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The effect of ultraviolet radiation-induced suppressor cells on T-cell activity

S. E. ULLRICH *Department of Immunology, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas, U.S.A.*

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SUMMARY

The suppression of contact hypersensitivity (CHS) after a single exposure to ultraviolet (UV) radiation provides an excellent model system with which to study both the activation and the mode of action of suppressor T cells. Suppression of CHS after UV radiation is mediated by hapten-specific suppressor T cells (UVTs). These cells have a broad range of activity: CHS and antibody production *in vivo* and the generation of cytolytic T lymphocytes (CTL) and T-cell proliferative responses *in vitro* are suppressed by UVTs. The present study is concerned with determining the target of UVTs. The UVTs could suppress the response to hapten-modified T-dependent antigens, such as trinitrophenyl (TNP)-modified sheep erythrocytes (TNP-SRBC) or TNP-conjugated bovine serum albumin (TNP-BSA), but had no suppressive effect on the response to a T-independent antigen, TNP-conjugated lipopolysaccharide (TNP-LPS). The UVTs also suppressed the generation of interleukin-2 (IL-2) *in vitro*. The suppression of CTL generation *in vitro* and CHS *in vivo* could be overcome by the addition of exogenous IL-2. These data suggest that UVTs suppress the immune response by affecting T-helper cell function.

INTRODUCTION

The cellular interactions involved in the regulation of the immune response are complex and, at the present time, are not completely understood. Considerable progress has been made in understanding the signals required to activate suppressor cell pathways by studying the suppression of the immune response by ultraviolet (UV) radiation. Exposure to UV radiation causes an impairment in the ability of splenic antigen-presenting cells (APC) to initiate an immune response (Greene *et al.*, 1979; Noonan *et al.*, 1981). The defect in APC function is also associated with the development of hapten-specific suppressor T lymphocytes. It was suggested that the activation of these suppressor cells was associated with a loss of the ability of UV-treated APC to present antigen to helper cells. Recent studies (Granstein, Lowy & Greene, 1984; Granstein, 1985; Granstein & Greene, 1985; Noma, Usui & Dorf, 1985) have demonstrated, however, that activation of the suppressor cell pathway is an active process involving the participation of a novel APC present in the skin and spleen. The activity of this I-J+ cell is resistant to the dose of UV radiation that inactivates normal APC activity (i.e. presentation of antigen to Lyt 1^+ helper cells), and the net result is the activation of hapten-specific suppressor T cells.

Our studies have been concerned with how the suppressor cells induced after a single exposure to UV radiation (UVTs) regulate the immune response. An appreciation of the mechanism by which UVTs suppress immune reactivity is important for a number of reasons. First, a greater understanding of how UVTs function should expand our knowledge of suppressor cell networks. More important, the involvement of UV-induced suppressor T cells in the development of cutaneous neoplasms is well documented (Spellman & Daynes, 1977; Fisher & Kripke, 1977, 1982). Studies aimed at determining the mechanisms by which UVTs suppress the immune response should aid in our understanding of the pathogenesis of skin cancer. Our previous studies have demonstrated that suppressor cells generated after a single exposure to UV are Lyt 1^+2^- cells (Ullrich & Kripke, 1984) that can suppress contact hypersensitivity (CHS) and hapten-specific antibody production *in vivo* (Ullrich, Yee & Kripke, 1986). These Lyt 1^+2^- cells can also suppress the generation of hapten-specific cytolytic T lymphocytes (CTL) and the proliferation of T cells in response to hapten-modified splenic-adherent cells *in vitro* (Ullrich, 1985; Ullrich *et al.*, 1986). At least two signals are required to induce the UVTs: UV radiation followed by hapten sensitization. Neither UV radiation alone nor simply hapten sensitization can induce the suppressor cells (Kripke, Morison & Parrish, 1983; Ullrich,

Abbreviations: APC, antigen-presenting cells; CHS, contact hypersensitivity; CTL, cytolytic lymphocytes; IL-2, interleukin-2; NR, non-irradiated controls; TNP, 2, 4, 6, trinitrophenyl; TNP-BSA, TNP-conjugated bovine serum albumin; TNP-LPS, TNP-conjugated lipopolysaccharide; TNP-SAC, TNP-modified splenic adherent cells; UV, ultraviolet; UVTs, ultraviolet radiation-induced suppressor T cells.

