MHC Class I^+/II^- Dendritic Cells Induce Hapten-Specific Immune Responses *In Vitro* and *In Vivo*

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Activation requirements and biologic properties of hapten-specific, major histocompatibility complex class I-restricted $CD8^+$ T lymphocytes are not fully understood. To address this issue, a novel $CD45^+$/major histocompatibility complex class $I^+(H\text{-}2^k)/II^-/CD80^+$ dendritic cell line, termed 80/1, which is capable of stimulating naïve, allogeneic $CD8^+$ but not $CD4^+$ T cells *in vitro*, was derivatized with trinitrobenzenesulfonic acid and co-cultured for 4 d with syngeneic, naïve $CD8^+$ T cells. Results obtained showed that trinitrophenyl-derivatized, but not underivatized 80/1 dendritic cells, can induce vigorous proliferation of $CD8^+$ T cells. T-cell blasts generated in this fashion were able to lyse syngeneic, trinitrophenyl-derivatized targets but failed to lyse underivatized or trinitrophenyl-derivatized syngeneic, major histocompatibility complex class I^- mutant cells or allogeneic targets. The ability of 80/1 dendritic cells to prime naïve, syngeneic T cells *in vivo* was tested in a contact hypersensitivity model. C3H/HeN mice were injected subcutaneously with identical numbers of (i) trinitrophenyl-derivatized 80/1 dendritic cells; (ii) trinitrophenyl-derivatized 80/1 dendritic cells fragmented by freeze-thawing cycles; (iii) trinitrophenyl-derivatized fibrosarcoma L929; and (iv) trinitrophenyl-derivatized lymphoma R1.1 cells. Whereas live trinitrophenyl-derivatized 80/1 dendritic cells were able to sensitize for contact hypersensitivity, killed hapten-derivatized 80/1 dendritic cells or control cells failed to do so. Thus, we conclude that 80/1 dendritic cells, when compared with major histocompatibility complex class I^+ non-dendritic cells, can effectively prime naïve, syngeneic $CD8^+$ T cells for hapten-specific responses, probably due to their better costimulatory and migratory properties. ***Key word: contact hypersensitivity. J Invest Dermatol 109:580–585, 1997***

Contact hypersensitivity (CHS) is a T-cell-mediated immune response to reactive haptens that have penetrated the skin. When haptens, not antigenic by themselves, bind to resident proteins, they serve as target(s) for lymphocyte attack, leading to intense inflammation. Numerous investigations have identified dendritic cells (DC) of the skin (epidermal Langerhans cells and dermal DC) as critical in the induction of CHS. It has been demonstrated that (i) mouse skin naturally deficient in Langerhans cells (tail skin) or experimentally depleted of functional Langerhans cells prior to immunization (by ultraviolet irradiation or topical steroid treatment) fails to induce CHS (Toews *et al*, 1980; Lynch *et al*, 1981); (ii) hapten-derivatized Langerhans cells, but not other hapten-conjugated epidermal cells (keratinocytes, dendritic epidermal T cells), induce sensitization even when immunizing conditions are chosen that, in the case of other major histocompatibility complex (MHC) class II-bearing antigen-presenting cells (APC), result in antigen-specific immune tolerance (Ptak *et al*, 1980; Tamaki *et al*, 1981; Sullivan *et al*, 1986); (iii) T cells stimulated *in vitro* by hapten-derivatized Langerhans cells mediate CHS when injected into syngeneic recipients (Hauser, 1990); and (iv) intradermal injection of hapten-derivatized MHC class II^+ dermal cells initiates the development of delayed type hypersensitivity (Tse and Cooper, 1990).

The T-cell response to haptens appears to be more complex than originally believed. Several laboratories have provided evidence that $CD4^+$ T cells represent the effector cells of CHS (Hauser, 1990; Kapsenberg *et al*, 1992; Kondo *et al*, 1996). By contrast, studies in mice depleted of either $CD4^+$ or $CD8^+$ T cells suggest that $CD8^+$ T cells mediate CHS and that the magnitude of the response is regulated by $CD4^+$ T cells (Gocinski and Tigelaar, 1990). Similarly, recent data using mice that are genetically deficient in MHC class I and/or class II molecules, demonstrate that MHC class I-restricted $CD8^+$ T cells mediate CHS to dinitrofluorobenzene (DNFB), and that MHC class II-restricted $CD4^+$ T cells downregulate this inflammatory response (Bour *et al*, 1995). This fits the recent observation that T cells in the draining lymph nodes of hapten-painted mice show polarized patterns of cytokine production, with $CD8^+$ T cells that preferentially produce interferon-γ, and interleukin (IL)-4/IL-10-secreting $CD4^+$ T cells (Xu *et al*, 1996). In accord with the murine studies, clones of T cells isolated from lesional skin of patients with allergy to urushiol were found to be of the $CD8^+$ phenotype (Kalish and Johnson, 1990), implying an effector role for $CD8^+$ T cells in humans as well.

