The Journal of Immunology

RESEARCH ARTICLE | JANUARY 15 1991

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J Immunol (1991) 146 (2): 485–491.
https://doi.org/10.4049/jimmunol.146.2.485

0022-1767/91/1462-0485$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 485–491, No. 2, January 15, 1991
Printed in U.S.A.

ULTRAVIOLET B RADIATION CONVERTS LANGERHANS CELLS FROM IMMUNOGENIC TO TOLEROGENIC ANTIGEN-PRESENTING CELLS

Induction of Specific Clonal Anergy in CD4+ T Helper 1 Cells[1]

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We have recently demonstrated that a single dose (200 J/m^2) of UVB radiation abrogates the capacity of mouse epidermal Langerhans cells (LC) or splenic adherent cells (SAC) to present keyhole limpet hemocyanin (KLH) to Ag-specific, MHC-restricted CD4+ Th1 cells. In the present study we determined whether such Th1 unresponsiveness represented long-lasting immunologic tolerance. To address this question, Th1 were preincubated with KLH-pulsed UVB-LC or UVB-SAC, then isolated and restimulated with unirradiated APC (LC or SAC) plus KLH or with exogenous rIL-2 in the absence of APC. Preincubation with KLH and UVB-LC or UVB-SAC rendered Th1 unresponsive to subsequent restimulation with APC and KLH. In addition, such Th1 were defective in their autocrine IL-2 production, but could respond normally to exogenous rIL-2, indicating that unresponsiveness was due to functional inactivation and not to cell death. Th1 unresponsiveness was Ag-specific, MHC-restricted, and long lasting (>16 days). In addition, it appears that Th1 unresponsiveness is not due to the release of soluble suppressor factors from UVB-LC or UVB-SAC because supernatants from such cells had no effect on Th1 proliferation. Addition of unirradiated allogeneic SAC during preincubation prevented the induction of unresponsiveness by UVB-LC or UVB-SAC, suggesting that UVB interferes with the capacity of LC or SAC to deliver a costimulatory signal(s) that can be provided by allogeneic SAC. We conclude that UVB can convert LC or SAC from immunogenic to tolerogenic APC.

Cutaneous exposure to UVB[3] can change the outcome of Ag-specific T cell-mediated immune responses in skin, including the reversal of CH from immunity to specific tolerance (1–4). Toews et al. (1) showed that exposure of mouse skin to UVB not only prevents the induction of CH to epicutaneously applied hapten, but also induces specific tolerance when the same hapten is applied to unirradiated sites at a later date (1). These findings were remarkable in that they closely parallel the extent of UVB-induced damage to epidermal LC, as determined by loss of dendritic morphology and surface ATPase activity (1). Subsequently, Cruz et al. (5) identified LC as the relevant epidermal target of this UVB-induced down-regulation of CH; they demonstrated that LC treated with UVB in vitro and then derivatized with hapten were capable of initiating specific tolerance rather than CH, when injected i.v. into syngeneic mice. Little, however, is known about the mechanism(s) by which UVB-LC induce tolerance of Ag-specific T cells.

Current models indicate that T cell tolerance can be induced directly through the interaction of T cells with the MHC/Ag complex or indirectly via Ts cells (6, 7). The former model can be generated in at least two ways: 1) by physical elimination (clonal deletion) and 2) by functional inactivation (clonal anergy) (6, 7). In particular, clonal anergy has been produced in vitro using several techniques, including treatment of T cells before Ag-specific stimulation with excess Ag, anti-CD3 mAb, anti-CD2 mAb, or exogenous IL-2 (8–13); stimulation of T cells with modified APC (i.e., treatment with chemical fixatives (14–18)); or Ag presented by purified Ia molecules on planar lipid membranes (19). Clonal anergy has also been produced in vivo in a population of autoreactive T cells that recognize Mls or IE Ag; such cells were functionally inactivated rather than physically deleted (20–22).

Very recently, we observed a single dose of UVB (200 J/m^2) to produce disparate effects on the capacity of LC to present KLH to Th1 and Th2 cells: UVB-LC stimulated proliferation of Th2, but not Th1 (23). Inasmuch as Th1 cells are important mediators of DTH reactions such as CH (24–26), these findings have direct implications for the regulation of T cell-mediated immune responses in vivo. We have postulated UVB-LC to alter the ratio of Th1 and Th2 cells generated during DTH. In this context, UVB-LC may suppress DTH by inducing two distinct down-regulatory events: 1) a long lasting immunologic tolerance in Th1, and 2) the release of Th2 factors, i.e., cytokine synthesis inhibitory factor (27) that can inhibit

Received for publication August 30, 1990.
Accepted for publication October 26, 1990.
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[1] This work was supported by the National Institute of Health Grants AI 27404, AR 40042, AR 35068, and by a research grant from Mary Kay Cosmetics, Inc., Dallas, TX. J.C.S. has been supported by Grant Si 397/1-1 from the Deutsche Forschungsgemeinschaft and by the Ken Burdick Memorial Research Fellowship Grant from the Dermatology Foundation.
[2] Address correspondence and reprint requests to Dr. Jan C. Simon, Department of Dermatology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9069.
[3] Abbreviations used in this paper: UVB, low-dose UVB-irradiation; CH, contact hypersensitivity, DTH, delayed-type hypersensitivity; ECDI, 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide; SAC, splenic adherent cells; LC, epidermal Langerhans cells; IEC, interface epidermal cells enriched for LC by density gradient centrifugation; KLH, keyhole limpet hemocyanin; SAC, Splenic adherent cells; SC, spleen cells; SN, supernatant; IP, inositol phosphate; PKC, protein kinase C.