Correspondence: Dr S. E. Ullrich, Dept. of Immunology, HMB 178, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, TX 77030, U.S.A.

1985). The suppressive activity of the UVTs is specific for the hapten used to sensitize the UV-irradiated animal. For example, the suppressor cells isolated from UV-irradiated mice that were sensitized with trinitrochlorobenzene (TNCB) could suppress the generation of CTL specific for the hapten trinitrophenyl (TNP) but had no suppressive effect on the generation of CTL specific for the hapten dinitrophenyl (DNP). Conversely, UVTs generated by sensitizing a UV-irradiated mouse with dinitrofluorobenzene (DNFB) could suppress on anti-DNP response but had no effect on the anti-TNP response (Ullrich *et al.*, 1986). In addition, while the UVTs that were specific for TNP could suppress the anti-TNP antibody response, they had no effect on the generation of antibody to the carrier, sheep erythrocytes (Ullrich *et al.*, 1986). What is not apparent from these studies is the target of the UVTs. However, the finding that antibody production is suppressed by UVTs provides an opportunity to examine the effect UVTs have on helper T-lymphocyte function. This was accomplished by measuring the effect of UVTs on the response of thymus-dependent and thymus-independent antigens. The data presented here suggest that UVTs affect helper T-cell activity, in that the response to trinitrophenyl-modified lipopolysaccharide (TNP-LPS), a T-independent antigen, is not suppressed by UVTs but the response to T-dependent antigens is. In addition, the generation of interleukin-2 (IL-2) *in vitro* is suppressed by UVTs, and the suppression mediated by UVTs can be abrogated by the addition of exogenous IL-2. These data suggest that UVTs mediate their suppressive activity by inhibiting the generation of T-helper cell activity.

MATERIALS AND METHODS

Animals

Specific pathogen-free female C3H/HeNCr (MTV$^-$) or BALB/c AnN mice were supplied by the NCI-Frederick Cancer Research Facility Animal Production Area (Frederick, MD). The mice were between 8 weeks and 12 weeks old at the beginning of each experiment. The animals were housed and cared for according to the guide-lines set forth in *The Guide for the Care and Use of Laboratory Animals*, Department of Health and Human Services, publication number (NIH) 78-23.

Induction of suppressor cells by ultraviolet radiation

The method of inducing suppressor cells has been published in detail elsewhere (Ullrich, 1985). In brief, the mice were irradiated with 30 kJ/m^2 UVB (280–320 nm) radiation during a single 3-hr exposure. Five days later, they were sensitized by the application of 100 μl of a 3% solution of 2, 4, 6 trinitrochlorobeneze (TNCB) on the ventral, unirradiated skin. Six days following sensitization, their ears were measured with a micrometer (Swiss Precision Instruments, Los Angeles, CA), and they were challenged by the application of 10 μl of a 1% solution of TNCB to each ear. Twenty-four hours later, the ears were remeasured and the ear swelling was determined. Control mice (NR) were sensitized and challenged as described above, but were not exposed to UV radiation. If suppression of the CHS response was observed in the UV-irradiated mice, their spleens were removed, and T cells were enriched by passage over nylon wool (Julius, Simpson & Herzenberg, 1973). Spleen cells from NR mice were treated similarly and were used as control cells in the subsequent assays.

Determination of antibody production

The slide modification (Mishell & Dutton, 1967) of the Jerne & Nordin (1963) plaque assay was used to determine the number of antibody-forming cells in the spleens of immunized mice. The mice were injected intravenously (i.v.) with either 10 μg trinitrophenyl-coupled bovine serum albumin (TNP-BSA) prepared according to the method of Little & Eisen (1967), or 10 μg TNP coupled to lipopolysaccharide (TNP-LPS; Sigma Chemical Co., St Louis, MO), or 0·1 ml of a 1% (v/v) solution of TNP-modified sheep erythrocytes (TNP-SRBC) (Rittenberg & Pratt, 1969). Four days after immunization, the number of direct plaque-forming cells in the spleens of the animals was determined. The effect of UVTs on antibody production was measured by injecting recipient mice i.v. with 10^8 UVTs immediately prior to immunization.

