MHC class II-deficient mice exhibit enhanced MHC class I expression upon short-term culture. Detection of MHC class I antigens and CD45 on MHC class II-deficient and wild-type epidermal cell suspensions by two color flow cytometry (data are plotted as intensities of FITC fluorescence on the *x* axis *versus* phycoerythrin fluorescence on the *y* axis). Epidermal cells were studied in freshly prepared suspensions (fEC) and after 3 d of culture (cEC).

100 μl 3% TNCB on the abdomen or with 2×10^6 TNP-epidermal cells injected in the footpad, and challenged 6 d later on the right ear with 20 μl 1% TNCB. Hapten-derivatized Langerhans cell-enriched epidermal cells from MHC class II-deficient mice induce CHS to TNCB in MHC class II-deficient mice (**Fig 8**). Uncoupled epidermal cells or FITC-coupled epidermal cells did not induce any significant ear swelling. Similar results were obtained when TNP-coupled epidermal cells from MHC class II-deficient mice were injected into naïve wild-type mice (data not shown). These results indicate that hapten-derivatized class II-deficient Langerhans cells can prime T cells *in vivo*.

DISCUSSION

In this study, we demonstrate that MHC class II-deficient mice have enhanced CHS reactions to TNCB *in vivo* compared with wild-type controls. $CD8^+$ T cells appeared to be the effector cells of the CHS as shown by the results of the *in vivo* CD8 depletion studies. $CD4^+$ T cells seemed to regulate CHS responses as indicated by the transfer of hapten-specific $CD4^+$ T cells from wild-type controls into MHC class II deficient mice that resulted in diminution of CHS. These results confirm experiments reported by Gocinski and Tigelaar (1990), Bour *et al* (1995), and Xu *et al* (1996). Miller and Jenkins (1985) have previously reported opposite findings. After injection of anti-CD4 antibody, they had observed inhibition of CHS responses. Gocinski and Tigelaar demonstrated that both $CD4^+$ T cells and $CD8^+$ T cells were sufficient to induce CHS to dinitroflurobenzene and that there were two populations of $CD4^+$ T cells, effector and suppressor cells. Enhanced contact sensitivity reactions to DNFB have been reported as well in MHC class II-deficient mice (Bour *et al*, 1995). Since we began our studies it has been reported by Xu *et al* that T cell populations primed by hapten sensitization are distinguished by two polarized patterns of cytokine production: interferon γ producing effector $CD8^+$ T cells and interleukin 4/interleukin 10 producing negative regulatory $CD4^+$ T cells. Altogether these results, consistent with our work,

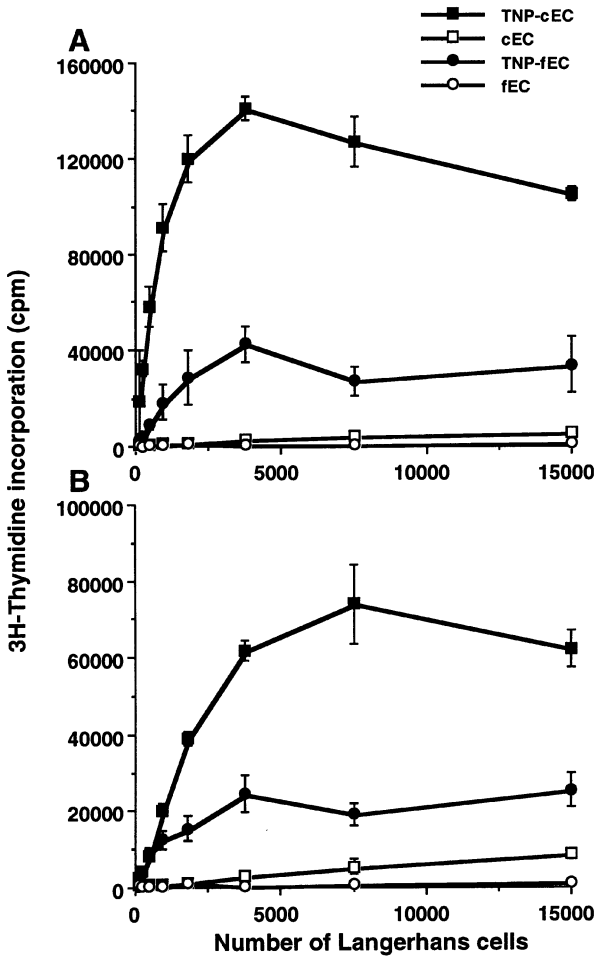

Figure 7. Hapten-derivatized epidermal cells from MHC class II-deficient mice can stimulate (*A*) hapten-specific CD8$^+$ T cells from MHC class II-deficient mice and (*B*) hapten-specific T cells from wild-type mice. (*A*) *In vivo* primed TNP-specific CD8$^+$ T cells were cocultured with fresh epidermal cells, TNP-fresh epidermal cells, cultured epidermal cells, or TNP-cultured epidermal cells. T cell proliferation was determined by the incorporation of [^{3}H]thymidine (1 μCi per well) during the last 16 h of culture. Results are presented as the mean (±SD) of proliferation assays performed in triplicate. The data are representative of two separate experiments. (*B*) *In vivo* primed TNP-specific T cells were cocultured with fresh epidermal cells, TNP-fresh epidermal cells, cultured epidermal cells, or TNP-cultured epidermal cells. T cell proliferation was determined by the incorporation of [^{3}H]thymidine (1 μCi per well) during the last 16 h of culture. Results are presented as the mean (±SD) of proliferation assays performed in triplicate. The data are representative of two separate experiments.