production of Th1-derived lymphokines important for the generation of DTH (i.e., IFN-γ). In the present study, we demonstrate that UVB-LC (and UVB-SAC) induce long lasting, Ag-specific clonal anergy in Th1 cells, consistent with the possibility that UVB-LC cause Ag-specific tolerance in DTH responses by inducing clonal anergy of Th1 cells.

MATERIALS AND METHODS

Animals. Female BALB/c and C3H/HEN mice, 8 to 16 wk of age, were purchased from Simonsen Laboratories (Gilroy, CA).

Media and reagents. RPMI 1640 (GIBCO, Grand Island, NY) was supplemented with 10% heat-inactivated FCS, 25 mM HEPES (Sigma Chemical Co., St. Louis, MO), 1 mM nonessential amino acids (GIBCO), 2 mM L-glutamine (GIBCO), 1 nM 2-ME (Sigma), Indomethacin (1 μg/ml), and 1% penicillin-streptomycin (GIBCO). MEM (GIBCO) was supplemented with DNAse I (1.3 U/ml) (ICN Biomedicals, Inc., Costa Mesa, CA), 10% FBS, and 1% penicillin-streptomycin (GIBC). HBSS (GIBCO) was supplemented with 1% penicillin-streptomycin (GIBCO). KLH and ECDI were purchased from Calbiochem-Behring Corp., La Jolla, CA, and Indomethacin from Merck, Sharp & Dohme, Rahway, NJ. [^{3}H]-TdR (sp. act. 6.7 Ci/mM) was obtained from ICN Radiochemicals, Irvine, CA. Human rIL-2, (5000 U/ml) was obtained from Amgen, Thousand Oaks, CA. mAb 11B11 (anti-IL-4) and S4B6 (anti-IL-2) (28, 29) were gifts from Dr. E. S. Vitetta, UT Southwestern, Dallas, TX.

Isolation of epidermal cells and purification of LC. Epidermal cell suspensions were obtained as described previously (5, 30, 31). Briefly, mice were killed by ether euthanasia, and trunk skin was removed surgically. After mechanical removal of s.c. tissue, the skin was cut into 1 × 5 cm pieces and floated in 0.3% trypsin (type XI, Sigma) in GNK buffer solution (0.17% glucose, 0.88% NaCl, and 0.04% KCl) supplemented with 0.1% DNAse I for 16 h at 4°C. Epidermis was separated from dermis, placed into 0.3% supplemented trypsin for 20 min at 37°C, and then washed in supplemented MEM. Stratum corneum, hair, and other debris were removed by filtration through Sera-Sepra columns (Evergreen Scientific, Los Angeles, CA). Disaggregated cells were washed and resuspended in supplemented RPMI and then subjected to density gradient centrifugation (Histopaque: 1.083, Sigma) to enrich for LC. Cells from the interface were harvested, washed, and resuspended in supplemented RPMI; hereafter the interface population will be referred to as IEC. IEC viability as assessed by trypan blue exclusion ranged from 85 to 97%. IEC were then sorted into Ia$^+$ (that is LC) and Ia$^-$ populations by flow cytometry, as described previously (30). Briefly, IEC were stained at 4°C with anti I-A^d mouse IgG2a mAb (Becton Dickinson, Mountain View, CA), followed by a FITC-conjugated F(ab)'2 rabbit-anti-mouse (H + L) secondary Ab (Zymed, San Francisco, CA). Stained cells were resuspended in supplemented MEM and then sorted using a Becton Dickinson FACstar. This procedure routinely yielded 95 to 100% Ia$^+$ LC.

Spleen cell suspensions. Mice were killed by ether euthanasia; spleens were removed surgically and then disaggregated by gentle teasing and pipetting in supplemented HBSS. Clumps and other debris were removed by filtration through Sera-Sepra columns. Cells were washed and resuspended in supplemented RPMI. Viability as determined by trypan blue exclusion ranged from 90 to 100%.

T cells. The KLH-specific, Ia^d-restricted Th1 clone, HDK-1 was provided by Dr. T. Mosmann (University of Alberta, Edmonton, Canada). These cells secrete IFN-γ and IL-2 in response to KLH presented by an Ia$^+$ accessory cell (32).

UVB radiation. SC and LC were suspended in supplemented RPMI and placed into 35-mm^2 petri dishes (Falcon, Oxnard, CA). The UV light source was a bank of four unfiltered FS20 fluorescent tubes ("Sunlamp," Westinghouse, Pittsburgh, PA) placed 46 cm over the target. Emission was of a broad band spectrum (250 to 400 nm) with a peak at 313 nm (1, 5, 33). UVB was administered as a single dose of 200 J/m^2, as determined at the surface of the cell suspension with an IL 700 Research radiometer (Newburyport, MA) equipped with a SEE 240 photodetector. Sham-treated cell populations served as control. After irradiation, the viability of washed cells ranged from 80 to 90% for both irradiated and unirradiated cells as determined by trypan blue exclusion.