Measurement of suppressor cell activity in vitro

The ability of UVTs to suppress the generation of anti-TNP cytolytic T lymphocytes (CTL) was used to assess suppressor cell activity *in vitro*. A modification of the procedure of Shearer (1974) was used. Stimulator cells were prepared by modifying normal spleen cells (NSC) with 10 mM trinitrobenzene sulphonic acid (TNBS). The cells were washed three times and cultured with an equal number (5×10^6) of NSC in 24-well dishes. The culture medium used was RPMI-1640, supplemented with 10% fetal calf serum, 2-mercaptoethanol (5×10^{-5} M), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamine, 10 mM HEPES buffer, and 1 × vitamins and non-essential amino acids (Gibco, Grand Island, NY). Generally 1×10^7 UVTs or NRT cells were added to the cultures, bringing the final volume to 2 ml. After 5 days in culture, the cells were used as effector cells in a 4-hr ^{51}Cr-release assay. A TNP-modified fibrosarcoma was labelled with ^{51}Cr (ICN Radiochemical, Irvine, CA), and the cells were used as the target cells. Various numbers of effectors were added to 10^4 target cells and cultured in a 96-well V-bottomed microtitre dish. The radioactivity present in the supernatant after 4-hr was converted to percentage cytotoxicity using the following formula:

$$\% \text{ cytotoxity} = \left(\frac{\text{experiment release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \right) \times 100$$

where experimental release equals counts per minute (c.p.m.) present in the supernatants of wells with effector cells; total release equals the c.p.m. present in the supernatants of target cells incubated with 1% Triton X-100; and spontaneous release equals the c.p.m. present in the supernatants of target cells incubated with medium.

In certain experiments, TNP-modified splenic adherent cells were used as stimulator cells. Normal spleen cells were separated into adherent and non-adherent cell populations by a 1-hr incubation at 37° on plastic dishes. The adhereent cells were removed with 12 mM lidocaine (Astra Pharmaceutical Products, Worcester, MA) and further fractionated on a 50% discontinuous Percoll density gradient (Sigma Chemical Co.). The cells at the interface were collected, washed, and conjugated with TNP, as described above.

Effect of UVTs on the generation of IL-2

The ability of supernatants from the *in vitro* culture system to support the proliferation of an IL-2-dependent cell line was used to measure IL-2 activity. Supernatants were harvested after a 3-

day culture period, and serial dilutions of the supernatants were added to 5000 HT-2 cells [originally described by Watson (1979), kindly provided to us by Dr Susan Rich, Baylor College of Medicine, Houston, TX] in a final volume of 0·2 ml in a 96-well microtitre dish. The cells were cultured overnight, after which 1 μCi/well tritiated thymidine ([^{3}H]TdR, ICN Radiochemical) was added. After 6 hr, the amount of radioactivity incorporated by the cells was measured by harvesting the DNA onto glass fibre filters followed by liquid scintillation counting. A partially purified rat IL-2 (Collaborative Research, Lexington, MA) was used as a positive control.

Depletion of lymphocyte subpopulations

Monoclonal antibodies specific for the murine T-cell antigen Thy 1.2 (Becton Dickinson, Mountain View, CA) and the lymphocyte differentiation antigens Lyt 1.1 and Lyt 2.1 (New England Nuclear, Boston, MA) were used as described previously to deplete T-cell populations expressing these antigens (Ullrich & Kripke, 1984). Cell suspensions containing 1×10^7 cells/ml were treated with a 10^{-4} dilution of anti-Lyt 1.1 or Lyt 2.1 or a 1:150 dilution of anti-Thy 1.2 for 30 min at 4°. The cells were washed three times and then treated with a 1:8 dilution of rabbit complement (Pel-Freeze, Rodgers, AK) at 37°. After a 1-hr incubation, the cells were washed three times in Hanks' balanced salt solution.