We have previously shown that the MHC class $I^+/II^-/CD80^+$ DC line 80/1 induces proliferation of naïve, allogeneic $CD8^+$ but not $CD4^+$ T cells *in vitro*. $CD8^+$ T cells thus activated lyse target cells expressing the MHC class I-specificity of the stimulator cell (Elbe *et al*, 1994). The sensitizing power of 80/1 DC *in vivo* is evidenced by the recent finding that allo-class I expression on 80/1 DC is sufficient to

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Abbreviations: APC, antigen-presenting cell(s); CHS, contact hypersensitivity; DC, dendritic cell(s); Eu^{3+}, Europium; MHC, major histocompatibility complex; MoAb, monoclonal antibody; NCM, normal culture medium; sc, subcutaneously; TNBS, 2,4,5-trinitrobenzenesulfonic acid; TNCB, trinitrochlorobenzene; TNP, trinitrophenyl; ip, intraperitoneal; iv, intravenous;

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sensitize H-2-disparate recipients for accelerated rejection of skin grafts (Lenz *et al*, 1996). Thus, we felt that this DC line could be an ideal tool to investigate the activation requirements of hapten-specific, MHC class I-restricted $CD8^+$ T lymphocytes.

MATERIALS AND METHODS

Animals C3H/HeN (C3H) (H-2^k) and C57BL/6 (B6) (H-2^b) inbred mice (6–10 wk old) were obtained from the Research Institute for Experimental Animal Breeding and Containment of the Vienna University, Himberg, Austria. For *in vivo* experiments only female mice were used. All animal procedures were approved by the Austrian Ministry of Science, Transportation, and Art (GZ 66.009/160-Pr/4/95).

Cell lines and monoclonal antibodies (MoAb) The DC line 80/1 (MHC class $I^+/II^-/CD45^+/CD3^-/CD54^+/CD80^+/CD86^-$) was generated from skin of day 15 C3H mouse fetuses and maintained in long-term culture in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 25 mM HEPES, 50 μg gentamycin per ml, 1 × antibiotic/antimycotic solution, and 50 μM 2-ME (all from GIBCO Life Technologies, Grand Island, NY) [normal culture medium (NCM)]; the cultures were, in addition, supplemented with IL-2 [5 U recombinant human IL-2 per ml (Amersham, Buckingamshire, U.K.) and murine IL-2 (0.3% culture supernatant of the IL-2-producing myeloma cell line X63Ag8–653; kindly provided by Dr. F. Melchers, Basel Institute for Immunology, Basel, Switzerland)], and 2 μg Concanavalin A (Con A; Pharmacia, Uppsala, Sweden) per ml. The fibrosarcoma cell line L929 (H-2^k; CCL 1) and the T-cell lymphoma line EL4 (H-2^b; TIB 39) were purchased from the American Type Culture Collection (Rockville, MD). The T-cell lymphoma lines R1.1 (H-2^k) and R1E (H-2^{k-}) (Hyman and Stallings, 1976) were generously provided by Dr. F. Karlhofer (*DIAID*, Department of Dermatology, University of Vienna Medical School, Vienna, Austria). MoAb 16–10A1 (anti-CD80), 3E2 (anti-CD54), 15–5–5S (anti-H-2D^k-α1/α2), 53–6.7 (anti-CD8), and isotype control antibodies were purchased from PharMingen (San Diego, CA). Purified hamster IgG was obtained from Cappel (West Grove, PA). Hybridoma 15–3–1S (anti-H-2K^{k}D^k-α1/α2; HB13) and 16–3–1N (anti-H-2K^k; HB25) were obtained from the American Type Culture Collection.

Hapten-modification of cells Cells were washed twice in Hank's balanced salt solution without Ca^{2+} and Mg^{2+} and then incubated (10^7 cells per ml) with 2,4,5-trinitrobenzenesulfonic acid (TNBS; Sigma Chemical Co., St. Louis, MO) at a concentration of 5 mM (pH 7.2) for 10 min at 37°C, followed by three washings in a 10-fold excess of RPMI 1640 containing 10% FBS (viability > 90% as measured by trypan blue exclusion).

T-cell proliferation assay 80/1 DC and L929 cells were x-irradiated (1.5 Gray per min, Philips RT 305, Philips, Vienna, Austria) at 40 and 60 Gray, respectively, and a portion thereof was derivatized with TNBS. Naïve, syngeneic $CD8^+$ T cells were obtained according to a protocol described elsewhere (Elbe *et al*, 1994) (97–99% purity) and cultured at 10^5 cells per well with stimulator cells (10^4 cells per well) in 96-well round-bottom plates (Costar) in either NCM or NCM supplemented with 50 U recombinant human IL-2 per ml. After 3, 4, and 5 d, cells were pulsed with [^{3}H]thymidine (TdR) (37 kBq per well; Amersham) for 8 h and incorporation of the radionucleotide was assessed by β-scintillation spectroscopy (Packard Instruments, Meriden, CT). Data are expressed as mean values ± SD of six replicates per group. Inhibition of T-cell proliferation with MoAb was performed by their addition as previously described (Elbe *et al*, 1994).

Cytolytic T lymphocyte assay Purified $CD8^+$ T cells (2 × 10^6 per well) were co-cultured with trinitrophenyl (TNP)-derivatized or, as a control, underivatized, syngeneic, irradiated 80/1 DC (2 × 10^5 per well) in 24-well flat-bottom culture plates (Costar). After 4 d of culture in NCM or medium containing 50 U recombinant human IL-2 per ml, cells were pooled and enriched for viable cells using a Lympholyte-M gradient (OD 1.087; Cederlane Laboratories, Hornby, Ontario, Canada). Their cytotoxic potential was measured in an Europium (Eu^{3+}) release assay that was performed essentially as described (Elbe *et al*, 1994). Varying numbers of effector cells were mixed with fixed numbers of Eu^{3+}-labeled, hapten-derivatized or underivatized target cells (R1.1, R1E, EL4, 80/1 DC, L929, or spleen cells from C3H and B6 mouse strains cultured for 72 h in NCM containing 2 μg Con A per ml). The percentage of specific lysis was calculated by the formula:

$$\%\ \text{specific lysis} = \frac{\text{experimental } Eu^{3+} \text{ release} - \text{spontaneous } Eu^{3+} \text{ release}}{\text{maximum } Eu^{3+} \text{ release} - \text{spontaneous } Eu^{3+} \text{ release}} \times 100$$

In all instances, spontaneous Eu^{3+} release was between 10 and 20% of the maximum release obtained by 1% Triton X-100 lysis. SD of the means of triplicates were less than 5% and are omitted from the figures for clarity.