ECDI fixation of APC. SC were fixed with ECDI either before or after Ag-pulsing as described previously (14, 34). SC were incubated for 1 h at 4°C in 0.44 ml of 0.9% NaCl containing 75 mM ECDI; cells suspended in NaCl alone served as control. Subsequently, SC were washed extensively in serum-free RPMI; viability (by trypan blue exclusion) of treated or untreated cells ranged from 85 to 95%.

Ag pulsing. APC were pulsed with KLH (100 μg/ml) in supplemented RPMI for 1 h at 37°C in 5% CO_2 and then washed four times in supplemented HBSS to minimize Ag carry-over. Cells incubated in RPMI alone served as control. After Ag pulsing, SC were γ-irradiated (3000 rad, ^{137}Cs). Based on preliminary experiments that showed similar background proliferation of γ-irradiated or unirradiated LC, γ-irradiation was omitted in subsequent experiments.

Preincubation of Th1 with APC and Ag. After UVB irradiation and Ag pulsing, SC, or LC, suspended in supplemented RPMI, were seeded into 24-well flat-bottom plates (Corning, Corning, NY) at densities of 6.4 × 10^6 or 4 × 10^4 cells/dish, respectively. SC were allowed to adhere to the dish for 2 h at 37°C in 5% CO_2; nonadherent cells were removed by gentle pipetting. The remaining adherent cells are referred to hereafter as SAC. Th1 in supplemented RPMI (2 × 10^5 cells/dish) were then added to SAC or LC. In some experiments, allogeneic SAC from C3H/HEN mice were added to the preincubation cultures at 3.2 × 10^6 cells/dish.

Restimulation of Th1 with normal APC and Ag. After 16 to 20 h of preincubation at 37°C in 5% CO_2, Th1 were recovered by density gradient centrifugation (Histopaque 1.083, Sigma) and washed extensively in HBSS. Th1 were either restimulated immediately or rested for 1 to 16 days in RPMI supplemented with rIL-2 (2 U/ml) in the absence of APC. Th1 (5 × 10^4 cells/dish) were then restimulated either with unirradiated, KLH-pulsed APC (SAC or IEC at 8 × 10^5 or 1.2 × 10^5 cells/dish) or without APC in RPMI supplemented with IL-2 (100 U/ml) in flat-bottom 96-well microtiter plates (Corning). After 48 h of incubation at 37°C in 5% CO_2, [^{3}H]TdR (1 μCi/well) was added for the final 16 h of culture to assess Th1 proliferation. Cells were lysed with distilled water and collected onto nitrocellulose filter paper using a PHD cell harvester (PHD, Cambridge, MA). Incorporated [^{3}H]-TdR was determined by liquid scintillation spectroscopy. Cultures were carried out in triplicate and results were calculated as mean cpm ± SEM.

SN. SN from unirradiated or UVB-irradiated IEC or SAC, either cultured alone or preincubated with Th1 in the presence of KLH as described above, were harvested after 20 h of culture and stored at −70°C until use. SN were then added at a 1/1 dilution to cocultures of resting Th1 and unirradiated IEC or SAC described previously. At 64 h, Th1 proliferation was determined by [^{3}H]-TdR incorporation.

Measurement of IL-2 production. IL-2 activity was assayed by its ability to induce proliferation of IL-2-dependent HT-2 cells (gift of Dr. E. S. Vitetta, UT Southwestern, Dallas) as described (28, 35). Briefly, SN were harvested at 20 h from preincubation or restimulation cultures of Th1 and APC were stored at −70°C until use. HT-2 in RPMI were plated into 96-well round-bottom well microtiter plates (Corning) (5 × 10^3 cells/dish) in the presence or absence of anti-IL-4 (11B11) or anti-IL-2 mAb (S4B6) (each at 2 μg/ml), and SN were added at a 2:1 ratio. HT-2 incubated in RPMI alone served as negative control and HT-2 cultured with graded doses of rIL-2 served as positive control. After 36 h of incubation at 37°C in 5% CO_2, HT-2 proliferation was determined by measuring [^{3}H]-TdR incorporation.

Statistical analysis. Mean values ± SEM were calculated from triplicate cultures. Student's *t*-test was performed to determine statistical significance.

RESULTS

Low-dose UVB-irradiated LC or SAC induce Ag-specific unresponsiveness in Th1. To test whether UVB-APC would induce proliferative unresponsiveness, Th1 were preincubated with irradiated or unirradiated APC (LC or SAC) in the presence or absence of KLH. Th1 were then isolated and restimulated either with unirradiated, KLH-pulsed APC (IEC or SAC) or with rIL-2 in the absence of APC; alternatively, Th1 were not restimulated at all. Preincubation with UVB-LC or UVB-SAC reduced the proliferative response of Th1 to subsequent restimulation by 70 and 85%, respectively (Fig. 1 *A* and *C*). However, such Th1 were fully responsive to stimulation with rIL-2 (Fig. 1 *B* and *C*).