Statistical analysis

The two-tailed Student's *t*-test was used to assess the significance of differences between control and experimental groups. All percentages were first converted into a normal distribution by the arcsine transformation before analysis by the Student's *t*-test. Data from representative experiments are shown; each experiment has been repeated independently two or three times.

RESULTS

The effect of UVTs on the antibody response to a thymus-independent antigen and thymus-dependent antigens

The effect of UVTs on the anti-TNP antibody response generated by immunizing mice with a T-independent antigen and T-dependent antigens is shown in Table 1. Transfer of 10^8 UVTs into normal mice suppressed the generation of anti-TNP antibody when the recipient mice were immunized with T-dependent antigens. However, when the recipient mice were immunized with TNP-LPS, a T-independent antigen that does not require T-helper cell activity, no suppression of the anti-TNP antibody response was observed. A possible interpretation of these data is that the UVTs may be suppressing T-helper cell activity. To examine this possibility further, the effect of UVTs on IL-2 activity was measured.

Suppression of IL-2 activity by UVTs

In order to measure the effect of UVTs on IL-2 activity, advantage was taken of the fact that UVTs suppress the generation of anti-TNP CTL *in vitro* (Ullrich *et al.*, 1986). A representative experiment demonstrating this phenomenon is found in Fig. 1a. The addition of UVTs to cultures containing TNP-conjugated stimulator cells and normal responder cells significantly suppressed the generation of anti-TNP CTL. Supernatants from these cultures were removed at 72 hr and IL-2 activity was measured. These data are shown in Fig. 1b. The addition of UVTs caused a significant suppression in the generation of IL-2 activity in the culture supernatants. The addition of an equivalent number of NRT had no suppressive effect either on the generation of CTL or on IL-2 activity in the supernatants.

Table 1. Effect of UVTs on the generation of a primary antibody response

Cell transferred*	Antigen†	Anti-TNP PFC/10^6‡	Suppression§	*P*¶
None	TNP-SRBC	592 ± 146	—	
NRT	TNP-SRBC	556 ± 76	0	
UVTs	TNP-SRBC	152 ± 18	73	<0·001
None	TNP-BSA	500 ± 26	—	
NRT	TNP-BSA	562 ± 44	0	
UVTs	TNP-BSA	154 ± 6	73	<0·005
None	TNP-LPS	288 ± 18	—	
NRT	TNP-LPS	272 ± 100	0	
UVTs	TNP-LPS	320 ± 34	0	NS

* 1×10^8 UVTs or NRT were injected i.v. prior to immunization.

† 10 μg/mouse of TNP-BSA and TNP-LPS; 0·1 ml of a 1% (v/v) solution of TNP-SRBC.

‡ TNP-HRBC were used as indicator cells. Three mice per group.

§ $\left(1 - \frac{\text{response of mice injected with UVTs}}{\text{response of mice injected with NRT}}\right) \times 100.$

¶ Probability of no difference from response of mice injected with NRT cells. Students' *t*-test (two-tailed): NS = not significant, $P > 0{\cdot}01$.