Sensitization and elicitation of CHS C3H mice were anesthetized intraperitoneally (ip) with tribromoethanol (Aldrich Chemical Company, Steinheim, Germany; 2.5% in isotonic saline, 350 μl per mouse). A positive control panel of mice was painted on dry shaved abdominal skin with either 50 μl of 2% trinitrochlorobenzene (TNCB; TCI, Tokyo Kasei, Tokyo, Japan) or 25 μl of 0.5% DNFB (Sigma-Aldrich, Vienna, Austria) in a 4:1 acetone and olive oil carrier solution (epicutaneous control panel). Other panels of mice were injected with selected hapten-derivatized and, for control purposes, underivatized cells (titrated cell numbers: 10^4–10^7 cells per mouse) in 100 μl HBSS with Ca^{2+} and Mg^{2+}, either subcutaneously (sc; into the base of the tail), intravenously (iv; into the tail vein) or ip. Five days later, these three panels and a naïve, negative control panel were challenged on the dorsal and ventral surfaces of their right ears with 20 μl of 0.5% TNCB. In some experiments, as a specificity control, the other ear was painted with 0.2% DNFB. Ear thickness was measured with an engineer's micrometer (Hahn und Kolb, Stuttgart, Germany) before challenge and at certain intervals up to 72 h after challenge. The prechallenge value was subtracted from the value assessed at selected time points after challenge. Experimental groups consisted of four mice each.

Histologic analysis of challenged ear tissue Following euthanasia, ears were removed from mice 24 h after challenge and fixed in 10% buffered formalin. The tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Statistical analysis Ordinary one-way ANOVA was used to evaluate the significance of experimental *versus* control group data.

RESULTS

TNP-derivatized 80/1 DC prime naïve, syngeneic $CD8^+$ T cells *in vitro* Using a hapten model, we attempted to determine whether 80/1 DC can serve as APC for the stimulation of naïve, syngeneic $CD8^+$ T cells. For this purpose, 80/1 DC were either derivatized with TNBS or not and co-cultured for 3–5 days with naïve, syngeneic $CD8^+$ T cells. We found that TNP-derivatized 80/1 DC, but not underivatized 80/1 DC, induce vigorous proliferative responses in $CD8^+$ T cells with peak responses occurring on day 4 (**Fig 1**). Inspection of culture wells 1–3 d after initiation revealed cluster formation between stimulator and responder cells, indicating T-cell proliferation and lymphokine production. In an attempt to generate higher numbers of proliferating T cells, we added recombinant human IL-2 to the co-cultures of 80/1 DC and $CD8^+$ T cells. This led to a more vigorous T-cell response to both underivatized and hapten-derivatized 80/1 DC, but the proliferation induced by TNP-derivatized 80/1 DC remained significantly higher (data not shown). To determine whether other MHC class I^+ cells, when derivatized with TNBS, are also able to stimulate naïve, syngeneic $CD8^+$ T cells *in vitro*, we tested the MHC class I^+ fibrosarcoma L929 cell line in a similar experimental setting as described above. Derivatized L929 cells, which express levels of MHC class I molecules comparable with those of 80/1 DC, failed to induce a significant proliferative response of naïve, syngeneic $CD8^+$ T cells (**Fig 2**). This shows that MHC class I expression on stimulator cells does not suffice to induce productive T-cell responses and implies that the ability of 80/1 DC to stimulate T-cell proliferation in the absence of MHC class II molecules is presumably due to the additional delivery of co-stimulatory signals. To test this possibility, we performed the co-cultures of TNP-80/1 DC with $CD8^+$ T cells in the presence of antibodies that block the function of different co-stimulatory molecules. **Table I** shows that the anti-CD80 MoAb 16–10A1, but not its isotype control, essentially abrogated the T-cell proliferative response. The inhibition induced by anti-CD54 MoAb was much less pronounced. These observations point to the critical involvement of the B7-CD28/CTLA-4 receptor–counter-receptor pairs for the productive presentation in our system.

The generation of hapten-specific T cells is MHC class I-dependent In order to determine whether the $CD8^+$ T-cell response to TNP-derivatized 80/1 DC is regulated by MHC class I molecules, selected MoAb directed against different MHC class I determinants were added to the cultures. As shown in **Table I**, the continuous presence of the MoAb 15–3–1S that is directed against H-2K^k and