Preincubation with UVB-LC or UVB-SAC in the absence of KLH did not reduce Th1 proliferation (Fig. 1 *A* and *C*), indicating that the development of Th1 unresponsiveness required Ag and that it was not due to nonspecific suppressor mechanisms induced by irradiated APC. Th1 preincubated with KLH-pulsed, unirradiated SAC proliferated to a greater degree than Th1 preincubated with SAC or UVB-SAC in the absence of KLH (Fig. 1 *A*

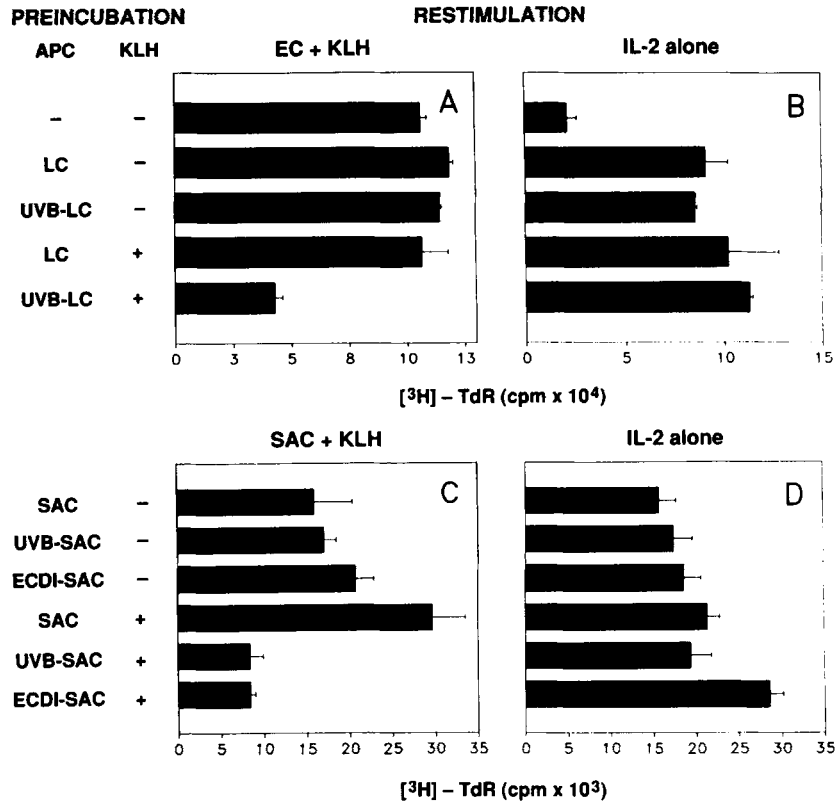

Figure 1. UVB-LC or UVB-SAC induce Ag-specific unresponsiveness in Th1. Th1 (2×10^5 cells/dish) were preincubated (37°C, 5% CO_2, 20 h) in 24-well plates with unirradiated or UVB-irradiated LC or SAC (4×10^4 or 6.4×10^6 cells/dish, respectively). Th1 incubated for 20 h in the absence of APC served as negative, and Th1 cocultured with ECDI-treated SAC served as positive controls. After preincubation, Th1 were isolated and restimulated with either unirradiated IEC (*A*) or SAC (*C*) or without APC in rIL-2 alone (100 U/ml) (*B* and *D*). After 48 h, [^{3}H]-TdR (1 μCi/well) was added for the final 16 h of the cultures to determine Th1 proliferation. Proliferation of LC, SAC, or Th1, when cultured alone, was <400 cpm. As another control Th1 were preincubated with unirradiated LC or SAC in the presence of KLH and then not restimulated; proliferation of these Th1 was modest: 12,300 ± 1,540 (*A*) or 2,800 ± 430 cpm (*C*). Data are expressed as the mean cpm ± SEM of triplicate measurements. Figure 1 shows results representative of five separate experiments.

and *C*), suggesting that 20 h of preincubation in the presence of KLH had activated Th1. However, proliferation of Th1 preincubated with KLH-pulsed, UVB-SAC was reduced by 50% over that of TH1 preincubated with either unpulsed SAC or unpulsed UVB-SAC. These results do not represent an immunologic null event, rather they indicate that KLH-pulsed UVB-LC or UVB-SAC actively induce T cell anergy.

The anergy induced by UVB-irradiated APC was never complete (Fig. 1 *A* and *C*). Two explanations may account for this residual proliferation: 1) UVB-irradiated APC are incapable of inducing complete unresponsiveness, or 2) it is impossible to achieve complete unresponsiveness in our HDK-1 Th1 clone with any type of modified APC. To distinguish between these possibilities, we compared the level of HDK-1-unresponsiveness achieved by UVB-SAC with that of ECDI-SAC, a method known to induce complete anergy in the Th1 clones used by Jenkins and Schwartz (14). Inasmuch as ECDI-SAC reduced HDK-1 responsiveness to the same degree as UVB-SAC (Fig. 1 *A* and *C*), we favor the second alternative.

Additional experiments revealed that after preincubation with UVB-APC, Th1 were unresponsive to restimulation with various concentrations of unirradiated KLH-pulsed APC (0.6, 1.2, 2.4×10^5 IEC or 4, 8, 16×10^5 SAC, data not shown).