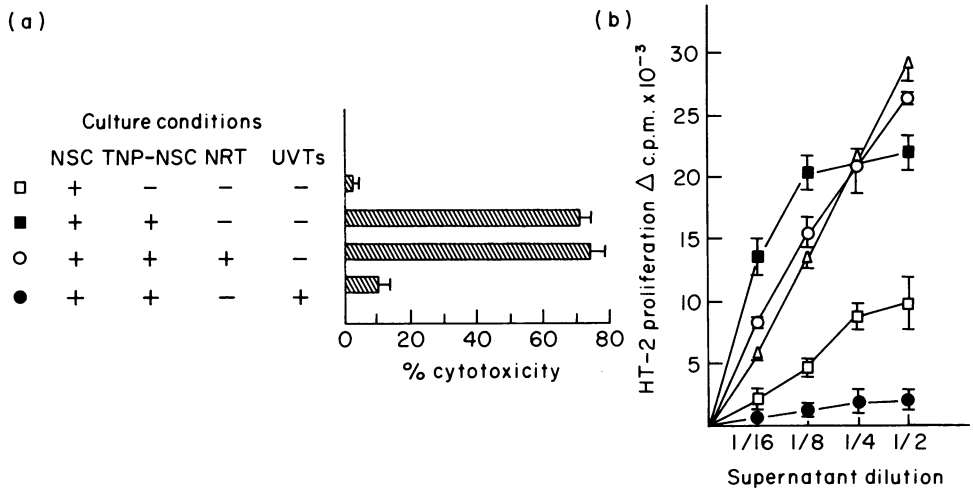

Figure 1. Suppression of CTL generation and IL-2 activitiy by UVTs. Normal spleen cells were cultured alone (□) or in the presence of TNP-NSC (■), TNP-NSC + NRT (○), or TNP-NSC + UVTs (●). Generation of CTL that lyse TNP-modified targets is shown in (a). The ability of supernatants from these cultures to support the proliferation of HT-2 cells is shown in (b). The response of HT-2 to partially purified IL-2 is also shown (△). The 1:2 supernatant dilution corresponds to 0·1 units/well of IL-2. All samples were run in triplicate.

The addition of exogenous IL-2 can overcome the suppressive effects of UVTs

It is not clear from the data presented in Fig. 1 if the suppression of CTL by UVTs is related to the reduced IL-2 activity in these cultures. To examine this point, an attempt was made to restore CTL activity by the addition of IL-2. The results from this experiment are found in Table 2. Various amounts of partially purified rat IL-2 were added to cultures containing TNP-modified stimulator cells, responder cells, and UVTs. The addition of 1 unit/ml of IL-2 totally overcame the suppressive effect of the UVTs; the amount of killing of the TNP-labelled targets by the CTL in these cultures was indistinguisable ($P > 0{\cdot}05$) from that observed in control cultures. This effect of IL-2 was dose dependent. Although effective killing of the TNP-modified targets was achieved in these cultures, no significant killing of Yac-1 cells, a cell sensitive to lymphokine-activated killer cells (LAK), was observed.

The effect of IL-2 on the suppression of CHS *in vivo* was also examined. In these experiments, suppressor cells were injected into normal recipient mice as described previously (Ullrich & Kripke, 1984). Immediately after injection of the suppressor cells, these mice were sensitized with hapten. One group of mice that received UVTs as well as a group of mice that received cells from control non-irradiated animals (NRT) were injected intraperitoneally with 100 units of IL-2. The IL-2 was administered on the day of sensitization and 1 day after sensitization. Six days after sensitization, the mice were challenged with hapten and the ear swelling was measured 24 hr later. The data in Table 3 demonstrate that the injection of IL-2 can totally overcome the suppressive effect of the UVTs.

Effector cell function is normal in UV-irradiated mice

The suppression of IL-2 activity and the failure of UVTs to suppress T-independent responses suggest that the immunological defect in UV-irradiated mice may be in the generation of T-cell help. However, due to the fact that exposure to UV radiation has a wide range of effects (reviewed by Kripke, 1984), we wanted to determine if effector cell function is affected by a single exposure to UV radiation. To do this, we took advantage of two previous findings. First, that the suppressor cells are Lyt 1^+2^- (Ullrich & Kripke, 1984), and second that Mizuochi *et al.* (1985) have demonstrated that the helper cells for the generation of anti-TNP CTL are L3T4$^+$ and Lyt2$^-$, and the effector cells are Lyt 2^+. Therefore, spleen cells from the UV-treated mice were separated according to their Lyt phenotype, and their functional capability was measured. Data from this experiment are shown in Table 4. In this experiment, the stimulator cells, TNP-conjugated splenic-adherent cells (TNP-SAC), were treated with monoclonal anti-Thy 1.2 to remove residual T cells. Addition of the stimulator cells to NR Lyt 1^+ and NR Lyt 2^+ cells resulted in the generation of anti-TNP CTL (Group 1). The use of UV Lyt 1^+ and UV Lyt 2^+ cells generated a cytolytic response that was slightly above background (Group 2 *vs* Group 5). The use of UV Lyt 1^+ and NR Lyt 2^+ cells also generated a response indistinguishable from background (Group 3 *vs* 6). However, when NR Lyt 1^+ cells were cultured with UV Lyt 2^+ cells in the presence of TNP-SAC, a CTL response similar to the positive control was generated (Group 4 *vs* 1). A low amount of cytotoxicity was generated when TNP-SAC were cultured with only Lyt 1^+ cells (Groups 7 and 9) or only Lyt 2^+ cells (Groups 8 and 10), indicating the efficacy of the monoclonal antibody depletions.