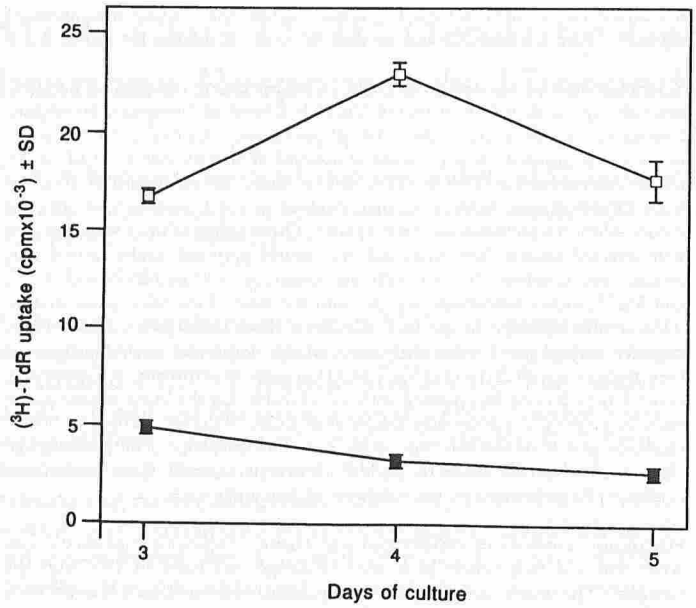


Figure 1. TNP-derivatized 80/1 DC induce vigorous proliferative responses in naïve, syngeneic CD8$^+$ T cells. Irradiated, underivatized (■) or TNP-derivatized (□) 80/1 DC (10^4 per well) were co-cultured with naïve, syngeneic CD8$^+$ T cells (10^5 per well) in 96-well round-bottom culture plates. On days 3–5, cells were pulsed with [^{3}H]TdR for an additional 8 h. Data are expressed as mean ± SD of six replicates per group. When cultured alone, T cells (nonstimulated or Con A-activated), as well as irradiated stimulators, did not proliferate. Experiments were repeated four times, yielding comparable results.

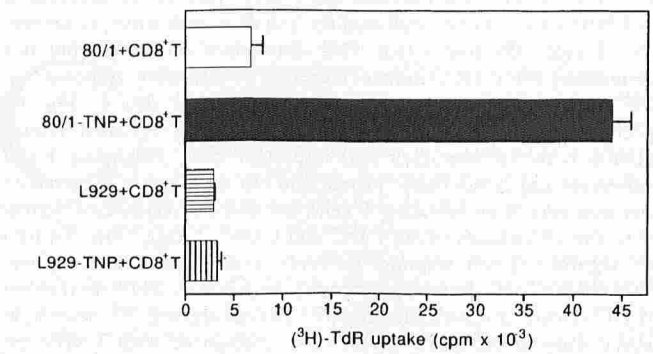


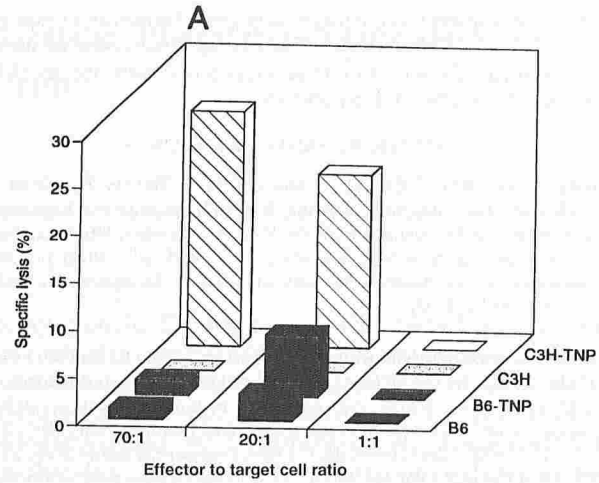
Figure 2. TNP-derivatized 80/1 DC, but not L929 cells, induce proliferation of syngeneic T cells. Irradiated, underivatized, or TNP-derivatized 80/1 DC or L929 cells were co-cultured with naïve, syngeneic CD8$^+$ T cells as described in **Fig 1.** On day 4, cells were pulsed with [^{3}H]TdR for an additional 8 h. Data are expressed as mean ± SD of six replicates per group.

Table I. Inhibition of TNP-80/1 DC-induced CD8$^+$ T-Cell proliferation by MoAb[a]

MoAb added	(^{3}H)-TdR uptake (cpm) ± SD
none	163,273 ± 13,020[b]
hIgG (isot. ctrl.)	172,430 ± 8,443
CD80 (16–10A1)	11,099 ± 854
CD54 (3E2)	96,300 ± 11,181
mIgG2a (isot. ctrl.)	149,121 ± 15,570
K^kD^k (15–3–1S)	33,663 ± 4,550
K^k (16–3–1N)	39,433 ± 5,919
D^k (15–5–5S)	177,677 ± 9,015
rIgG2a (isot. ctrl.)	174,501 ± 15,234
CD8 (53–6.7)	13,007 ± 1,620

[a]Irradiated, TNP-derivatized 80/1 DC and naïve, syngeneic CD8$^+$ T cells were co-cultured for 4 days in the continuous presence of the indicated MoAb (1–10 μg per ml). Cell proliferation was measured by [^{3}H]TdR incorporation.

[b]Values represent means of nine replicates per group ± SD.