Unresponsiveness induced by UVB-LC or UVB-SAC is long lasting. The duration of Th1 unresponsiveness was determined by resting Th1 after preincubation for various periods of time before restimulation. After preincubation with UVB-LC or UVB-SAC, Th1 remained unresponsive to restimulation with unirradiated APC for up to 16 or 8 days, respectively, whereas retaining their responsiveness to exogenous rIL-2 (Fig. 2).

Unresponsive Th1 exhibit defect in autocrine IL-2 production. Th1 activation by normal APC results in a complex set of premitotic events, which include the production of T cell growth factors, including IL-2 (10). To explore the cause for the proliferative unresponsiveness of Th1 cells, we assessed their capacity to produce IL-2, which is an essential Th1 growth factor (35). IL-2 secretion by Th1 was examined during preincubation with UVB-LC or UVB-SAC and during restimulation with unirradiated APC. At both time points, the detectable IL-2 activity in SN derived from such Th1 was significantly reduced (Table I).

SN from UVB-irradiated APC fail to induce Th1 unresponsiveness. To determine whether soluble suppressor factors released by UVB-irradiated APC cause Th1 unresponsiveness. SN from KLH-pulsed, UVB-irradiated or unirradiated APC were added to cocultures of Th1 and KLH-pulsed unirradiated IEC (Fig. 3*A*) or SAC (*B*). Proliferation of these cells was not different from that of Th1 cultured without SN. This result does not exclude the possibility that UVB-irradiated APC could produce suppressor factors during interaction with T cells. However, SN from cocultures of Th1 cells with KLH-pulsed, UVB-IEC (Fig. 3*C*) or UVB-SAC (*D*) also failed to suppress Th1 proliferation. We therefore conclude that soluble suppressor factors released by irradiated APC are probably not responsible for Th1 unresponsiveness.

Allogeneic SAC prevent induction of unresponsiveness by UVB-LC or UVB-SAC. Jenkins et al. (16) reported that normal allogeneic APC can restore the responsiveness of T cells stimulated with ECDI-treated APC when such cells were present during preincubation. We therefore asked whether allogeneic SAC could also prevent UVB-induced Th1 anergy. Addition of unirradiated allogeneic SAC to preincubation cultures of Th1 with UVB-LC (Fig. 4*A*) or UVB-SAC (*B*) restored completely the

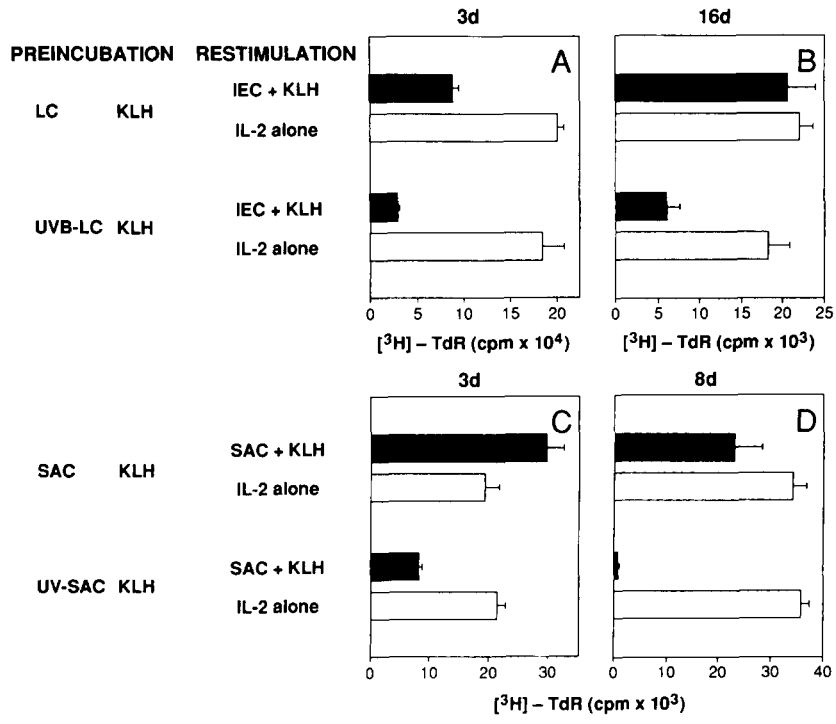

Figure 2. The unresponsiveness induced by UVB-LC or UVB-SAC is long lasting. Th1 were preincubated with unirradiated or UVB-irradiated LC (*A* and *C*) or SAC (*B* and *D*) and then isolated as described in Figure 1. Such Th1 were then rested in RPMI supplemented with rIL-2 (2 U/ml) for 3 days (*A* and *C*), 8 days (*D*), or 16 days (*C*). As described in Figure 1, Th1 were then washed, restimulated either with APC plus KLH (*closed bar*) or with rIL-2 without APC (*open bar*). Proliferation was determined at 64 h by [^{3}H]-TdR incorporation and data are expressed as the mean cpm ± SEM of triplicate measurements. A set of controls were performed: Th1 were preincubated with APC or UVB-APC in the absence of KLH and then restimulated with unirradiated APC and KLH. Such Th1 gave an optimal proliferative response of 49,590 ± 3,100 or 53,030 ± 5,070 (*A*) or 23,600 ± 2,790, 19,730 ± 4,000 (*C*). These results were also not different from Th1 that had been preincubated in the absence of APC: 63,400 ± 9,800 cpm (*A*) and 30,400 ± 2,570 cpm (*C*). Figure 2 shows results representative of three separate experiments.