DISCUSSION

The data presented here suggest that UVTs function by interfering with helper cell activity. This is indicated by the data presented in Table 1. The effect of UVTs on the antibody response to TNP-LPS was examined because this response is not influenced by regulatory T cells (Braley-Mullen, 1982). If UVTs affect T-helper cell activity, then the response to TNP-LPS should be normal, whereas the response to TNP-BSA should be suppressed. As demonstrated by the data presented in Table 1, UVTs suppressed the response to TNP-BSA but had no effect

Table 2. Effect of the addition of exogenous IL-2 on the activity of UVTs *in vitro*

Culture conditions*	IL-2 (units/ml)†	Specific cytotoxicity of target cells‡	
		TNP-MCA 113	Yac-1
TNP-NSC+NSC	—	39·0±5·5	3·8±1·3
TNP-NSC+NSC+UVTs	—	8·4±0·9	5·0±4·0
TNP-NSC+NSC+UVTs	1·0	38·1±4·3	7·2±0·4
TNP-NSC+NSC+UVTs	0·5	18·7±2·4	4·1±0·8
TNP-NSC+NSC+UVTs	0·1	17·3±1·9	4·8±1·5
TNP-NSC+NSC+NRT	—	41·4±1·5	4·9±0·7
TNP-NSC+NSC+NRT	1·0	54·3±0·4	11·1±2·6
TNP-NSC+NSC+NRT	0·5	52·4±3·4	9·1±2·4
TNP-NSC+NSC+NRT	0·1	53·8±3·6	4·8±0·8

* Five million NSC were cultured with 5×10^6 TNP-NSC and 1×10^7 UVTs or NRT.

† Partially purified rat IL-2.

‡ Specific cytotoxicity was determined as described in the Materials and Methods. 50:1 effector to target ratio was employed. Samples were run in triplicate.

Table 3. Effect of the addition of exogenous IL-2 on the suppressive activity of UVTs *in vivo*

Group	Treatment of recipient mice*	ΔEar thickness	Specific ear swelling†	% suppression
1	No sensitization	4·3±1·9	0	—
2	No cells	13·3±2·0	9·0	0
3	UVTs	5·7±3·9	1·4	84
4	UVTs+IL-2‡	17·0±4·7	12·7	0
5	NR T	14·9±3·9	10·6	0
6	NR T+IL-2	13·6±4·5	9·3	0

* 1×10^8 UVTs or NR T were injected i.v. immediately prior to sensitization with 0·3% DNFB. Six days later the mice were challenged with 0·2% DNFB. Δear thickness was read 24 hr IL-2/mouse later. Units = cm × 10^{-3}.

† Specific ear swelling = ear swelling of mice sensitized and challenged minus the response of mice not sensitized but challenged. Five animals per group.

‡ 100 units IL-2/mouse.

on the response to TNP-LPS, suggesting that the UVTs suppress the generation of T-helper cell activity.