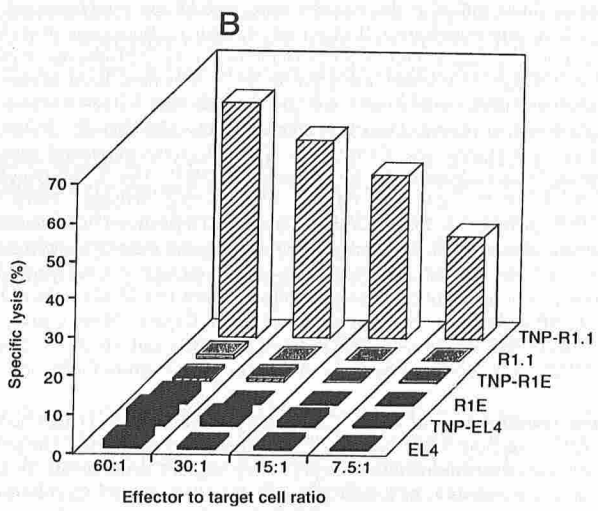


Figure 3. Cytotoxicity of CD8$^+$ T cells after stimulation with TNP-derivatized, syngeneic 80/1 DC. Irradiated, TNP-derivatized 80/1 DC (2×10^5 per well) were co-cultured with naïve, syngeneic CD8$^+$ T cells (2×10^6 per well) in 24-well flat-bottom culture plates in the presence of recombinant human IL-2 (50 U per ml). After 4 d of culture, a varying number of effector cells was mixed with fixed numbers of Eu^{3+}-labeled, underivatized, or TNP-derivatized (*A*) syngeneic (C3H) or allogeneic (B6) Con A-stimulated spleen lymphoblasts or (*B*) syngeneic (MHC class I$^+$ R1.1, MHC class I$^-$ R1E) or allogeneic (MHC class I$^+$ EL4) tumor cell lines. After a 4-h incubation, supernatants were harvested for determination of percentage specific lysis. Data shown are representative from one out of two (*A*) or six (*B*) experiments.

also cross-reacts with H-2D^k determinants, greatly diminished the hapten-specific T-cell response. A similar result was obtained when the H-2K^k-specific MoAb 16–3–1N was added to the primary cultures. In contrast, the addition of the H-2D^k MoAb 15–5–5S did not show an inhibitory effect. These results demonstrate that, in our system, H-2K^k rather than H-2D^k moieties are involved in hapten recognition. We also observed that the addition of the anti-CD8 MoAb 53–6.7, but not its isotype control, almost entirely prevented T-cell proliferation. In summary, these data show that the priming of CD8$^+$ T cells by hapten-derivatized 80/1 DC occurs via the MHC class I-CD8 pathway in a helper cell-independent fashion.

TNP-derivatized, but not underivatized, 80/1 DC stimulate naïve, syngeneic CD8$^+$ T cells for cytotoxicity To study the cytolytic activity of T-cell lymphoblasts that had been generated in a 4-d co-culture of TNP-derivatized 80/1 DC and syngeneic CD8$^+$ T cells, we performed an Eu^{3+}-release assay, using selected TNP-derivatized or underivatized syngeneic and allogeneic cells as targets. We observed in repeated experiments that TNP-80/1 DC-primed CD8$^+$ T cells display significant lysis of TNP-derivatized-H-2^k targets

(Con A-stimulated spleen lymphoblasts, R1.1, 80/1 DC, L929) but not of underivatized (Con A-stimulated spleen lymphoblasts, R1.1, 80/1 DC, L929), TNP-derivatized syngeneic MHC class I^- targets (R1E), and H-2^b targets (Con A-stimulated spleen lymphoblasts, EL4) (**Fig 3*A*** and ***B*** and data not shown). Taken together, these data provide evidence that 80/1 DC can induce MHC class I-restricted cytotoxic T-lymphocyte activity in primed $CD8^+$ T cells in the absence of help by $CD4^+$ T cells.

TNP-derivatized 80/1 DC immunize for CHS *in vivo* To determine whether 80/1 DC are also able to prime naïve T cells *in vivo*, we used the CHS model. We found that TNP-derivatized 80/1 DC, introduced sc, induce a CHS response that was similar in magnitude and kinetics to that elicited by epicutaneous sensitization (**Fig 4**). In contrast, when TNP-derivatized 80/1 DC were administered iv or ip, they failed to sensitize at any cell concentration tested (data not shown).

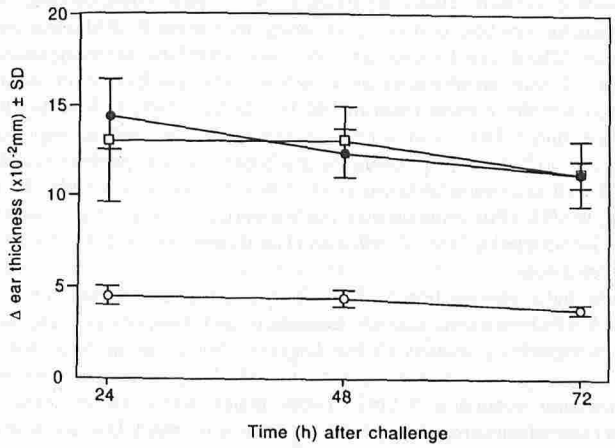


Figure 4. TNP-derivatized 80/1 DC prime for CHS *in vivo*. On day 0, C3H mice were injected sc with TNP-derivatized 80/1 DC (□) or painted on abdominal skin with TNCB (●). The negative control panel (○) received no treatment. On day 5 all mice were challenged with TNCB on both sides of one ear. Ear thickness was measured 24, 48, and 72 h later.

Histologic examination of ear biopsies from TNCB-sensitized mice (**Fig 5*A***) and from mice injected sc with TNP-80/1 DC (**Fig 5*B***) showed a pronounced inflammatory reaction that was characterized by interstitial oedema and infiltration of inflammatory cells. TNCB-challenged ear tissue from unsensitized mice did not exhibit marked oedema or cellular infiltrate (**Fig 5*C***).