TABLE I

Unresponsive Th1 are defective in their autocrine IL-2 production[a]

Source of SN		HT-2 Proliferation (cpm ± SEM) in Presence of	
		anti-IL-4	anti-IL-2
Controls			
		600 ± 120	610 ± 100
rIL-2 (1 U/ml)		93,100 ± 9,630	—[b]
rIL-2 (10 U/ml)		97,150 ± 5,380	—[b]
Preincubation			
IEC		38,980 ± 8,470[c]	650 ± 100
UVB-IEC		2,880 ± 600	650 ± 50
SAC		40,500 ± 5,410[c]	1,250 ± 320
UVB-SAC		1,900 ± 80	—
Preincubation: Restimulation			
IEC	IEC	12,680 ± 720[c]	1,340 ± 200
UVB-IEC	IEC	7,220 ± 650[d]	840 ± 80
SAC	SAC	5,150 ± 490[c]	640 ± 50
UVB-SAC	SAC	800 ± 130[d]	690 ± 80

[a] SN from preincubation or restimulation cultures were harvested at 20 h and added to HT-2 cells in the presence or absence of anti-IL-4 (11B11) or anti-IL-2 mAb (S4B6). As negative control, HT-2 were incubated in RPMI alone; HT-2 cultured with graded doses of human rIL-2 served as the positive control. After 36 h HT-2 proliferation was determined by measuring [^{3}H]-TdR incorporation.

[b] Not performed since S4B6 is unable to neutralize human rIL-2.

[c] In the absence mAb HT-2 proliferation was 38,540 ± 2,250; 42,450 ± 1,320; 11,090 ± 350; 5,400 ± 980, respectively.

[d] Statistical significance $p < 0.01$ and 0.001, respectively. Data are expressed as the mean cpm ± SEM of triplicate measurements and are representative for two separate experiments.

responsiveness of such Th1 to subsequent restimulation. Restoration of Th1 responsiveness was not due to direct presentation of KLH by allogeneic SAC, because KLH-pulsed allogeneic SAC failed to induce Th1 proliferation (570 ± 100 cpm). Similar results were obtained when Th1 were rested for 3 days before restimulation (data not shown). These data strongly suggest that UVB perturbs the capacity of APC to deliver a costimulatory signal(s) (presumably supplied by unirradiated allogeneic SAC) essential for Th1 proliferation.

DISCUSSION

The present studies are the first to demonstrate that UVB-irradiation confers upon APC (LC or SAC) the capacity to induce long-lasting, Ag-specific tolerance in CD4$^+$ cells of the Th1 subset. This tolerant state is due to functional inactivation rather than physical deletion of Th1, thus resembling in many aspects the T cell anergy induced by chemically fixed APC (14–19).

How do UVB-irradiated APC induce Th1 anergy? We first considered the possibility that UVB-LC or UVB-SAC release suppressor factors and tested the effects of SN from irradiated APC on Th1 proliferation. The inability of SN to reduce proliferation makes it unlikely that soluble factors released by UVB-APC are the principal cause for Th1 unresponsiveness.

A second possibility is that UVB alters an APC function that is essential for the induction of Th1 proliferation. In previous studies we have shown that unresponsiveness of Th1 to KLH presented by UVB-LC or UVB-SAC is not due to modification of MHC class II-complexes on such APC (23). The present studies have extended these observations with the demonstration that addition of normal allogeneic APC during preincubation with UVB-LC or UVB-SAC will prevent the development of anergy. These results parallel those obtained previously with ECDI-fixed APC (6), and are consistent with the hypothesis that UVB alters or deletes an essential costimulatory function of LC or SAC distinct from the interaction of MHC class II-associated Ag with the TCR. In this model, allogeneic APC (themselves incapable of presenting KLH) appear to prevent unresponsiveness by providing the costimulatory signal(s) that is missing from LC or SAC after UVB irradiation. Additional preliminary experiments have suggested that allogeneic APC require close physical contact with Th1 for this effect to occur (data not shown), consistent with the possibility that the putative costimulatory activity is membrane bound or acts over a short