A second example of the effect of UVTs on T-helper cell function is the suppression of IL-2 activity. One potential problem with measuring IL-2 activity in a supernatant fluid is that the activity is a measure of the net balance between IL-2 production and consumption. While it is possible that the decreased level of IL-2 in the supernatant may reflect an increased consumption of the lymphokine, it does not appear to be the case for the following reasons. First, the addition of an equal number of NRT cells (isolated from non-irradiated but TNCB-sensitized mice) to the cultures did not result in a decrease in IL-2 activity. Second, the supernatants that had no IL-2 activity were isolated from cultures that had no CTL activity. We have shown previously that the UVTs can also suppress the proliferation of T cells in response to TNP-SAC (Ullrich, 1985). It seems unlikely that IL-2 would be consumed in cultures where CTL generation and T-cell proliferation are suppressed. Third, previous studies have clearly demonstrated the hapten specificity of these suppressor cells (Ullrich *et al.*, 1986). If the UVTs were simply consuming the IL-2 generated in the cultures, it would seem unlikely that their suppressive action would be specific for the hapten used to sensitize the UV-irradiated mouse. Finally, the use of glutaraldehyde-treated UVTs, a procedure that is reported to kill cells without affecting their ability to absorb IL-2 (Lominitzer, Phillips & Rabson, 1984), did not cause any suppression of CTL activity (data not shown). Therefore, the conclusion is that UVTs are acting to inhibit production and/or release of IL-2.

In order to test this hypothesis directly, the ability of exogenous IL-2 to overcome the suppressive effect of UVTs was measured (Table 2). The finding that IL-2 can overcome the suppressive activity of UVTs is consistent with the conclusion that UVTs inhibit the generation of T-cell help. Note that the addition of up to 1 unit/ml of IL-2 to the cultures did not generate LAK cell killing as measured by lysis of Yac-1 cells, a LAK cell-sensitive target. This is an essential control because Grimm & Wilson (1985) have demonstrated that LAK cells can kill TNP-modified targets. From these data we conclude that the increased killing of the TNP-modified target cells after culturing

Table 4. Effector cell function in UV-irradiated mice

Accessory cells* (TNP-SAC)		Responder cells† Lyt 1+	Lyt 2+	% cytotoxicity‡	$P<$§
1	+	NR	NR	36·3±2·0	
2	+	UV	UV	15·0±4·4	0·05
3	+	UV	NR	10·7±2·9	0·005
4	+	NR	UV	30·4±4·2	NS
5	−	UV	UV	8·6±2·6	0·005
6	−	NR	NR	7·3±2·1	0·001
7	+	NR	—	15·3±2·2	0·001
8	+	—	NR	13·6±3·4	0·05
9	+	UV	—	8·7±0·5	0·001
10	+	—	UV	10·0±0·8	0·001
11	+	—	—	0·3±0·4	0·01

* Adherent normal spleen cells were treated with anti-Thy 1.2 and complement and conjugated with 10 mM TNBS.

† Nylon wool non-adherent cells from UV or NR mice were separated into Lyt 1+ and Lyt 2+ cells by monoclonal antibody treatment. 5×10^6 TNP-SAC were cultured with $2{\cdot}5\times10^6$ Lyt 1+ cells and $2{\cdot}5\times10^6$ Lyt 2+ cells for 5 days.

‡ 50:1 effector to target ratio.

§ Probability of no difference form Group 1. Not significant (NS) = $P>0{\cdot}05$.

UVTs with stimulators and responder cells in the presence of IL-2 is not due to the generation of non-specific LAK cell killing by the IL-2 but a reversal of the suppressive effect of UVTs by IL-2. This conclusion is also confirmed by the data presented in Table 3. The injection of IL-2 *in vivo* could overcome the suppression of CHS by UVTs. Because the generation of CHS is not dependent upon the generation of cytolytic cells, these data support the contention that the introduction of the IL-2 is indeed overriding the suppressive effects of UVTs and not simply an *in vitro* artifact.

It is interesting to note that others have demonstrated that the UV-induced impairment in APC function can be abrogated by the addition of exogenous IL-2. Tominaga *et al.* (1983) and Granstein *et al.* (1984) have demonstrated that culturing UV-treated APC with immune effector cells in the presence of IL-2 could overcome the defect in APC function and generate anti-hapten CTL. While it is clear that the phenomenon described by Tominaga *et al.* (1983) and Granstein *et al.* (1984) is quite different from that described in this paper, it is noteworthy that the addition of IL-2 can overcome both the UV-induced impairment in APC function, and UVTs activity.