To test the specificity of the TNP-induced immune response, mice were sensitized with TNP-derivatized 80/1 DC and then challenged with either TNCB or DNFB. As seen in **Fig 6**, recipients of TNP-derivatized, but not underivatized, 80/1 DC mounted a significant CHS response when challenged with TNCB on the right ears. By contrast, the same experimental animals, when challenged with DNFB on the left ears, did not develop an ear swelling response to this hapten. These data indicate that the CHS response induced by TNP-derivatized 80/1 DC is restricted to the initial hapten-self determinants seen upon primary immunization.

To investigate whether the strong sensitizing capacity of 80/1 DC is an intrinsic property of all MHC class I^+ cells, we tested two other cell lines of the same haplotype and similar MHC class I expression levels (L929, R1.1) for their capacity to prime for CHS. 80/1 DC, L929, and R1.1 cells that had been TNP-derivatized were inoculated sc (10^6 cells per mouse), and 5 days later all mice and the negative control panel were challenged with TNCB on both sides of their right ears. **Figure 7** documents the sensitizing power of haptenized 80/1 DC, as well as the inability of nonprofessional APC (TNP-derivatized L929 and R1.1 cells) to prime for CHS.

Haptens are directly presented by 80/1 DC To determine whether sensitization for CHS, under the experimental conditions chosen in this study, results from direct hapten-presentation by 80/1 DC to naïve T cells in lymphoid organs or, alternatively, from indirect hapten-presentation by host APC, we compared the sensitizing

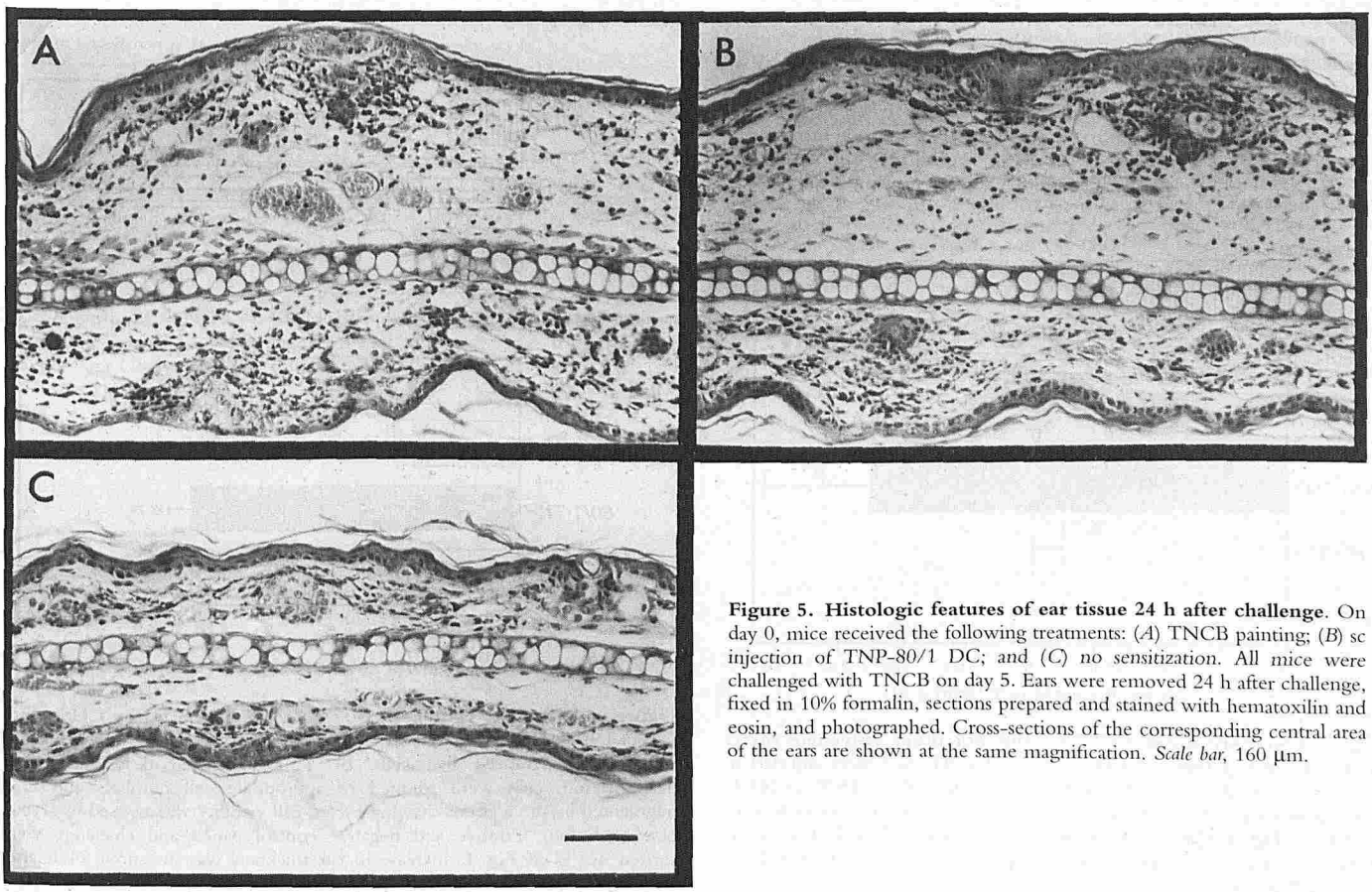


Figure 5. Histologic features of ear tissue 24 h after challenge. On day 0, mice received the following treatments: (*A*) TNCB painting; (*B*) sc injection of TNP-80/1 DC; and (*C*) no sensitization. All mice were challenged with TNCB on day 5. Ears were removed 24 h after challenge, fixed in 10% formalin, sections prepared and stained with hematoxilin and eosin, and photographed. Cross-sections of the corresponding central area of the ears are shown at the same magnification. *Scale bar*, 160 μm.