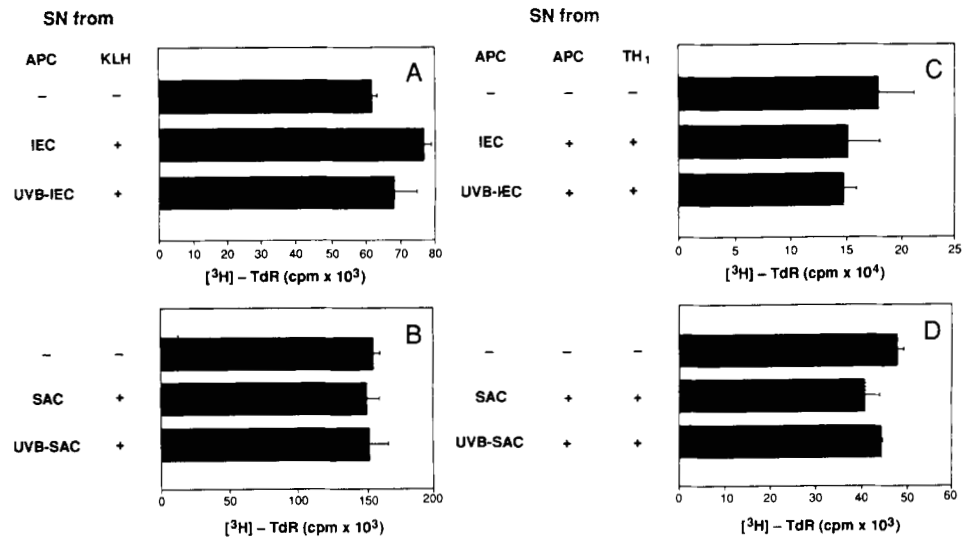

Figure 3. SN from UVB-LC or UVB-SAC do not mediate Th1 unresponsiveness. SN were obtained from KLH-pulsed, unirradiated or UVB-irradiated IEC or SAC (1×10^6 or 6.4×10^6 cells/24-well dish, respectively) cultured either alone (*A* and *B*) or together with Th1 cells (2×10^5 cells/well) (*C* and *D*). After 20 h, SN were harvested and added (1/1 dilution) to Th1, cocultured with unirradiated IEC (*A* and *C*) or SAC + KLH (*B* and *D*). After 64 h, Th1 proliferation was determined by [^{3}H]-TdR incorporation. Data are expressed as the mean cpm ± SEM of triplicate measurements. Figure 3 shows results representative of three separate experiments.

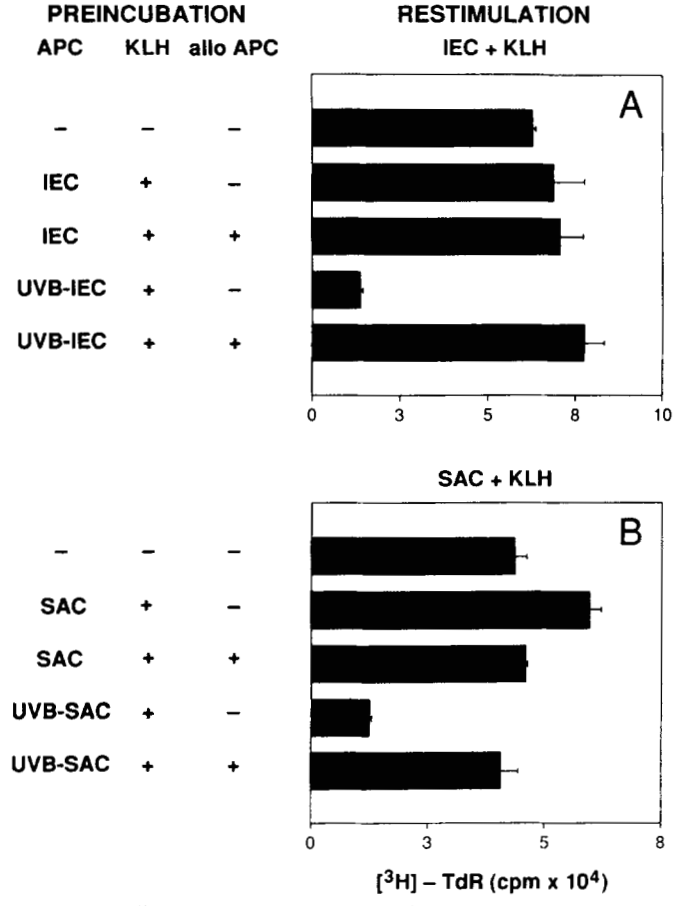

Figure 4. Allogeneic SAC prevent the induction of unresponsiveness by UVB-LC or UVB-SAC. Th1 cells were preincubated with unirradiated or UVB-irradiated IEC (*A*) or SAC (*B*) as described in Figure 1. In some panels, allogeneic unpulsed SAC (3.2×10^6 cells/dish) were added during preincubation. After 20 h of preincubation, Th1 were isolated and restimulated with unirradiated IEC or SAC as described in Figure 1. After 64 h, Th1 proliferation was determined by [^{3}H]-TdR incorporation. Data are expressed as the mean cpm ± SEM of triplicate measurements. Proliferation of IEC, SAC, or Th1, when cultured alone, was >400 cpm. Proliferation of Th1 cocultured with allogeneic SAC was 570 ± 100. Figure 4 shows results representative of three separate experiments.

distance (6).

Whatever the UVB-sensitive costimulatory function on LC or SAC is, it is probably required specifically for activation of Th1 cells, because we have previously shown UVB-LC to be fully capable of inducing proliferation of Th2 cells (23). These findings support the concept that Th1 and Th2 differ in their response to tolerogenic signals (18). One candidate for the Th1-specific costimulator is a membrane-bound, non-IL-1 activity described by Weaver et al. (36, 37). Alternative candidates are the accessory molecules LFA-1, ICAM-1, CD2, LFA-3, CD28, CD45, each of which are known to deliver costimulatory signals through interaction with ligands on T cells (38–53).