The data presented in Table 4 demonstrate that UV-irradiated mice can generate normal effector cell function. CTL were generated in cultures that contained Lyt 2+ cells from UV-treated mice and Lyt 1+ cells from NR control mice. This finding demonstrates that effector cell function is normal in these mice. This demonstration of normal effector cell function rules out 'clonal inhibition' as described by Miller, Sy & Claman (1977) after i.v. injection of free hapten as a possible mechanism for the immunological unresponsiveness found after a single exposure to UV radiation.

The suppressive activity of UVTs is reminiscent of the activity of the suppressor cells generated by painting mice with supraoptimal doses of hapten or by the i.v. injection of hapten-modified cells (Claman *et al.*, 1980; Monroe *et al.*, 1984), in that the UVTs are afferent suppressor cells (Ullrich, 1985), and they suppress a variety of responses to the hapten (Ullrich *et al.*, 1986). The major difference between the immunosuppression induced by UV radiation and the induction of suppressor cells by the i.v. injection of hapten or the use of supraoptimal amounts of hapten is the dose of hapten used and the route of sensitization. In these studies, the mice are sensitized with immunogenic doses of hapten by a route that normally induces an immune response. Pretreatment with UV radiaition modifies the immune system in such a way that sensitization with the immunogenic dose of hapten now induces suppression rather than immunity. Monroe *et al.* (1984) have proposed that the reason i.v. administration of antigen favours the induction of suppression rather than immunity is due to compartmentalization of APC in the immune system. They hypothesize that antigen injected i.v. comes into contact with the I-J+ UV-resistant splenic APC described in the literature (Granstein & Greene, 1985; Noma *et al.*, 1985), and that this interaction leads to the activation of suppressor cells. While the data presented here cannot prove or disprove this hypothesis, the many similarities between the suppressor T cells induced by i.v. immunization with hapten-modified spleen cells and those induced by UV radiation may lend some support to this contention. In addition, a recent report from this laboratory suggests that prior UV irradiation facilitates the entry of epicutaneously applied hapten into the circulation (M. L. Kripke and E. McClendon, submitted for publication). It is not inconceivable that the hapten is presented to the suppressor cell pathway by the I-J+ UV-resistant splenic APC. This may account for the many similarities between UVTs and those induced by i.v. immunization.

The data presented in this paper suggest that UVTs function by inhibiting the generation of T-helper cell activity. It is not apparent at this time exactly where the UVTs act to suppress the generation of T-cell help. It is conceivable that the UVTs may impair the proper presentation of antigen to helper T cells. We believe that the UVTs function by blocking an early step in the immune response. It is possible that the TNP-specific suppressor cells are recognizing TNP determinants on the TNP-conjugated antigen-presenting cells, and preventing the proper presentation of this antigen to helper T cells. This hypothesis is consistent with the observations that UVTs are afferent, hapten-specific suppressor cells (Kripke *et al.*, 1983; Ullrich *et al.*, 1986) that interfere with the release of IL-2. Studies measuring the binding of UVTs to TNP-coated dishes are in progress to examine this possibility. In order to examine these questions properly in detail, a cloned suppressor T cell is required, and attempts to clone this cell are in progress.

The phenotype of these cells, Lyt 1^+2^-, suggests that they may be 'suppressor/inducer' cells, and could function by inducing other cells to become the ultimate suppressor cell. An alternative possibility is that these Lyt 1^+2^- cells are similar to those described by Cantor *et al.* (1978) and function by a feedback inhibitory mechanism.

The finding that UVTs impair T-helper cell function may have important implications in uderstanding the immunological suppression of tumour rejection in UV-irradiated mice. It is possible that the impairment of APC function and the activation of UVTs are acting in concert and effectively shutting down the generation of antigen-specific T-helper cell activity in these mice. This may help to explain how these highly antigenic tumour cells (Kripke, 1974) can escape the immune system of the primary host. Experiments are currently in progress in our laboratory to measure T-helper cell activity in mice treated with a prolonged exposure to UV radiation.

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