capacities of viable and fragmented 80/1 DC. We found that, in contrast to recipients of live TNP-modified 80/1 DC, animals injected with cell fragments of 10^6 TNP-derivatized 80/1 DC did not develop a CHS response when ear-challenged with TNCB (**Fig 8**). These results lend support to the concept that the sensitizing power of hapten-modified 80/1 DC resides in their capacity to approach and directly interact with naïve T cells.

DISCUSSION

The question whether professional and/or semiprofessional APC can directly prime $CD8^+$ T cells in the absence of $CD4^+$ T cells has long been a matter of debate (Elbe and Stingl, 1995). In this study, we have shown that TNP-derivatized 80/1 DC can generate hapten-specific, MHC class I-restricted $CD8^+$ cytotoxic T cells *in vitro* in the absence of help. We undertook great efforts to exclude an influence of $CD4^+$ T cells and MHC class II molecules on our *in vitro* system. These included a very stringent purification procedure of $CD8^+$ lymph node T cells and the use of stimulator cells lacking not only constitutive, but also cytokine-inducible (A. Elbe-Bürger, manuscript in preparation) MHC class II expression. Still, we observed abundant cluster formation between TNP-derivatized 80/1 DC and $CD8^+$ T cells. This interaction is mediated and restricted by MHC class I antigens as evidenced (i) by the capacity of an anti-MHC class I ($H\text{-}2K^k$ but not $H\text{-}2D^k$) mAb to almost completely abolish TNP-80/1 DC-induced syngeneic $CD8^+$ T-cell proliferation, and (ii) by the cytotoxic activity of TNP-80/1-primed $CD8^+$ lymphoblasts against syngeneic, TNP-derivatized but not syngeneic, MHC class I^- or allogeneic, TNP-derivatized target cells.

Whether processing of TNP is required for hapten presentation by 80/1 DC remains to be elucidated. Due to their chemical structure, aromatic haptens can be delivered via different presentation pathways. Because of lipid-solubility, they may enter the cell, react with cytoplasmic proteins, and – after processing by the endogenous route – be presented to MHC class I-restricted $CD8^+$ T cells. Alternatively, direct binding of haptens, without processing, to a peptide in the groove of surface MHC class I molecules may also contribute to recognition by $CD8^+$ T cells. Both routes of hapten presentation have been demonstrated for the hapten urushiol (Kalish *et al*, 1994). It has also been shown that $CD4^+$ and $CD8^+$ T-cell clones recognize haptenated peptides and that the position of the hapten in the peptide is critical for T-cell activation (Martin *et al*, 1993; Cavani *et al*, 1995; Kohler *et al*, 1995), thus emphasizing the existence of both hapten-specific class I-restricted $CD8^+$ T cells and class II-restricted $CD4^+$ T cells for a given hapten.

We have demonstrated that TNP-derivatized $CD80^+$ DC, but not TNP-derivatized $CD80^-$ fibroblasts and lymphoma cells, when administered sc, induce CHS. Together with the finding that the continuous presence of the anti-CD80 MoAb inhibits the TNP-80/1 DC-driven syngeneic $CD8^+$ T-cell proliferation by $> 90\%$, and experiments showing that CD80 expression on 80/1 DC is absolutely required for the induction of accelerated skin graft rejection (Lenz *et al*, 1996), these data emphasize the necessity of a second signal for productive presentation of hapten in our system.

Our *in vivo* data demonstrating that haptenized MHC class II^- 80/1 DC, when administered sc, can prime for CHS are in keeping with results by other investigators, showing that $CD4^+$ T-cell-depleted or MHC class II-deficient animals exhibit enhanced ear swelling responses

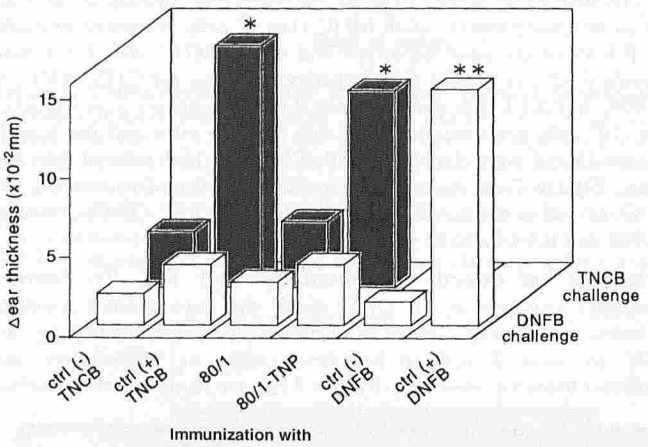


Figure 6. Mice injected with TNP-derivatized 80/1 DC respond to relevant, but not irrelevant hapten. C3H mice were injected sc with underivatized or TNP-derivatized 80/1 DC or painted on abdominal skin with either TNCB or DNFB. Two negative control panels did not receive any treatment. On day 5, mice were challenged with TNCB (*black bars*) on one ear and with DNFB (*white bars*) on the other ear. Ear swelling response was measured after 24 h. $*p < 0.05$; $**p < 0.01$.