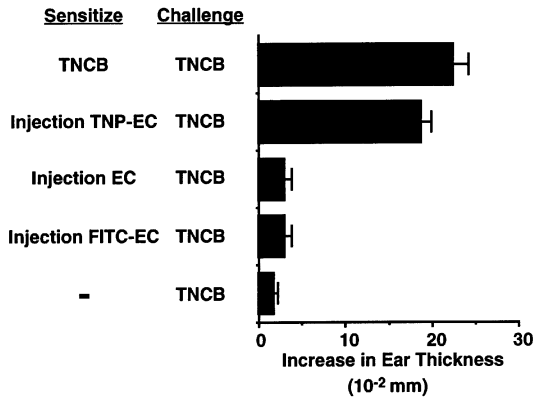

Figure 8. Hapten-derivatized epidermal cells from MHC class II-deficient mice induce CHS to TNCB in MHC class II-deficient mice. Mice were sensitized with either 100 μl 3% TNCB on the abdomen or with 2×10^6 TNP-epidermal cells or FITC-epidermal cells injected in the footpad and challenged 6 d later on the right ear with 20 μl 1% TNCB (four mice per group). The results are expressed as the mean of increase of ear swelling (±SD) 24 h after challenge.

indicate that CD8$^+$ T cells are effector cells in contact hypersensitivity. It should be noted that contact hypersensitivity differs from delayed type hypersensitivity to cellular or protein antigens, where only CD4$^+$ T cells have been reported to be the effector cells (Cher and Mosmann, 1987).

Langerhans cell-enriched epidermal cells from MHC class II-deficient mice also induced CHS to TNCB after *in vivo* transfer, showing that antigen-presenting cells can prime CD8$^+$ T cells in the absence of CD4$^+$ T helper cells. It had been shown that fetal skin derived class I$^+$, class II$^-$ dendritic cells can stimulate unprimed CD8$^+$ T cells (Elbe *et al*, 1994). It had also been demonstrated that in CD4 deficient mice cytotoxic T cell responses could be observed (Buller *et al*, 1987) and that the development of CD8$^+$ T cells and the cytotoxic functions were normal (Rahemtulla *et al*, 1991).

Another issue that is addressed in this study is the role of CD4$^+$ T cells and MHC class II antigens in Langerhans cells activation. Epidermal Langerhans cells are bone marrow-derived cells and as the primary antigen-presenting cell population in the skin are responsible for initiation of immune responses. They have been implicated as pivotal antigen-presenting cells in the induction and expression of CHS (Haftek, 1988). They are the first cells of the skin immune system to react with a contact allergen, transporting the antigen to the draining lymph node for presentation to T lymphocytes. Cell surface expression of MHC molecules and costimulatory molecules are central for their function (Stingl and Bergstresser, 1995). MHC class I and class II antigens (Tamaki *et al*, 1979; Lenz *et al*, 1989; Sharrow *et al*, 1994), as well as costimulatory and adhesion molecules such as ICAM-1 (Tang and Udey, 1991), LFA-3 (Teunissen *et al*, 1990), B7–1, and B7–2 (Larsen *et al*, 1992; Lee *et al*, 1993), have been identified on the surface of Langerhans cells. The increased accessory cell activity of cultured Langerhans cells relative to fresh Langerhans cells is thought to reflect the increased expression of major histocompatibility complex antigens and costimulatory molecules. Here we show that, except from lacking MHC class II molecules, Langerhans cells from MHC class II-deficient mice had otherwise normal phenotypic characteristics. They upregulated B7–1 and B7–2 costimulatory molecules and exhibited enhanced MHC class I expression and increased antigen-presenting cell activity after short-term culture. The full expression of CHS is usually thought to require that epidermal Langerhans cells present antigen in the context of MHC class II molecules to specifically sensitized T lymphocytes. It has been suggested that the CD4$^+$ T cell-derived cytokines may be essential for terminal differentiation of Langerhans cells into lymphoid dendritic cells (Kitajima *et al*, 1996). Our data indicate that Langerhans cells from MHC class II-deficient mice had normal functional characteristics *in vivo* as well as *in vitro*. One possibility is that terminal differentiation of Langerhans cells *in vivo* is T cell independant. Another possibility is that both CD8$^+$ T cells and CD4$^+$ T cells may induce these important functional changes. Additional experiments will be required to differentiate between these two alternatives.

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