In light of reports by Jenkins et al. (16), that increasing the concentration of Ag during restimulation assays could partially overcome Th1 anergy in their system, future experiments might examine effects of KLH concentration when added directly to the restimulation culture.

We also investigated changes within Th1 that are associated with unresponsiveness. Our current hypothesis is that UVB-APC possess unaltered MHC class II molecules, which in association with processed Ag can effectively engage the TCR on Th1, but lack an essential costimulatory function. In the absence of this costimulatory function, TCR occupancy leads to an alternative activation program that produces unresponsiveness rather than proliferation. In line with this hypothesis, we examined the activation state of anergic Th1. Unresponsive Th1 exhibited deficient autocrine IL-2 production both during preincubation with UVB-LC or UVB-SAC and during restimulation with normal APC and KLH. This phenomenon has been described for T cells tolerized by other techniques (13, 15, 18, 20, 54, 55) and is associated with a defect in IL-2 transcription (6, 15). We have yet to determine whether the deficiency in IL-2 production induced by UVB-irradiated APC also occurs at this level.

Although unresponsive Th1 were unable to produce IL-2 and to proliferate upon stimulation with normal APC, such cells responded normally to exogenous rIL-2, indicating that these cells were viable and expressed sufficient amounts of IL-2R. Thus, Th1 anergy induced by UVB-irradiated APC is due to an inability of Th1 to produce IL-2 in amounts sufficient to drive their proliferation, and not to their inability to respond to IL-2. Once again, these results parallel observations made in other models of tolerance induction (13–17, 19). By contrast,

tolerance induced by UVB-APC differs from tolerance induced by ECDI-APC in that KLH-pulsed UVB-SAC does not augment responsiveness of Th1 to exogenous IL-2 whereas KLH-pulsed ECDI-SAC do (Fig. 1*D*). This difference suggests that ECDI-fixed, but not UVB-irradiated APC, up-regulate IL-2R expression on HDK-1, thereby raising the possibility that ECDI-fixed and UVB-irradiated APC deliver different activation signals that both lead to proliferative unresponsiveness in Th1.

Defective IL-2 production may result from impaired intracellular second messenger pathways within Th1; indeed, in other systems, unresponsive T cells possess decreased IP turnover and PKC activity (11, 15, 54). By contrast, other studies have shown unresponsive Th1 to proliferate (in the presence of allogeneic costimulators) despite continuous low levels of IP turnover and PKC activity, prompting Mueller et al. (17) to conclude that Th1 unresponsiveness is due to a defect in costimulatory signaling distinct from the IP/PKC signal transduction pathway. The CD28 molecule has been linked to such an alternative activation pathway (49–52), making CD28 a candidate for the putative ECDI- and/or UVB-sensitive costimulatory signal.

UVB-APC may also alter the expression of T cell molecules required for activation, as has been suggested for tolerant human T cells (12, 56). Evidence against this hypothesis comes from the observation that tolerant mouse Th1 cells express normal levels of Ag receptors and other accessory molecules (6, 13, 15, 19). We have yet to determine the expression of such molecules in our unresponsive Th1. Another possibility is that UVB-LC and UVB-SAC may induce Th1 to synthesize new proteins that prevent IL-2 production, a notion supported by the findings of Quill and Schwartz (19), who demonstrated cycloheximide to block unresponsiveness induced by purified Ia on planar membranes.

In summary, we have demonstrated that a single dose of UVB (200 J/m^2) converts the APC function of mouse LC (and SAC) for Th1 from one that is immunogenic to one that is tolerogenic. This finding has several important implications for the regulation of T cell-mediated immune responses in vivo because Th1 cells are important effector cells for DTH responses, a capacity that appears to be related directly to their production of IFN-γ (57). As cited previously, UVB-irradiated LC not only prevent the induction of CH to epicutaneously applied hapten but also induce tolerance to the same hapten (1, 5). We therefore propose that UVB-irradiated LC can produce Ag-specific T cell tolerance in DTH and CH reactions in vivo via functional inactivation of Th1 cells. This mechanism does not exclude concurrent generation of suppressor cells (e.g., Th2 and/or CD8$^+$ The cells) or production of soluble suppressor factors (2, 3).

UVB radiation is among the most ubiquitous agents encountered in the environment, and humans are frequently exposed to the relatively low fluences of UVB used in our experiments. It is, therefore, not unreasonable to speculate that tolerogenic LC play a relevant role in humans. UVB-irradiated tolerogenic LC may be detrimental to homeostasis in skin with respect to immunosurveillance against tumor or viral Ag. However, these cells may subserve a physiologic function as peripheral inactivators of autoreactive T cells.

Acknowledgments. We thank Drs. E. S. Vitetta, T. R. Mosmann, and D. Fiorentino for cell lines or antibodies, Drs. R. Fernandez-Bottran, L. S. Davis, T. S. Geppert, G. Girolomoni, A. Takashima for helpful discussions, and Dr. D. L. Mueller for carefully reviewing this manuscript. The expert technical assistance of L. Siverio-Horna was highly appreciated.

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