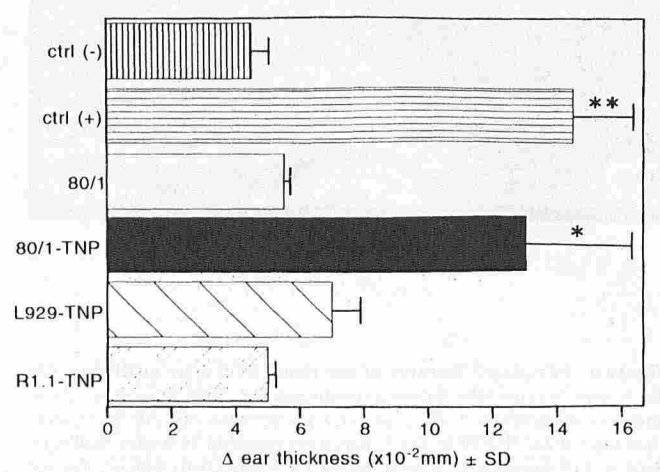


Figure 7. TNP-derivatized 80/1 DC, but not other syngeneic MHC class I^+/II^- cells prime for CHS. Panels of C3H mice were injected sc either with one of the three TNP-derivatized cell lines 80/1, L929, or R1.1, or with underivatized 80/1 DC. The positive and negative control panels were treated as in **Fig 4**. On day 5 all mice were challenged with TNCB on both sides of one ear. Changes in ear thickness were measured 24 h later. $*p < 0.05$; $**p < 0.01$.

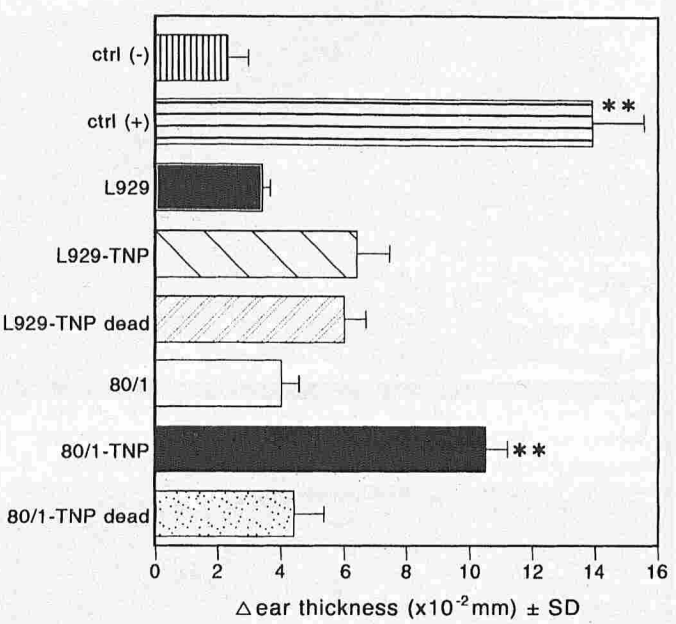


Figure 8. Only live TNP-derivatized 80/1 DC prime for CHS. 80/1 DC or L929 cells (10^6 each) were injected sc into C3H mice as underivatized cells, TNP-derivatized live cells, or TNP-derivatized dead cells (after derivatization, cells were adjusted to appropriate cell numbers and then fragmented by three freeze-thawing cycles; cell viability was assessed by trypan blue exclusion). Positive and negative control panels and challenge were carried out as in **Fig 4**. Increase in ear thickness was measured 24 h after challenge. $**p < 0.01$.

(Gocinski and Tigelaar, 1990; Bour *et al*, 1995). The inability of fragmented TNP-derivatized 80/1 DC and haptenized fibroblasts or lymphoma cells to efficiently prime for CHS suggests that live 80/1 DC, and not host APC, directly present hapten to naïve $CD8^+$ T cells. Although the final experimental proof is still lacking, we propose that sc-injected 80/1 DC can migrate via the afferent lymphatics to reach the draining lymph nodes in numbers sufficient for sensitization. The similarities between 80/1 DC and cells of the Langerhans cell lineage (Elbe *et al*, 1994), as well as evidence obtained in preliminary migration studies (A. Kolesaric, unpublished observation), give support to this assumption.

Interestingly, TNP-derivatized 80/1 DC fail to induce CHS when injected iv or ip. Preliminary data indicate that, whereas their ip injection is an immunologic null event, the iv administration of haptenized 80/1 DC leads to hyporesponsiveness to subsequently painted hapten (A. Kolesaric, unpublished observation). We are currently investigating whether the differences in the sensitizing capacity of 80/1 cells applied via different routes are linked to their migratory properties and/or are due to the delivery of different maturational stimuli at different tissue compartments.

Our data imply that at least two components, i.e., co-stimulation and migration, are essential for the efficient induction of CHS. Similarly to other DC lines (K. Ariizumi *et al*, manuscript in preparation), easily accessible 80/1 DC could be a useful tool for a better understanding of both cellular and molecular events involved in this process. Considerable evidence suggests that, upon appropriate modulation (e.g., cytokine treatment; Enk *et al*, 1993;[1]) or other treatment modalities (Simon *et al*, 1991), immunogenic DC can be converted to tolerogenic APC. It is thus conceivable that, by the selective interference with components critical for sensitization, 80/1 DC could be a promising therapeutic vaccine for CHS and other T-cell-mediated skin diseases.

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